Downregulation of SNCA Expression by Targeted Editing of DNA Methylation: A Potential Strategy for Precision Therapy in PD

Author: Boris Kantor*

*Viral Vector Core, Duke University Medical Center, Durham, NC 27710, USA

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Background

Parkinson’s disease (PD)

- is the second most common neurodegenerative disorder in the world
- is a brain disorder that leads to shaking and difficulty with walking, balance, and coordination.
- Parkinson's symptoms usually begin gradually and get worse over time. As the disease progresses, people may have difficulty walking and talking.
Background

SNCA gene

- The SNCA gene has been implicated as a highly significant genetic risk factor for PD

- α-synuclein, encoded by the SNCA gene, is one of the most validated and promising therapeutic targets for PD.
Hypothesis

- They aimed to identify and validate a target for achieving tight regulation of SNCA transcription that will allow maintaining normal physiological levels of α-synuclein.
- The manipulation of gene expression is a valuable therapeutic strategy for neurological disorders, such as PD.
Graphical Abstract

All-in-one vector → Lentiviral particles → SNCA-intron 1 methylation → SNCA-mRNA expression → α-synuclein protein levels → Cellular phenotypes

Mitochondrial Superoxide & Cell Viability
Results

The Design of the SNCA Intron 1-Targeted Methylation System

(A) Schematic description of the targeted region in SNCA intron 1. Upper panel: the SNCA gene structure. Lower panel: the sequence in intron 1 that contains the CpG island (chr4: 89,836,150–89,836,593 [GRCh38/hg38]). The gRNA sequences are marked in green, the PAM in red, and the CpGs are numbered in blue according to Jowaed et al. and appear in uppercase letters.
The Design of the SNCA Intron 1-Targeted Methylation System

(B) A schematic map of the designed vector cassette. A lentiviral vector backbone was created to include a unique BsrGI restriction enzyme site flanked by two BsmBI sites to be used for cloning gRNAs. dCAS9-DNMT3A-fused transgene was integrated into the expression cassette downstream of EFS-NC promoter. The vector also expressed a puromycin selection marker.
The Design of the **SNCA Intron 1-Targeted Methylation System**

Repression of **SNCA** transcription by dCas9-DNMT3A in hiPSC-derived dopaminergic neurons from a PD patient with the **SNCA** triplication.

Upper panel: low level of methylation (open lollipops) within the **SNCA** intron 1 region corresponds to a high level of the gene expression (ON).

Lower panel: gRNA-dCAS9-DNMT3A system targeting the CpGs within **SNCA** intron 1 to enhance methylation (closed lollipops) results in downregulated expression (OFF).
Characterization of the Stably Transduced SNCA-Tri MD NPCs

Representative immunocytochemistry images of the SNCA-Tri MD NPCs carrying the gRNA-dCas9-DNMT3A transgene. (A–J) MD NPCs showed the expression of Nestin (A–E) and FoxA2 (F–J) with no gRNA (A and F), gRNA1 (B and G), gRNA2 (C and H), gRNA3 (D and I), or gRNA4 (E and J). Scale bars, 10 μm. (K and L) MD NPC markers were evaluated using real-time qRT-PCR for (K) Nestin and (L) FoxA2. The levels of mRNAs were measured by TaqMan expression assays and calculated relative to the geometric mean of GAPDH mRNA and PPIA mRNA reference controls using the 2^−ΔΔCT method. Each column represents the mean of two biological and technical replicates. The error bars represent the SEM.
Characterization of DNA Methylation at the SNCA intron1 CpG Island Region

The methylation levels (%) of the 23 CpG sites in the SNCA intron 1 in the four hiPSC-derived MD NPC lines carrying the gRNA-dCas9-DNMT3A transgenes and the control line with the no-gRNA transgene. DNA from each of the 5 cell lines was bisulfite converted, and the methylation (%) of the individual CpGs was quantitatively determined by pyrosequencing.

*Bars represent the mean of percentage methylated CpG for two independent experiments.*
SNCA mRNA and α-Synuclein Protein Levels in the MD NPC Lines Carrying the gRNA-dCas9-DNMT3A Transgenes

(C) Quantification of the α-synuclein protein signals for each MD NPC line using ImageJ.
(D–G) Representative immunocytochemistry images for the α-synuclein signal (D and F) and the α-synuclein and Nestin double-staining signals (E and G) of the MD NPC lines.
(H) Quantification of the α-synuclein protein signal in the MD NPC line carrying the gRNA4-dCas9-DNMT3A vector and the control line with the no-gRNA vector.
The Effect of the gRNA4-dCas9-DNMT3A Transgene on Mitochondrial Superoxide Production and Cell Viability

Mitochondrial superoxide production (A) and cell viability (B) were measured in SNCA-Tri MD NPCs carrying the gRNA4-dCas9-DNMT3A transgene and the control MD NPC line carrying the no-gRNA transgene.
Global DNA methylation (5-mC%) of the MD NPC line carrying the gRNA4 transgene showed no statistically significant difference compared to the original untransduced hiPSC-derived MD NPC line (p = 0.59).

In contrast, the line carrying the no-gRNA transgene showed a significant increase in global DNA methylation relative to the original untransduced MD NPC line.
They developed a novel all-in-one lentiviral vector, harboring gRNA, dCas9 nuclease, and the catalytic domain of the DNMT3A, to target specific hypomethylated CpG islands in the SNCA intron 1 region.

Delivery of the novel system into a patient with the triplication of the SNCA locus resulted in the downregulation of SNCA mRNA and protein expression and reversed disease-related phenotypic perturbations.
Their study provides a proof of concept that the manipulation of gene expression, e.g., reversing overexpression, by epigenome editing, is a valuable therapeutic strategy for neurological disorders, such as PD, that involve dysregulation of gene expression.
REFERENCES