(SD2022-222) Optimized CAG repeat-targeting CRISPR/cas13d designs

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ABSTRACT

Reseachers from UC San Diego demonstrated a proof of principle for a CAGEX RNA-targeting CRISPR–Cas13d system as a potential allele-sensitive therapeutic approach for HD, a strategy with broad implications for the treatment of other neurodegenerative disorders.

TECHNOLOGY DESCRIPTION

Researchers from UC San Diego leveraged the advantageous compact nature of Ruminococcus flavefaciens XPD3002 (Rfx) CRISPR/Cas13d and engineered a CRISPR/Cas13d-based gene therapy vector packaged into a single vector in both lentiviral and adeno-associated viral delivery vehicles, that silences mutant toxic CAG-expanded (CAGEX) RNA in both human patient iPSC models and an established mouse model of HD.

APPLICATIONS

1) Research tool. To target, locate, and track intracellular or extracellular CAG expansion-containing RNA transcripts in fixed or living cells and/or in vivo.

2) Biomarker. Can serve as a pharmacodynamic biomarker to assess efficacy of potential therapies that target CAG expansions including small molecules or natural/synthetic compounds and/or any DNA or RNA-targeting gene therapy approach.

3) Therapeutic for disease. To target and destroy disease-causing RNA transcripts harboring toxic CAG expansions including those that cause HD.

ADVANTAGES

OTHER INFORMATION

KEYWORDS

gene therapy, HD therapeutics, Huntington's disease, RNA-targeting CRISPR

CATEGORIZED AS

- **Biotechnology**
  - Health
- **Medical**
  - Disease: Central Nervous System
  - Gene Therapy
  - Therapeutics

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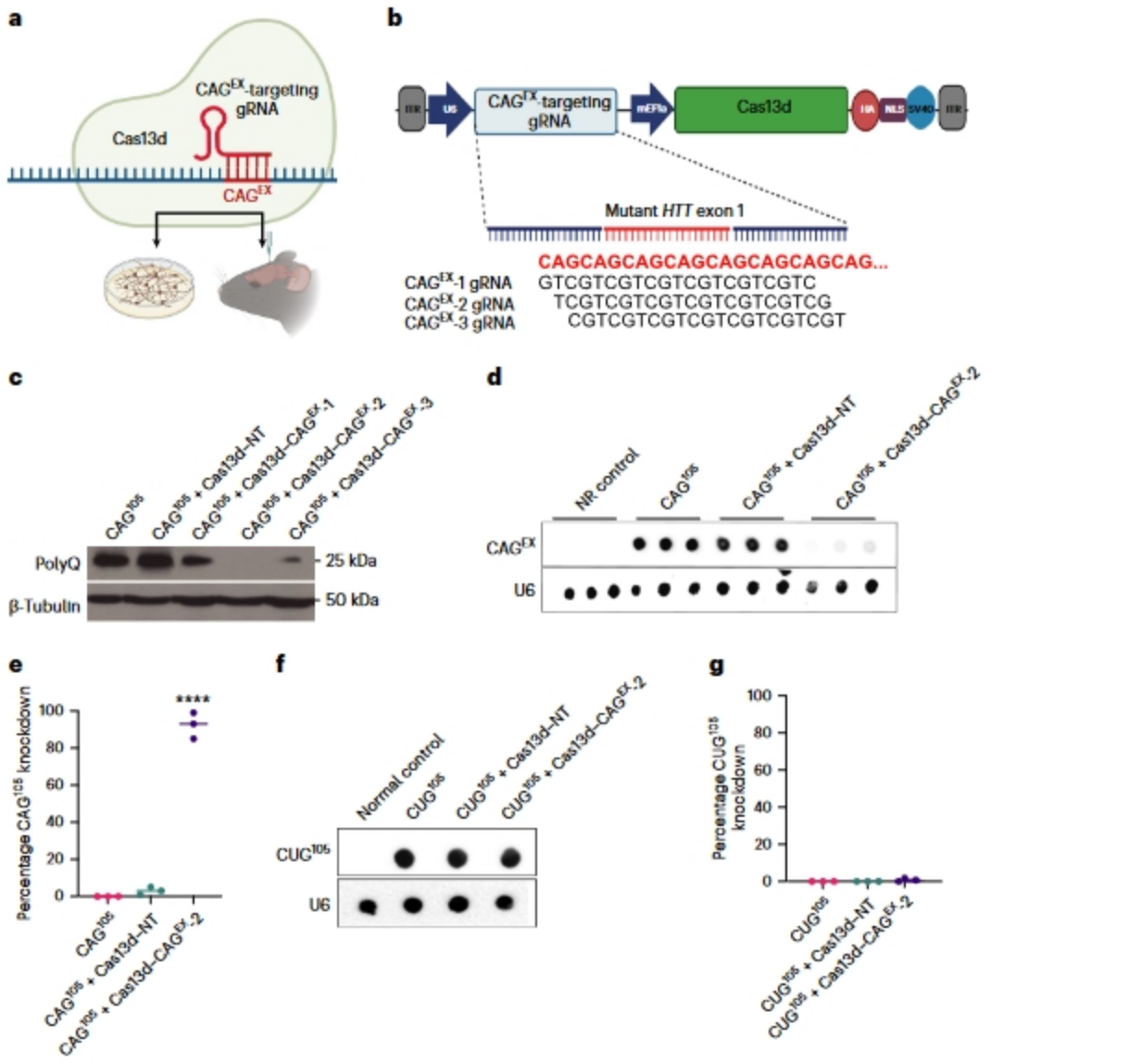

