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#02

Design and Construction of Human CRISPR Activation Guide RNAs for Epigenome Editing of Craniofacial Disorders

Silvia Baila[1], Michelle N. Skelton[1], Sunil Wadhwa*[1], Christopher L. Ricupero[1]

[1]College of Dental Medicine, Columbia University, New York, New York: [*]Faculty mentor

INTRODUCTION

- Current regenerative treatment strategies for many craniofacial disorders fail to restore tissues to their native state.
- Cell fate programming is an attractive approach; however, it will likely require a multi gene transcriptional control.
- Our objective is to design, construct and screen molecular components for a CRISPR activation (CRISPRa) platform for precise gene modulation.
- We present an innovative strategy to build and apply a modular dCas9-VPR CRISPRa platform to precisely target, epigenetically edit and stimulate essential genes in craniofacial, ligament/tendon and neurological disorders such as Angelman syndrome and periodontitis.
- We hypothesize that properly designed guide RNAs (gRNAs) with dCas9-VPR will target and stimulate endogenous gene expression.

OBJECTIVES

Design, construct and screen molecular components for a CRISPR activation platform for precise gene modulation.

METHODS

Design human gRNAs
- gRNAs were specifically designed for MKX, SCX, UBE3A, GABRB3.
- Used Genetic Perturbation Platform algorithms for design scoring.
- Ranked gRNAs for specificity and off target activity

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Target the gRNAs
- gRNAs were targeted within 250 base pairs of each promotor region

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Insert gRNAs into a GFP expressing vector pSB700 plasmid
- 4-5 gRNAs were chosen based on top scores per target gene
- Golden Gate cloning was preferred method of choice

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Plasmid transduction
- NEB stable E. coli were transduced, grown overnight and 4 colonies per gRNA were picked for sequence verification

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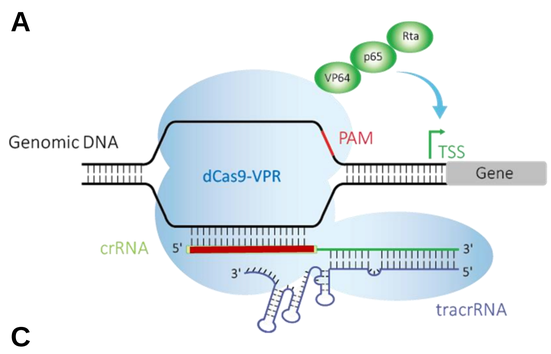
Utilize CRISPRa
- CRISPRa component dCas9-VPR was combined with each gRNA and transfected for transcriptional activation

RESULTS

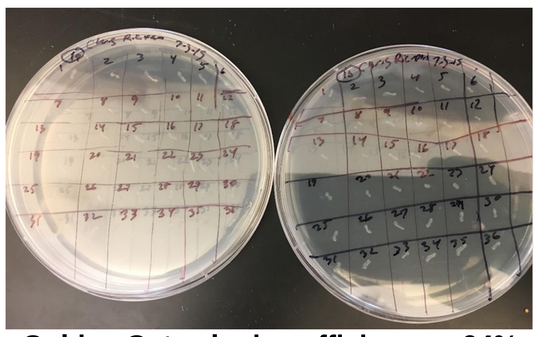
1. Human CRISPRa guide RNAs efficiently cloned into plasmid vectors and targeted to essential genes in craniofacial, ligament/tendon and neurological disorders.



B

Well Position	Name	Sequence
A1	hu_SCX_Guide1_FWD	CACCGCCCCCCACCCCCGCCAAAGCG
B1	hu_SCX_Guide1_REV	AAACCGCTTTGGCGGGGGTGGGGGC
C1	hu_SCX_Guide2_FWD	CACCGTCTGTGGCCACGCTTTGGCG
D1	hu_SCX_Guide2_REV	AAACCGCCAAAGCGTGGCCACAGAC
E1	hu_SCX_Guide3_FWD	CACCGGCGTGGCCACAGAAGGCCGA
F1	hu_SCX_Guide3_REV	AAACTCGGCCTTCTGTGGCCACGCC
G1	hu_SCX_Guide4_FWD	CACCGCGCCAAAGCGTGGCCACAGA
H1	hu_SCX_Guide4_REV	AAACTCTGTGGCCACGCTTTGGCGC
A2	hu_MCX_Guide1_FWD	CACCGCCCAGCCGGCGAACATCCTG
B2	hu_MCX_Guide1_REV	AAACCAGGATGTTCGCCGGCTGGGC
C2	hu_MCX_Guide2_FWD	CACCGGCGGTATCCACGTTCCCGGG
D2	hu_MCX_Guide2_REV	AAACCCCGGGAACGTGGATACCGCC
E2	hu_MCX_Guide3_FWD	CACCGGGCAGTCAGGAAACATGAGA
F2	hu_MCX_Guide3_REV	AAACTCTCATGTTTCCTGACTGCCC
G2	hu_MCX_Guide4_FWD	CACCGCCGCAGGATGTTCGCCGGCT

