Zinc Finger Protein Transcription Factors Targeting Alpha-synuclein - A Promising Therapeutic Strategy for Parkinson's Disease

Abstract #539

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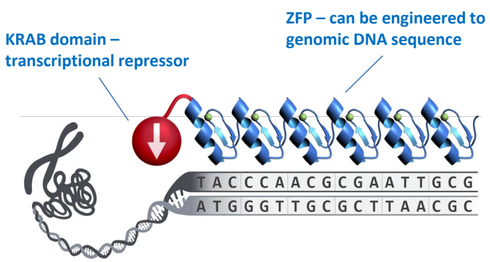
Abstract

Parkinson's disease (PD) is a progressive neurodegenerative disorder that leads to a wide range of motor and non-motor deficits, as well as dementia in ~50% of cases. Neuropathologically, PD is characterized by the loss of dopaminergic neurons in the substantia nigra and the appearance of intraneuronal inclusions called Lewy bodies and Lewy neurites, which contain alpha-synuclein (aSyn) aggregates. There is a large body of molecular and genetic evidence implicating aSyn as a key mediator of PD pathogenesis, and Lewy pathology is thought to propagate in the brain in a stereotyped prion-like manner as the disease progresses. Repressing the expression of aSyn in the brain thus has the potential to halt or slow PD progression.

Zinc finger proteins (ZFPs) are naturally occurring human DNA-binding proteins and may be engineered to specifically bind any genomic sequence. When fused to transcriptional regulatory domains to form ZFP transcription factors (ZFP-TFs), they can be used to modulate the expression level of the targeted gene. We produced ZFP-TFs comprising 1) a ZFP DNA-binding domain targeting the human SNCA sequence, and 2) the KRAB domain of the human Kox1 protein. These constructs were screened in human neuroepithelial cells transiently transfected with mRNA coding for each of 384 ZFP-TFs. The screen identified multiple ZFP-TFs with human SNCA repression activity ranging from ~40% to >99%. A subset of these constructs was further evaluated in human iPSC-derived and mouse primary neurons transduced with adeno-associated virus (AAV) coding for each of the ZFP-TFs (AAV-ZFP-TFs). Experiments in human iPSC-derived neurons confirmed the on-target activity of the constructs and the durability of the response for 30 days. Transcriptome-wide specificity analyses using the Affymetrix microarray platform were performed in both human iPSC-derived neurons and mouse primary neurons transduced with selected AAV-ZFP-TFs.

Highly specific ZFP-TFs were selected for an in vivo proof-of-concept study in the PAC synuclein mouse model, which expresses the full human SNCA sequence along with its upstream regulatory sequence on a mouse aSyn-null background. AAV vectors expressing each ZFP-TF were bilaterally administered to the striatum at two sites. Three weeks following AAV-ZFP-TF administration, the mice were euthanized and their brains were collected for molecular analyses. AAV-ZFP-TF administration was well-tolerated and led to statistically significant downregulation of aSyn mRNA expression in the brain. Further optimization of the specificity and on-target activity profiles of the ZFP-TFs is ongoing. Our findings support continued development of ZFP-TFs as a potential aSyn-targeted therapeutic approach for the treatment of PD.