Fig. 1 | Development of an RNA-targeting, Cas13d-based gene therapy approach for HD. a, Treatment scheme of our single gene therapy that expresses Cas13d and a gRNA designed to eliminate CAG-expanded HTT *HTT*RNA in both human striatal neuronal cultures derived from patient iPSCs and in the striatum of an established mouse model of HD, zQ175/+. **b**, Diagram of a series of CAG-expanded, RNA-targeting vectors that consists of (1) Cas13d tagged with an HA epitope and (2) one of three U6 promoter-driven Rfx CRISPR–Cas13d gRNAs (denoted as CAG^{EX} gRNA 1–3). **c**, Western blot analysis of polyQ protein from protein lysates isolated from HEK293 cells transfected with a CAG^{105} repeat plasmid and each candidate Cas13d vector. **d**,**e**, RNA dot blot analysis (**d**) and quantification (**e**) of CAG-expanded RNA within HEK293 cells transfected with a CAG^{105} repeat plasmid along with a nontargeting control (NT) or CAG^{EX}-2 vector (one-way ANOVA, Tukey post hoc test, $^{****}P < 0.0001$; $n = 1$ technical replicates, $n = 3$ biological replicates). **f**,**g**, RNA dot blot analysis (**f**) and quantification (**g**) of CUG-expanded RNA within HEK293 cells transfected with a CUG^{105} repeat plasmid along with a NT or CAG^{EX}-2 vector (one-way ANOVA, Tukey post hoc test, $n = 3$ technical replicates, $n = 3$ biological replicates).

STATE OF DEVELOPMENT

The UCSD researchers have collected extensive preclinical data in two established models of HD for a new gene therapy strategy that effectively eliminates CAG-expanded pre-mRNA with the use of RNA-targeting CRISPR/Cas13d technology. Specifically, they engineered a potent gene therapy vector that encases Ruminococcus flavefaciens Cas13d, an RNA-targeting enzyme only 2.4 kb in size, and a CAG-expansion RNA-targeting gRNA in a single viral delivery vehicle. They utilized multiple established preclinical models of HD including the Q175/+ mouse model which harbors one human allele of HTT with 175 CAG repeats in exon 1, and a series HD patient iPSC-derived striatal neurons to show both efficacy and safety of the new gene therapy approach both in vivo and in a humanized preclinical model.

Further details are in the manuscript.

INTELLECTUAL PROPERTY INFO

University is securing US patent rights, see:

https://patents.google.com/patent/WO2023154843A2/en?oq=US2023%2f062352

Please contact UCSD is you are interested in licensing this technology for commercial development.

RELATED MATERIALS

- Morelli KH, Wu Q, Gosztyla ML, Liu H, Yao M, Zhang C, Chen J, Marina RJ, Lee K, Jones KL, Huang MY, Li A, Smith-Geater C, Thompson LM, Duan W, Yeo GW. An RNA-targeting CRISPR-Cas13d system alleviates disease-related phenotypes in Huntington's disease models. Nat Neurosci. 2023 Jan;26(1):27-38 - 12/12/2022

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