Epigenetic Editing of Endogenous Periodontal Transcription Factors by CRISPR Activation

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INTRODUCTION

- Periodontitis is the eleventh most prevalent medical condition worldwide affecting 20-50% of the global population
- Current clinical treatments facilitate the attachment of long junctional epithelium to root surfaces rather than generate new connective periodontal tissues.
- Regeneration using stem cell programming is an attractive treatment approach for restoring periodontal 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 development.
- Simultaneous upregulation of genes may enhance periodontal ligament restoration. We take a multiplexed approach to precisely target and activate both periodontal and tendon master regulators.

HYPOTHESIS

We hypothesize that simultaneous upregulation of key transcriptional factors, SCX and MKX, using CRISPR epigenetic editing will activate downstream lineage pathways toward periodontal differentiation and stimulate periodontal ligament regeneration

METHODS

CRISPR activation system: Targeted CRISPRa gene activation of SCX and MKX was conducted using a SP-dCas9-VPR plasmid vector and designed gRNAs cloned into a pSB700 plasmid vector. CRISPR activation, a form of epigenetic editing is similarly programmed to target and bind specific genome regions. However, epigenetic editing does not cut the underlying DNA, instead recruiting chromatin modifiers to stimulate gene expression.

Guide RNA Design: Four gRNAs per gene, targeting the upstream promoter region of both SCX and MKX within 250 base pairs of the transcriptional start site were screened.

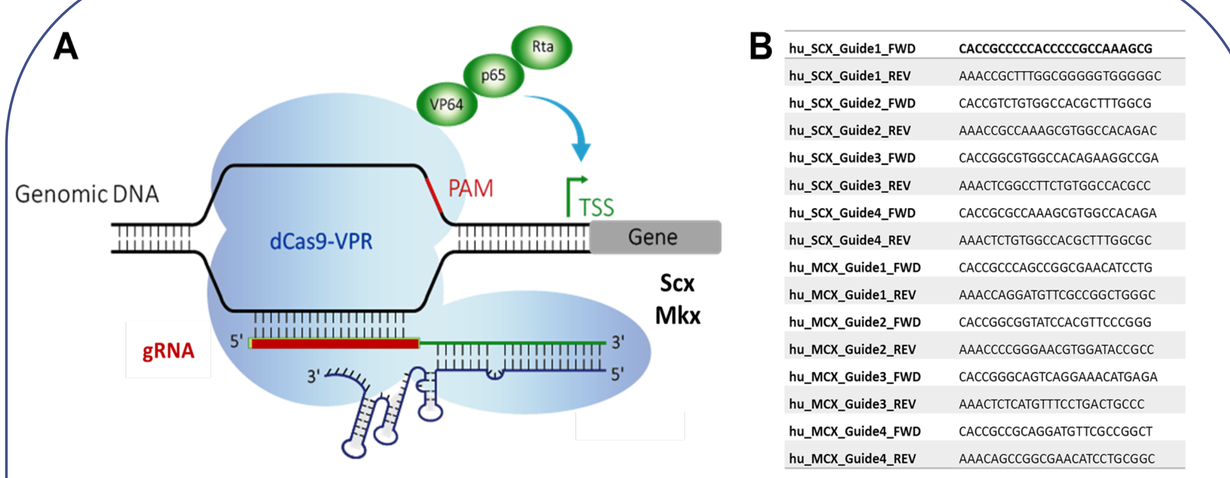
Gene expression: Cell samples were harvested after 48 hours, total RNA isolated, and cDNA synthesized from 0.1 – 1 µg of RNA. GAPDH was used as an endogenous control. qPCR was run on a ViiA 7 Real-Time PCR System.

3D Collagen Hydrogel Transfection: Dental pulp stem cells (DPSCs) were transfected with Lipofectamine 2000 and then encapsulated in collagen hydrogel just prior to deposition in a 24-well plate. Transfected cells were observed at 72 hours post transfection

Ex Vivo Transfection: Rat tendons were excised and transfected in 24 wells with Lipofectamine 2000. Transfected tissue was observed at 72 hours (not shown) and after 13 days.

