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AAV-BASED SPLIT-dCAS9 GENE ACTIVATOR SYSTEM FOR IN VIVO REPROGRAMMING APPROACHES

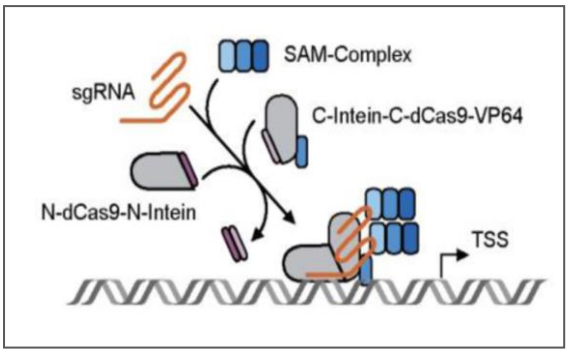
Keywords: *in vivo* reprogramming, CRISPRa, split-dCas9, gene activation, split-intein, Parkinson's Disease, AAV, gene therapy

INVENTION NOVELTY

This novel AAV-based split-dCas9 gene activator system (AAV-dCas9) allows endogenous gene activation of multiple genes and subsequent *in vivo* cell reprogramming. It has been validated for the reprogramming of striatal astrocytes to GABAergic neurons rescuing voluntary motor behavior in a toxin-induced murine model of Parkinson´s disease.

VALUE PROPOSITION

Parkinson's disease is the second most common neurodegenerative disorder. Current treatments focus on symptomatic disease management, either by pharmacological restoration of dopamine levels or electrophysiological pace making of downstream nuclei, which initially ameliorates motor symptoms. Alternative therapy options, aiming to replace lost neurons, have been explored with mixed beneficial outcome for patients, partly due to the lack of appropriate standardized tissue or alternative cell sources. Novel strategies based on efficient genetic tools for adjustable induction of multiple genes *in vivo* are offering novel options for therapeutic interventions via cellular reprogramming.



Scheme of the split-intein-dCas9 gene activator system [Giehrl-Schwab et al., 2022]

TECHNOLOGY DESCRIPTION

A split-intein-mediated dCas9 system for *in vivo* gene activation was developed for the reprogramming of striatal astrocytes to GABAergic neurons and it could be demonstrated that these GABAergic neurons functionally integrate into striatal circuits. For proper AAV packaging dCas9 is delivered by two AAVs as split-intein-fusions, which reconstitute dCas9 efficiently via intein-mediated protein trans-splicing. A third AAV is delivering the SAM activator, an optional fourth AAV could be used to deliver reporter sequences, while sgRNAs for the activation of target genes can be distributed between all vectors.
Multiplexed activation of Ascl1, Lmx1a, NeuroD1, Nr4a2, PITX3 and FoxA2 in primary astrocytic and Neuro2A cells was investigated for its ability to induce neurons.
It was demonstrated that AAV-mediated delivery resulted in reprogrammed neurons *in vivo* and could rescue voluntary motor behavior in a toxin-induced mouse model of Parkinson´s disease. Neuronal reprogramming was confirmed by electrophysiology, immuno-histochemistry and single cell RNA sequencing.

COMMERCIAL OPPORTUNITY

The technology is available for licensing or further co-development and includes access to material.

DEVELOPMENT STATUS

Established gene activator systems for Parkinson's Disease are available for research applications and further evaluation of therapeutic approaches.

PATENT SITUATION

A priority establishing application (LU102570) was filed in February 2021 followed by a PCT application (WO2022175381), entitled "CRISPR/Cas9-mediated means and methods for cell reprogramming".

FURTHER READING

Giehrl-Schwab et al., EMBO Mol Med (2022) Vol. 14(5):e14797. DOI: 10.15252/emmm.202114797

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