SNCA Gene Repression Mediated by Zinc Finger Repressors (ZFRs) as a Therapeutic Approach for Parkinson's Disease

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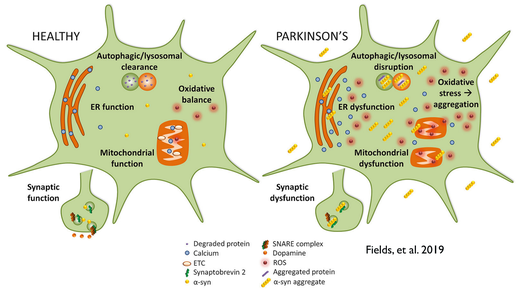
Introduction

- Molecular and genetic evidence implicate alpha-synuclein (SNCA) as a key mediator of Parkinson's disease (PD) pathogenesis and neuronal loss.
- Our goal was to identify a one-time therapeutic approach capable of robustly decreasing the expression of *SNCA* with high specificity for the target gene.
- We show that AAV packaged ZFRs decrease *SNCA* transcript in human cells lines with exquisite specificity.
- In addition, we demonstrate *SNCA* transcript reduction at the bulk tissue level within key brain regions using a humanized mouse disease model.

Alpha-synuclein's role in Parkinson's disease pathology



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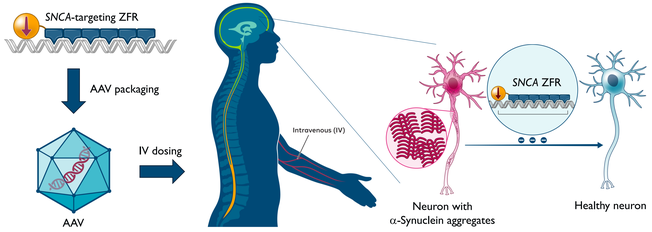
Recent advances in early detection of mutant SNCA in presymptomatic Parkinson's disease patients and its well-established role in disease pathology make it an attractive target for genomic medicine. Utilizing a blood brain barrier-penetrant AAV capsid would be optimal for systemic delivery of our *SNCA*-targeting ZFRs and brain-wide biodistribution to affected neurons.

Leveraging ZFRs for the potential treatment of Parkinson's disease



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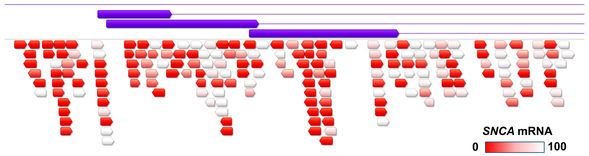
Large scale screening of human *SNCA*-targeting ZFRs

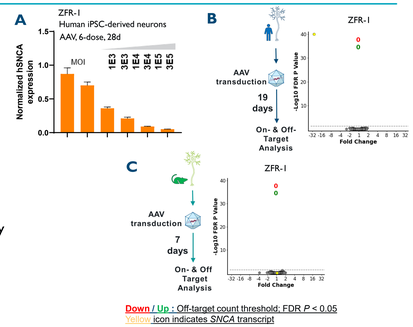


SNCA-targeting ZFRs were screened for human *SNCA* repression in the SK-N-MC human neuroepithelial cell line. ZFRs were designed against a sequence 500 bp upstream of the transcription start site (TSS) to 500 bp downstream of the TSS. Individual ZFRs showed a broad range of *SNCA* repression activity in the initial screen.

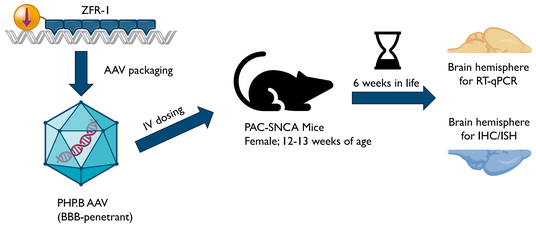
Select ZFRs show robust repression and high specificity *in vitro*

ZFR	*SNCA* Repression (max MOI)	Human DEGs	Mouse DEGs
ZFR-1	95%	0/0	0/0
ZFR-2	79%	1/0	0/1
ZFR-3	79%	2/0	3/0
ZFR-4	75%	1/0	1/0
ZFR-5	75%	1/0	1/0

Above: a table of select *SNCA*-targeting ZFRs that show robust on target activation and high specificity as seen in the # of differentially expressed genes (DEGs). On the left is the on-target dose response for ZFR-1 (A) and its DEG expression in volcano plots generated from microarrays following AAV transduction in human (B) and mouse neurons (C).



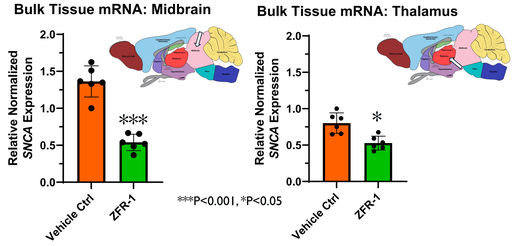
Approach for *in vivo* testing of ZFRs in a humanized mouse model



Following *in vitro* characterization of ZFR-1 indicating potent activity and high specificity, it was packaged into PHP.B AAV with a human synapsin promoter. AAV was administered via tail vein injection to PAC-SNCA mice at a dose of 1E14 vg/kg. After 6 weeks brain hemispheres were processed individually for molecular and histological analysis.

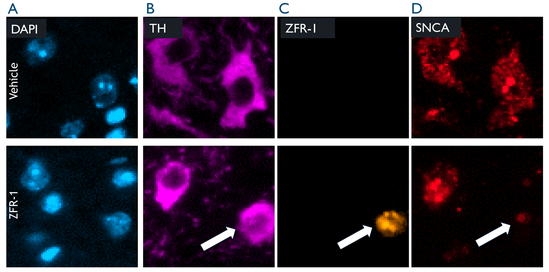
ZFR-1 represses human *SNCA* transcript in key brain regions

Tissue collected from the PAC-SNCA mice and analyzed by RT-qPCR showed a significant decrease in *SNCA* expression in the ZFR-1 treated group when compared with a vehicle treated control group. Black circles represent individual animals. Midbrain and thalamus are both critical centers of neurodegeneration in PD patients.



***P<0.001, *P<0.05

IHC/ISH processed tissue shows *SNCA* repression in TH+ neurons



Tyrosine hydroxylase (TH) positive cells in the substantia nigra are critical PD targets. Individual cells in the substantia nigra were identified by DAPI signal (A) and probed for TH (B), ZFR-1 (C) and SNCA (D). The white arrow indicates a TH+ neuron transduced by ZFR-1 that is not expressing SNCA. These findings support the bulk tissue RT-qPCR results from midbrain.

Conclusions and next steps

- We have identified potent and specific ZFRs capable of reducing human *SNCA* expression *in vitro* and *in vivo*.
- For next steps, we plan to quantify any changes in SNCA protein levels in neurons *in vitro* following treatment with select ZFRs.
- In addition, we plan to perform an *in vivo* proof of concept study using translationally-relevant, phenotypic endpoints with lead surrogate ZFRs in a rodent model of PD.

References

Carroll Rutherford Fields, Nora Bengoa-Vergiony and Richard Wade-Martins. Targeting Alpha-Synuclein as a Therapy for Parkinson's Disease Front. Mol. Neurosci., 05 December 2019 Sec. Brain Disease Mechanisms, Volume 12-2019. https://doi.org/10.3389/fnmol.2019.00299

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