RESULTS

1. CRISPR activation identifies best performing guide RNAs upregulating periodontal 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

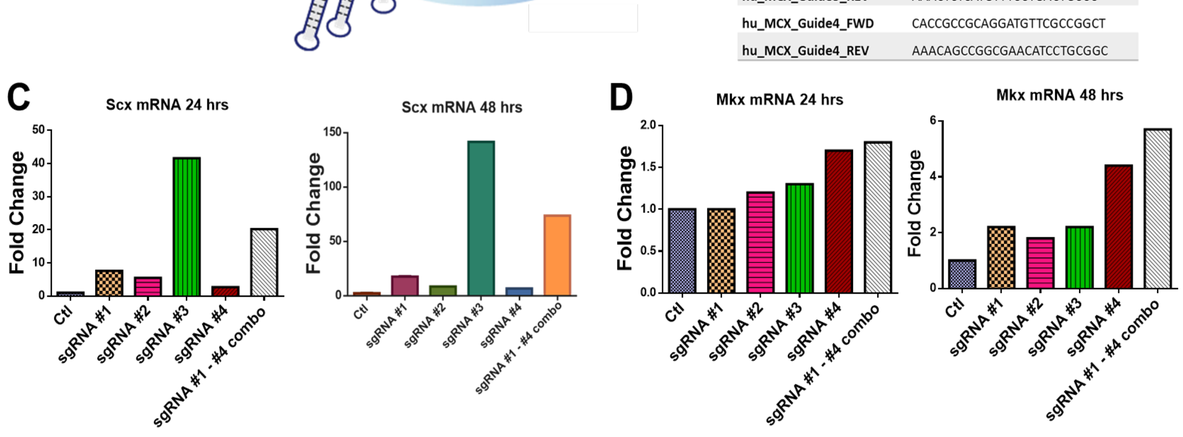


Figure 1. Targeted CRISPR activation system via dCas9-VPR upregulates specific gene targets guided by designed gRNAs.
(A) Model of CRISPR activation system **(B)** Sequences of gRNAs for SCX and MKX **(C)** Initial screening of gRNA activity of SCX **(D)** and MKX in HEK-293T cells.

2. Multiplex activation of SCX and MKX expression in HEK-293T cells using dCas9-VPR

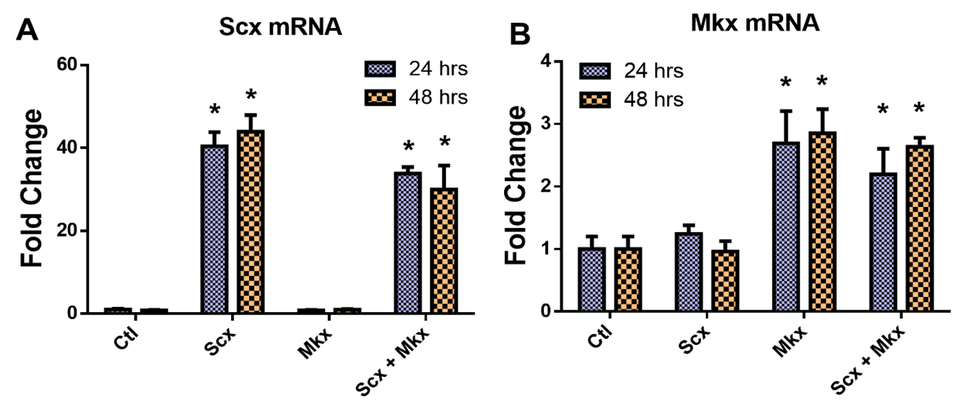


Figure 2. dCas9-VPR transfection with the best performing SCX and MKX gRNAs endogenously and concurrently upregulate gene expression (n = 3 per group: *:p<0.0001 compared to control).

