Zinc Finger Protein Transcription Factor-Mediated Repression of Alpha-Synuclein Expression as a Therapeutic Approach for Parkinson's Disease

Poster #P491

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Abstract

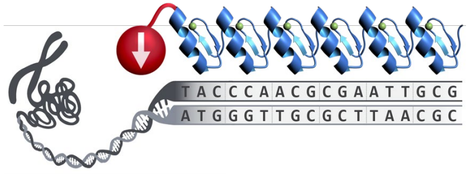
Aims: Molecular and genetic evidence implicate alpha-synuclein as a key mediator of Parkinson's disease (PD) pathogenesis. Reducing alpha-synuclein expression in neurons thus has the potential to halt or slow PD progression. Zinc finger proteins (ZFPs) are DNA-binding proteins that can be engineered to specifically bind any genomic sequence. When fused to transcriptional regulatory domains to form ZFP transcription factors (ZFP-TFs), they can modulate the expression level of the targeted gene. We engineered ZFP-TFs with human *SNCA* repression activity for use as potential therapeutics for PD.

Methods: ZFP-TFs were identified in a screen of human neuroepithelial cells. On-target activity was assessed in human iPSC-derived neurons transduced with AAVs expressing the ZFP-TFs (AAV-ZFP-TFs). Transcriptome-wide specificity analyses using Affymetrix microarrays were performed in human iPSC-derived and mouse primary neurons. ZFP-TFs with minimal off-target activity in both human and mouse neurons were administered to PAC synuclein mice, which express the full human *SNCA* sequence on a mouse *Snca*-null background. Animals were euthanized after 3 weeks and their brains were collected for molecular analyses.

Results: The initial screen identified ZFP-TFs with human alpha-synuclein repression activity ranging from ~40% to >99%. Experiments in human iPSC-derived neurons confirmed the on-target activity of the constructs and the durability of the response for 30 days. Alpha-synuclein AAV-ZFP-TFs with a range of repression activity and minimal off-target activity were well tolerated in vivo and led to significant downregulation of aSyn mRNA expression in the mouse brain.

Conclusions: The data presented here support continued development of ZFP-TFs as a potential therapy for PD.

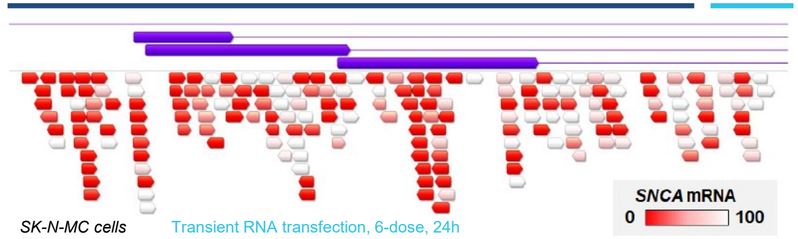
Gene regulation using ZFP-TFs



Human origin
ZFP and KRAB are from human genes, lower potential for adverse immune reactions
Compact
Easily packaged into AAV
High potency
2 targets per cell

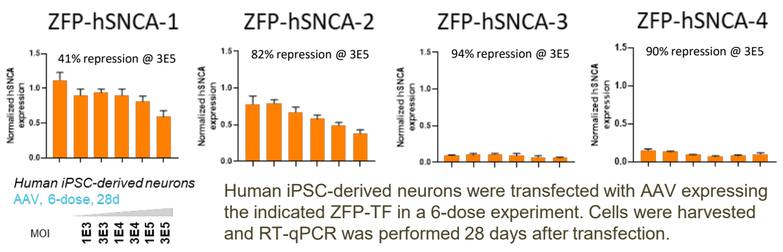
ZFP-TFs are comprised of 1) an engineered ZFP that binds the target genomic DNA sequence and 2) a functional domain to modulate gene expression .

>50% of screened ZFP-TFs repressed human α-synuclein



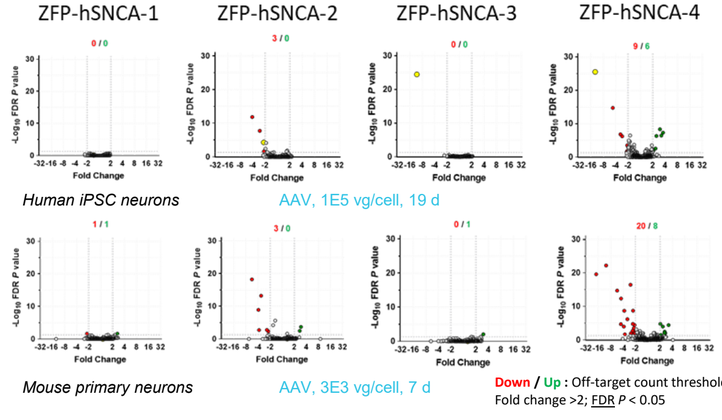
ZFP-TFs were screened for human alpha-synuclein repression in the SK-N-MC human neuroepithelial cell line. ZFP-TFs were designed against the sequence 500 bp upstream of transcription start site (TSS) 2a to 500 bp downstream of TSS 2b. There was a broad range of alpha-synuclein repression activity in the initial screen.

