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Precision Modulation of Neurodegenerative Disease-Related Gene Expression in Human iPSC-Derived Neurons

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Introduction

The ability to reprogram adult somatic cells into induced pluripotent stem cells (iPSCs) and the subsequent development of protocols for their differentiation into disease-relevant cell types have enabled in-depth molecular analyses of multiple disease states as hitherto impossible. Parkinson's disease (PD) is one such example, in which the dopaminergic neurons that manifest pathology are embedded in an inaccessible region of the midbrain, the substantia nigra pars compacta, making it unfeasible to obtain samples of the affected region from living patients. Overabundance of alpha-synuclein (SNCA) has long been implicated in the pathogenesis of PD. However, the precise mechanism by which overexpression of SNCA leads to the demise of dopaminergic neurons remains elusive. Neurons differentiated from PD patient-specific iPSCs carrying multiplications of the SNCA gene may thus provide a means to recapitulate molecular phenotypes of the disease in vitro. The application of CRISPR/Cas9 to mammalian systems is likewise revolutionizing the utilization of genome editing in the study of molecular contributors to pathogenesis of numerous diseases, including PD.

Methods

We applied the nuclease-null or "dead" Cas9 (dCas9), fused to transcriptional activators and repressors to exert precise control over gene expression, in the absence of permanent alterations to the genome.

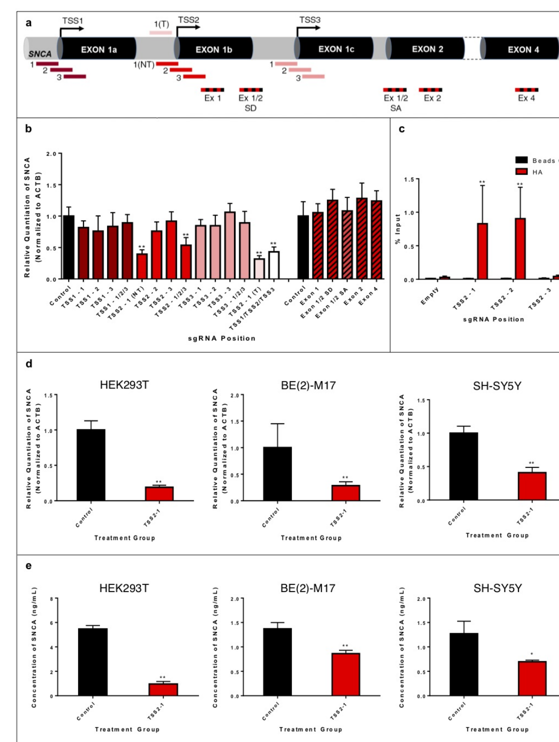
References

Heman-Ackah SM, Bassett AR, Wood MJA. Precision

Results

The transcriptional repressor, dCas9-KRAB, is used to silence SNCA gene expression in PD patient-specific iPSC-derived neurons harboring a triplication of the SNCA gene locus. In parallel, the transcriptional activator, dCas9-VPR, is used to activate endogenous SNCA expression in iPSC-derived neurons from a healthy control patient to levels that parallel SNCA overexpression due to triplication.

Screening of sgRNAs for CRISPRi Targeting SNCA Exons and Transcription Start Sites

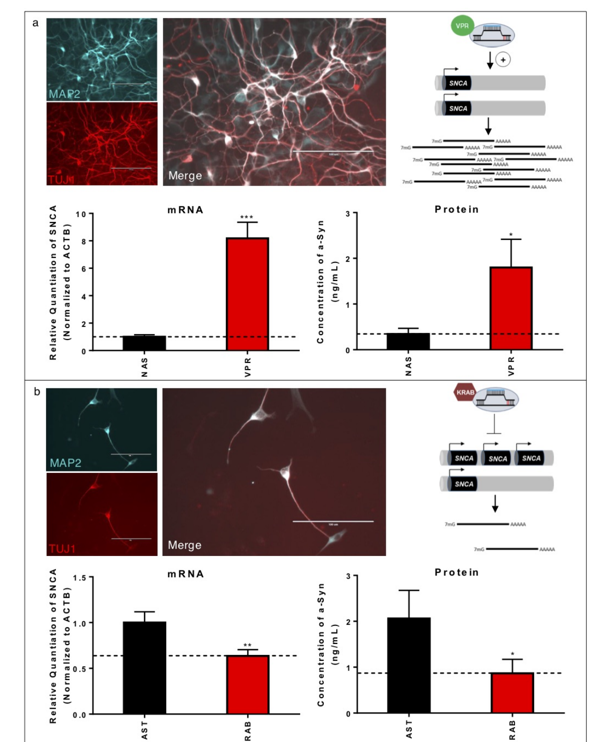


(a) Exon- and TSS-targeting sgRNAs. (b) qRT-PCR post-treatment. (c) HA ChIP of treated HEK293T cells (d) qRT-PCR of TSS2-1 sgRNA treated cells. (e) ELISA of TSS2-1 sgRNA treated cells. *p0.05, **p0.01.

Conclusions

The ability to execute precise transcriptional control over disease-associated gene expression in iPSC-derived neurons provides an unprecedented opportunity to evaluate the temporal course of pathogenic events contributing to development of neurodegenerative diseases, such as PD. This can be further developed for therapeutic purposes, so called "genome surgery", in the absence of permanent genome alterations, rendering this a safer platform for therapeutic development.

Modulation of SNCA Expression Levels in Human iPSC-Derived Neurons



(a) CRISPRa- and (b) CRISPRi-mediated control of SNCA expression.