3. CRISPR activation induces robust endogenous expression of Scleraxis in DPSCs

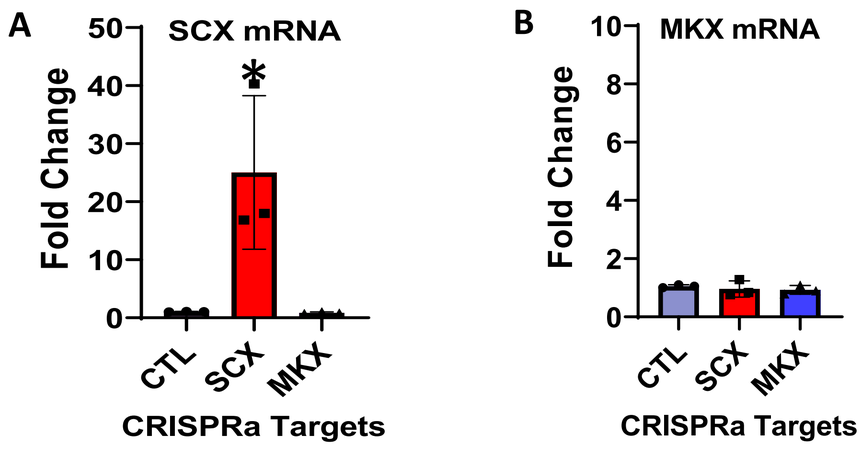


Figure 3. dCas9-VPR transfection with the best performing SCX #3 gRNA upregulates endogenous gene expression (A), however, MKX gRNA did not demonstrate gene induction (B). Gene expression analysis of transfected DPSCs demonstrate statistically significant increase in SCX. (n = 3 biological replicates *:p<0.0174 compared to the dCas9-VPR no insert control).

4. Moving towards *Ex Vivo* epigenetic editing

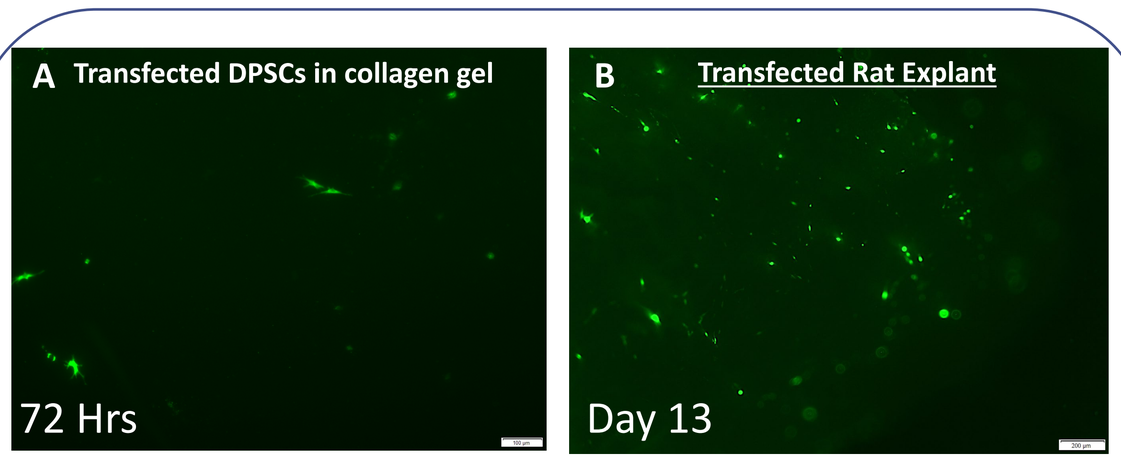


Figure 4. DPSCs encapsulated in a 3D collagen hydrogel (A) and rat tendon tissue explants (B) were successfully transfected, demonstrating the potential to transfect *ex vivo* tissues.
Fluorescence microscopy images show a sustained GFP plasmid expression of endogenous tendon tissue 13 days post transfection. (A) Scale bar = 100 µm (B) Scale bar = 200 µm.

FUTURE DIRECTIONS

- Future improvements include multiplexing of SCX and MKX in biologically relevant periodontal or dental pulp stem cells, leading to the multi-transcriptional network regulation.
- Each epigenetic locus is different and may require a customized approach to achieve the desired regulation.
- RNA sequencing (RNA-Seq) of CRISPRa edited DPSCs will characterize periodontal and tendon lineage programming, potentially regulated by multiplexed activation of SCX and MKX.

CONCLUSIONS

- Targeted gene activation, using the CRISPRa dCas9-VPR system, induced the endogenous activation of key tendon & periodontal ligament transcription factors, Scleraxis and Mohawk.
- We identified standout gRNAs for robust activation in HEK-293T and efficient transfection conditions for human DPSCs.
- Activation of SCX was significantly stronger compared to MKX, which could be due to guide RNA design, basal expression or the chromatin configuration of each gene promoter region
- Sufficient activation of these master regulators have the potential to activate necessary downstream targets to further stimulate periodontal ligament repair.
- Successful transfections of dCas9-VPR and gRNAs within a collagen hydrogel and rat tendon explants demonstrate a step towards ex vivo and in vivo clinical translation.

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