ZFP-TFs had a broad range of repression activity in human iPSC-derived neurons



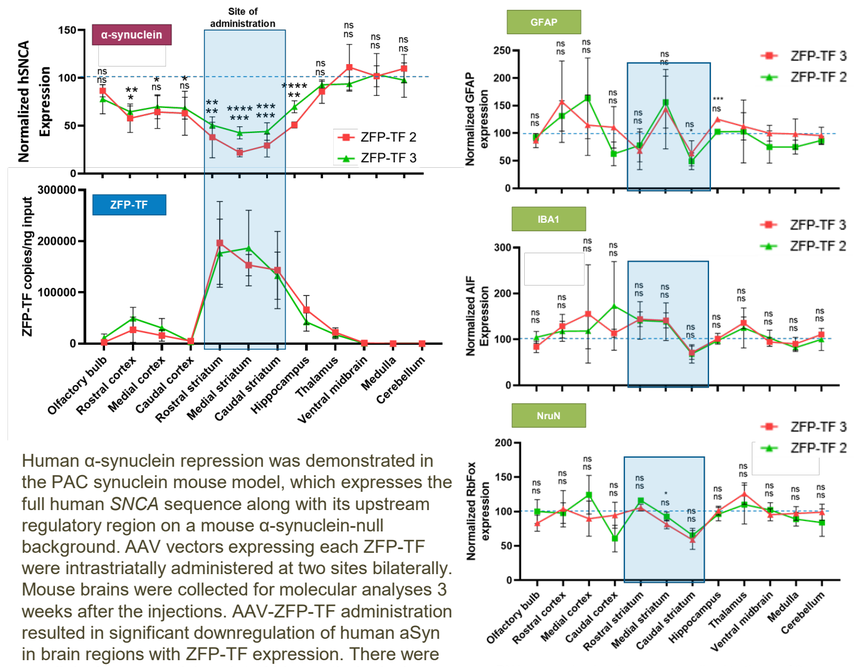
Human iPSC-derived neurons were transfected with AAV expressing the indicated ZFP-TF in a 6-dose experiment. Cells were harvested and RT-qPCR was performed 28 days after transfection.

Several ZFP-TFs had minimal to no detectable off-target activity



Off-target activity assessed using the Affymetrix platform. Human iPSC-derived neurons or mouse primary neurons were transfected with AAV expressing the indicated ZFP-TF.

ZFP-TFs reduced human alpha-synuclein expression *in vivo* and were well-tolerated



Human α-synuclein repression was demonstrated in the PAC synuclein mouse model, which expresses the full human *SNCA* sequence along with its upstream regulatory region on a mouse α-synuclein-null background. AAV vectors expressing each ZFP-TF were intrastriatally administered at two sites bilaterally. Mouse brains were collected for molecular analyses 3 weeks after the injections. AAV-ZFP-TF administration resulted in significant downregulation of human aSyn in brain regions with ZFP-TF expression. There were no clinical or test article-related histopathological findings, and no overt test article-related elevations in GFAP or IBA1 gene expression. NeuN expression indicates absence of neurotoxicity.

mRNA expression levels were normalized to those of 3 housekeeping genes. Gene expression levels in the ZFP-TF-treated groups were normalized to those in the vehicle control group. One-way ANOVA, Dunnett's multiple comparisons, n = 3, * p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001

Summary and Conclusions

- 55% of ZFP-TFs screened in SK-N-MC human neuroepithelial cells had >50% human alpha-synuclein repression activity
- Repression activity in the screen had a broad range, from 41% to >99%
- ZFP-TFs were selected for AAV production and displayed a range of human alpha-synuclein repression activity in human iPSC-derived neurons
- Several ZFP-TFs were shown to have no to minimal off-target activity in human iPSC-derived and mouse primary neurons in Affymetrix microarray experiments
- ZFP-TFs led to significant human alpha-synuclein repression and were well-tolerated *in vivo*
- The *in vitro* and *in vivo* data presented here support development of ZFP-TFs as a therapeutic strategy for Parkinson's disease and other synucleinopathies

Selected references

- Kingwell. Zeroing in on neurodegenerative α-synuclein. *Nat Rev Drug Disc.* (2017)
- Spillantini et al. alpha-Synuclein in filamentous inclusions of Lewy bodies from Parkinson's disease and dementia with lewy bodies. *PNAS.* (1998)

Author disclosures

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