C



Golden Gate cloning efficiency = 94%

D

Well Position	Name	Sequence
A3	hu_UBE3A_Guide1_FWD	CACCGCGGGGCCAAGACGCGCGGGA
B3	hu_UBE3A_Guide1_REV	AAACTCCCGCGCGTCTTGGCCCCGC
C3	hu_UBE3A_Guide2_FWD	CACCGTGGGGCGGGGCCAAGACGCG
D3	hu_UBE3A_Guide2_REV	AAACCGCGTCTTGGCCCCGCCCCAC
E3	hu_UBE3A_Guide3_FWD	CACCGGGGGCGGGGCCAAGACGCGC
F3	hu_UBE3A_Guide3_REV	AAACGCGCGTCTTGGCCCCGCCCCC
G3	hu_UBE3A_Guide4_FWD	CACCGAGGCCGGGACGCGGGGACGT
H3	hu_UBE3A_Guide4_REV	AAACACGTCCCCGCGTCCCGGCCTC
A4	hu_GABRB3_Guide1_FWD	CACCGCGGCTCCGCCTCCGACTCTC
B4	hu_GABRB3_Guide1_REV	AAACGAGAGTCGGAGGCGGAGCCGC
C4	hu_GABRB3_Guide2_FWD	CACCGGGAGGCCGGAGAGTCGGAGG
D4	hu_GABRB3_Guide2_REV	AAACCCTCCGACTCTCCGGCCTCCC
E4	hu_GABRB3_Guide3_FWD	CACCGGGGGGAGGAGGCCGGAGAGT
F4	hu_GABRB3_Guide3_REV	AAACACTCTCCGGCCTCCTCCCCCC
G4	hu_GABRB3_Guide4_FWD	CACCGGGGGAGGGAGGAGCGCGCGC
H4	hu_GABRB3_Guide4_REV	AAACGCGCGCGCTCCTCCCTCCCCC
A5	hu_UBE3A_Guide5_FWD	CACCGCGGCGCCCTCCCGCGCGTCT
B5	hu_UBE3A_Guide5_REV	AAACAGACGCGCGGGAGGGCGCCGC
C5	Sequencing Primer	GAGGGCCTATTTCCCATGATTCC

Figure 1. CRISPR activation system via dCas9-VPR upregulates specific gene targets guided by designed gRNAs.
(A) Model of CRISPRa system using dCas9-VPR transcriptional activator (blue) plus a designed gRNA (red) specifically targeted upstream of gene of interest. **(B)** 8 designed gRNA sequences for tendon related regulators-- SCX and MKX. **(C)** 72 molecularly cloned gRNA E. Coli colonies (n=4 per cloned gRNA) picked and sent for sequencing. Each gRNA was cloned into a GFP expressing vector pSB700 plasmid. **(D)** 9 designed gRNA sequences for Angelman Syndrome critical genes-- UBE3A and GABRB3.

