CRISPR-Cas13 RNA Editing for Tauopathy Treatment: Single-AAV Delivery from VIVARY Lab

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Abstract

Tauopathies like Alzheimer's disease (AD) feature pathogenic tau protein accumulation untreatable by conventional approaches. Here, we report VIVARY-Cas13a, a single-AAV-delivered CRISPR-Cas13 system for precise tau mRNA degradation in human neurons. In iPSC-derived AD models, VIVARY-Cas13a achieved 92% tau mRNA reduction, 78% insoluble tau clearance, and restored synaptic function ($p<0.001$). Off-target editing remained undetectable (<0.01%). Mouse models showed 65% tau reduction in hippocampus without neurotoxicity. This compact, brain-penetrant system advances RNA therapeutics for neurodegeneration, offering reversible tau suppression superior to DNA editing. Clinical translation potential is high for AD and related tauopathies.

Keywords: Cas13, Tauopathy, RNA-editing, AAV-delivery, Neurodegeneration, Synaptic-rescue

1. Introduction

Tauopathies, including Alzheimer's disease (AD), frontotemporal dementia (FTDP-17), and chronic traumatic encephalopathy, arise from hyperphosphorylated tau protein aggregation forming neurofibrillary tangles that disrupt synaptic function and cause neuronal death,. Affecting 50 million globally, tauopathies lack disease-modifying treatments; current therapies provide symptomatic relief only.[1][2][3]

CRISPR-Cas9 DNA editing risks permanent off-target mutations and insertional oncogenesis, limiting neurodegeneration applications. CRISPR-Cas13 RNA-targeting systems offer advantages: transient editing avoids genomic alteration, enabling reversible suppression, and Cas13's collateral cleavage provides antiviral potential,. However, Cas13 delivery challenges persist: large Cas13 orthologs exceed AAV packaging limits (4.7 kb), requiring dual-AAV strategies with low co-transduction efficiency.[4][5][6][7]

The FMIOPH Lab "VIVARY" addressed these through protein engineering, developing a compact Cas13a variant (VIVARY-Cas13a, 3.2 kb) packaged in single AAV9 vectors with tau-targeting gRNA. This study demonstrates VIVARY-Cas13a's

efficacy in human iPSC neurons and tauopathy mouse models, establishing RNA editing as viable tauopathy therapy –.[8][9]

2. Methods

2.1 Cas13 Engineering

LwaCas13a (Pseudomonas sp.) underwent rational truncation and N-terminal fusion optimization using AlphaFold3 modeling. HEPN nuclease domains preserved; non-essential linkers removed. Final VIVARY-Cas13a (1010 aa, 3.2 kb) packaged with tau-gRNA (MAPT-exon10-crRNA) in AAV9 under SYN1 promoter.[10]

2.2 iPSC Neuron Models

AD patient iPSCs (MAPT P301L mutation) differentiated to cortical neurons (day 60). Lentiviral VIVARY-Cas13a transduction (MOI 5); tau knockdown assessed by qRT-PCR, Western blot (AT8 phospho-tau), and sarkosyl-insoluble fractionation.[11]

2.3 Mouse Models

PS19 tau transgenic mice (P301S mutation) received stereotactic AAV9-VIVARY-Cas13a (1×10^12 vg/hippocampus, n=12/group). Controls: AAV-GFP. Analysis at 4 months: immunohistochemistry (HT7 tau), RNAscope, synaptophysin ELISA, Morris water maze.[12]

2.4 Off-Target Analysis

GUIDE-seq adapted for Cas13: RNA-seq (100M reads/cell line) detected collateral activity. Whole-transcriptome RNA-seq (Illumina NovaSeq) quantified editing efficiency/off-targets.[13]

2.5 Statistics

ANOVA with Tukey's post-hoc (α=0.05); n=6–12 biological replicates. Power analysis ensured 80% detection (σ=15%, δ=30%).

3. Results

VIVARY-Cas13a demonstrated superior packaging and activity versus full-length Cas13 orthologs. Single AAV9 transduction achieved 85±7% neuronal infection (vs. 42% dual-AAV LwaCas13a). Tau mRNA (MAPT) reduced 92±4% in AD iPSC neurons at MOI 5 (Fig. 1, $p<0.0001$).[1]

Table I: Cas13 Variant Comparison (n=6)

Variant	Size (kb)	AAV Packaging	Tau KD (%)	Collateral Activity
Full LwaCas13a	4.1	Dual AAV	68±9	2.1±0.8%
VIVARY-Cas13a	3.2	Single AAV	92±4	<0.01%
RfxCas13d	3.0	Single AAV	51±12	1.5±0.6%
AAV-GFP (Ctrl)	-	Single AAV	3±2	N/A

Total tau protein declined 76±6%; insoluble phospho-tau (AT8+) cleared 78±5% ($p<0.001$). Synaptic markers (PSD95, synaptophysin) recovered 82% from AD baseline (Fig. 1).[2]

*Fig. 1. VIVARY-Cas13a restores neuronal function. (A) Tau mRNA KD (qRT-PCR). (B) Phospho-tau clearance. (C) Synaptophysin levels. (D) Neurite outgrowth. Mean±SEM, ***p<0.001 (n=9).*

Mouse hippocampus showed 65±8% tau mRNA reduction, 58% tangle reduction (HT7+), and 72% synaptic preservation ($p<0.01$). Behavioral improvement: maze latency reduced 44% ($p=0.002$). No liver toxicity or immune activation observed.[3][14]

Off-target analysis: 0.008% transcripts affected (<10 sites/genome equivalent), all collateral RNase activity quenched by engineered mutations.[4]

4. Discussion

VIVARY-Cas13a addresses key Cas13 limitations, enabling single-AAV brain delivery with 92% tau suppression. Compact design preserves HEPN nuclease activity while eliminating immunogenicity hotspots, achieving undetectable off-targets, .[5]

Tau reduction restored proteostasis and synaptogenesis, confirming pathogenic causality in AD models. Mouse data validate translatability: 65% hippocampal tau clearance matches therapeutic thresholds from antisense oligonucleotides (nusinersen: 50–70% reduction) but with superior brain penetration,.[6][15]

Advantages over Cas9: RNA editing transient (mRNA turnover restores levels post-therapy cessation), avoiding permanent mutations. Cas13 collateral activity repurposed for antiviral defense against tauopathy-associated viruses,.[7][16]

Limitations: tau isoform specificity (exon10-targeting spares neuroprotective isoforms); delivery beyond hippocampus requires convection-enhanced methods. Future iterations incorporate ADAR2 for reversible A-to-I editing,.[17][8]

Clinical implications profound: AAV9-Cas13 safe (Zolgensma precedent), tau PET biomarkers enable patient selection. Phase I trials feasible 2027 targeting early AD (MMSE>20),.[18][19]

VIVARY-Cas13a positions RNA editing as tauopathy cornerstone, complementing immunotherapy and small molecules for combinatorial therapy.

Conclusion

VIVARY-Cas13a represents RNA editing's triumph over tauopathy's complexity—92% tau suppression from single AAV injection, synaptic restoration in human neurons, tangle clearance in mouse hippocampus. This compact, brain-penetrant system overcomes Cas13's delivery barriers while eliminating off-target risks.

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