ZFP-TFs – a differentiated genome engineering platform



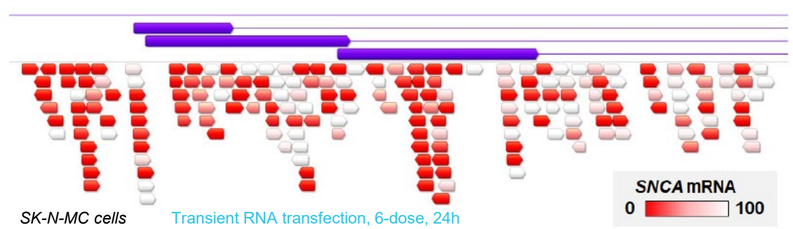
Advantages of ZFP-TFs

Human origin
ZFP and KRAB are from human genes, reduced immunogenicity

Compact
Easily packaged into AAV

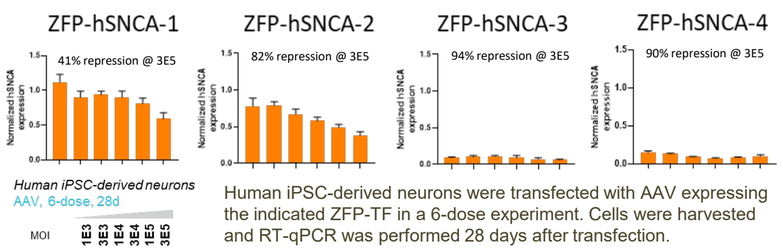
High potency
2 targets per cell

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



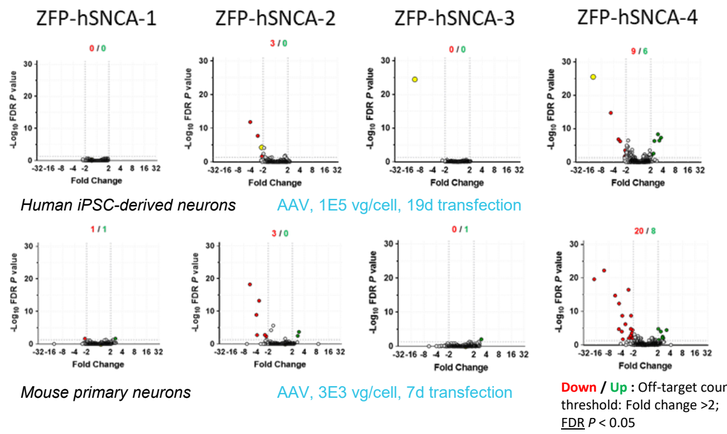
ZFP-TFs were screened for human alpha-synuclein repression in the SK-N-MC human neuroepithelial cell line. ZFP-TFs were designed against the sequence 500 bp upstream of transcription start site (TSS) 2a to 500 bp downstream of TSS 2b. There was a broad range of alpha-synuclein repression activity in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

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



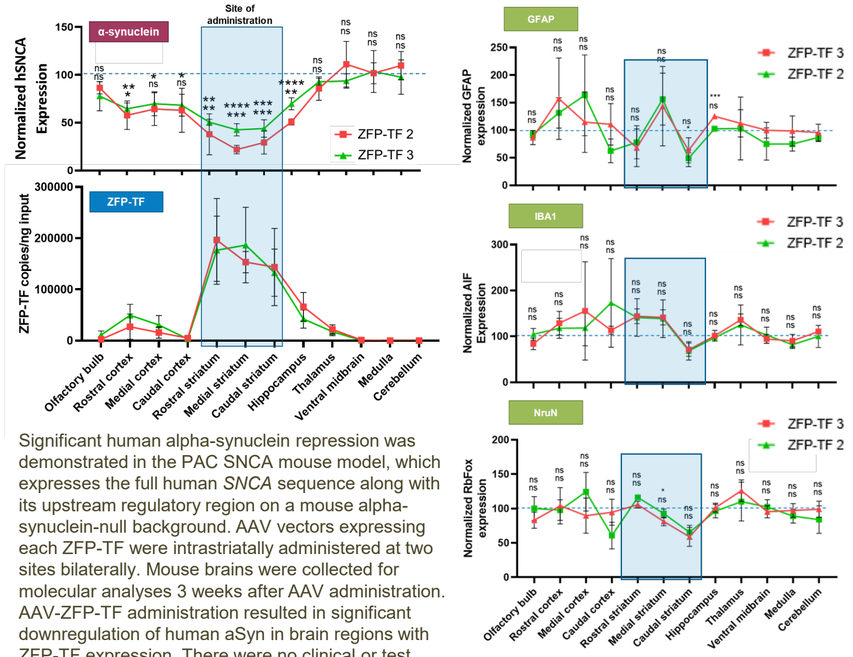
Human iPSC-derived neurons were transfected with AAV expressing the indicated ZFP-TF in a 6-dose experiment. Cells were harvested and RT-qPCR was performed 28 days after transfection.

Several ZFP-TFs had minimal to no detectable off-target activity in human iPSC-derived and mouse primary neurons



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



Significant human alpha-synuclein repression was demonstrated in the PAC SNCA mouse model, which expresses the full human *SNCA* sequence along with its upstream regulatory region on a mouse alpha-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 AAV administration. 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 the geometric mean of 3 housekeeping genes. hSNCA, GFAP, IBA1, and NeuN 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 repressed human alpha-synuclein expression by >50%
- 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

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- Spillantini et al. alpha-Synuclein in filamentous inclusions of Lewy bodies from Parkinson's disease and dementia with lewy bodies. *PNAS*. (1998)
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Author disclosures

All authors are or were employees of Sangamo Therapeutics.

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