2. Strong viability and efficiency of dCas9-VPR and gRNAs transfected into HEK-293 cells.

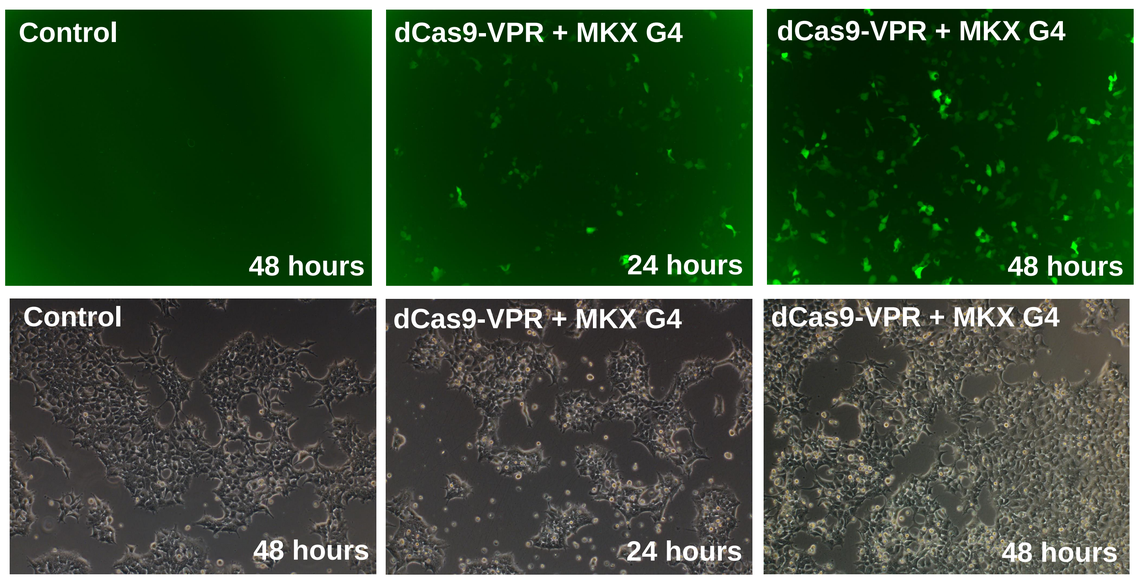


Figure 2. Increased gRNA expression observed over time. Transfection efficiency of HEK293-T cells was evaluated using dCas9-VPR and human gRNA targeting MKX. ~50k cells were transfected with 200 ng of dCas9-VPR and 10ng gRNA and evaluated at 24 and 48 hours. Cell viability and efficiency was robust and >80%..

3. CRISPR activation system utilizes designed gRNAs to upregulate UBE3A and the tendon transcription factors, SCX and MKX.

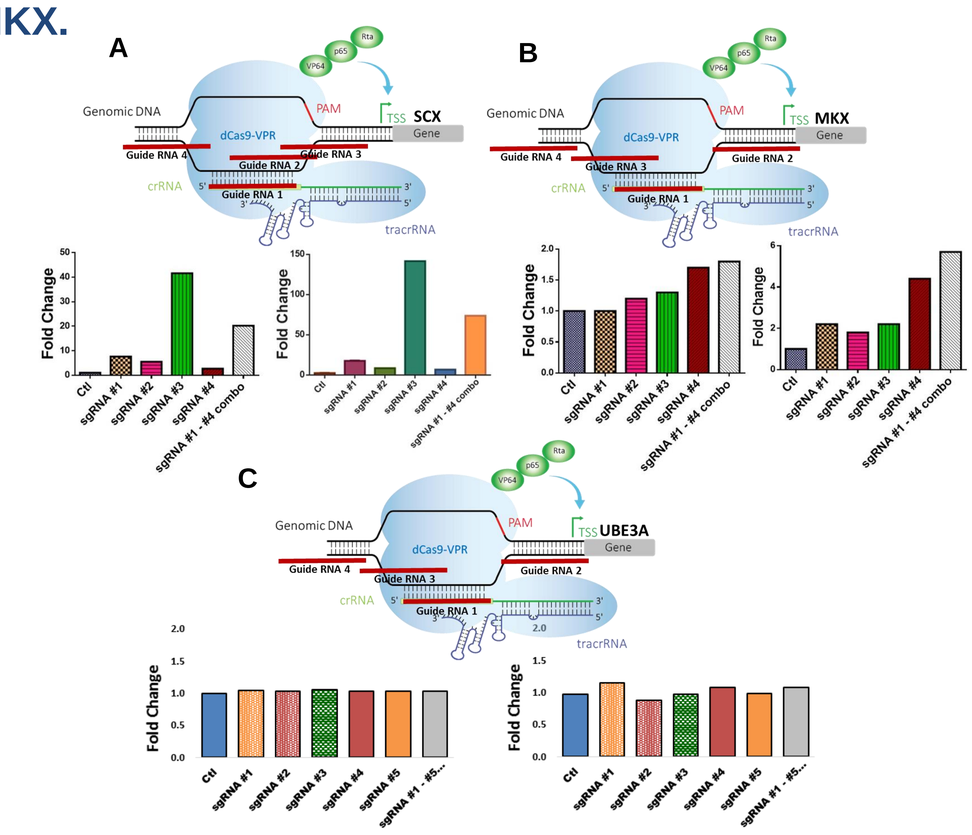


Figure 3. CRISPRa via dCas9-VPR and gRNA was utilized for upregulation of (A) SCX (B) MKX (C) UBE3A. Screening identified the best performing gRNAs for SCX and MKX, producing a 141 and 4.4 fold increase respectively post 48 hours. Interestingly, UBE3A did not show any significant upregulation at either time point.

DISCUSSION & CONCLUSIONS

- We successfully designed, cloned and evaluated 17 human guide RNAs for the CRISPR activation platform.
- These components precisely target and modulate endogenous gene expression through epigenetic editing.
- Screening identified the best performing gRNAs for SCX and MKX, producing a 141 and 4.4 fold increase respectively post 48 hours.
- Non-stimulating gRNAs may need to be redesigned due to the underlying chromatin architecture.
- Although gRNAs were designed to target two unrelated disorders, Angelman Syndrome and periodontitis/tendon injuries were selected because of their substantial health burdens on society.
- Therefore, this proof of principle platform highlights its modularity which can be utilized for multiple craniofacial disorders, disease or repair.

ACKNOWLEDGEMENTS

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