CRISPR-Cas System for RNA Targeting and Manipulation

INVENTION: RNA biology is an exciting and rapidly advancing area of biomedical research and therapeutic development. Investigators at Salk have identified and engineered a novel family of RNA-guided and RNA-targeting CRISPR-Cas enzymes, termed Cas13d. The optimized variant – CasRx – offers superior RNA knock-down efficiency and dramatically higher specificity compared to RNAi (**Figure 1**). As the smallest known family of CRISPR enzymes, CasRx can be readily delivered using a single-vector AAV system. As a proof of concept, AAV delivery of CasRx was used to disrupt the build-up of tau proteins in a neuronal model of frontotemporal dementia (**Figure 2**).

CasRx is a programmable RNA targeting system that can be customized to serve a variety of functions including knockdown of RNA, modulation of transcript splicing, and RNA base editing. CasRx therefore has potential for RNA-based therapeutic applications involving knockdown to degrade toxic RNAs or proteins, correction of mis-splicing, exon skipping, and correction of deleterious point mutations.

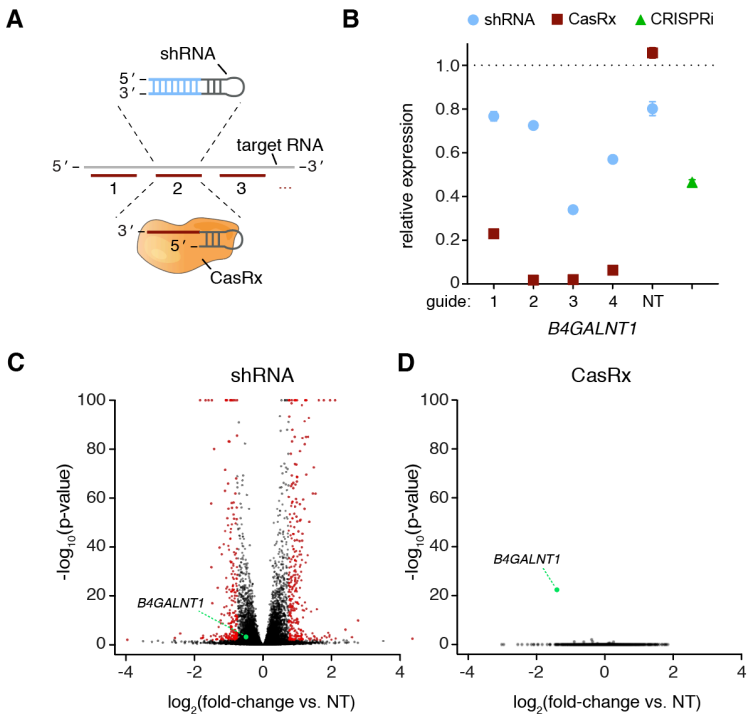

Figure 1: CasRx is an efficient and specific RNA-guided RNase in human cells. (A) Schematic of CasRx target sequences and matching shRNAs. (B) Relative target RNA knockdown by CasRx, shRNA, and CRISPRi. (C) Off-target effects of shRNA by RNA sequencing. (D) Off-target effects of CasRx by RNA sequencing.

ADVANTAGES:

- Superior knock-down efficiency compared to currently available methods (median knock-down >90% across diverse human transcripts)
- Off-target effects are essentially eliminated compared to RNA interference (shRNA)
- Ability to target nuclear RNAs (including non-coding RNAs)
- Manipulation of splicing including correction of mis-splicing defects and targeted exon skipping
- Small size enables single-vector AAV delivery of CasRx and guide RNA *in vivo*
- System is fully orthogonal to human cells (minimal potential for interference with endogenous cellular processes)

APPLICATIONS:

- Transcripts targeting, manipulation, modification, and detection

BACKGROUND:

The discovery of genome editing via CRISPR-Cas9 have created multiple new fronts in modern medicine and biology research. Scientists at Salk, through computational screening and modeling of previously uncharacterized families of CRISPR-Cas enzymes, sought to replicate the utilities of CRISPR-Cas9, but for RNA transcripts instead of DNA. One candidate identified from a bacterial strain of *Ruminococcus flavefaciens* displayed the desired RNA-targeting characteristics. This enzyme, named CasRx, can be engineered for programmable RNA targeting in mammalian cells.

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To evaluate CasRx, Salk scientists knocked down a diverse set of 14 endogenous mRNAs and lncRNAs, consistently achieving >90% knockdown with significantly greater efficiency relative to RNAi. Additionally, CasRx interference is markedly more specific than spacer-matching shRNAs, with no detectable off-target changes compared with hundreds for RNAi.

CasRx is a minimal two-component platform, consisting of an engineered CRISPR-Cas13d effector and an associated guide RNA, and can be fully genetically encoded. Cas13d enzymes are small at an average size of 930 amino acids, allowing CasRx effector domain fusions to be paired with a CRISPR array encoding multiple guide RNAs while remaining under the packaging size limit of AAV delivery.

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PATENT STATUS: Patent application filed

PUBLICATIONS:
Konermann *et al,* 2018. Cell S0092-8674(18)30207-1
http://www.cell.com/cell/fulltext/S0092-8674(18)30207-1

https://www.salk.edu/news-release/crispr-gene-editing-takes-another-big-step-forward-targeting-rna/

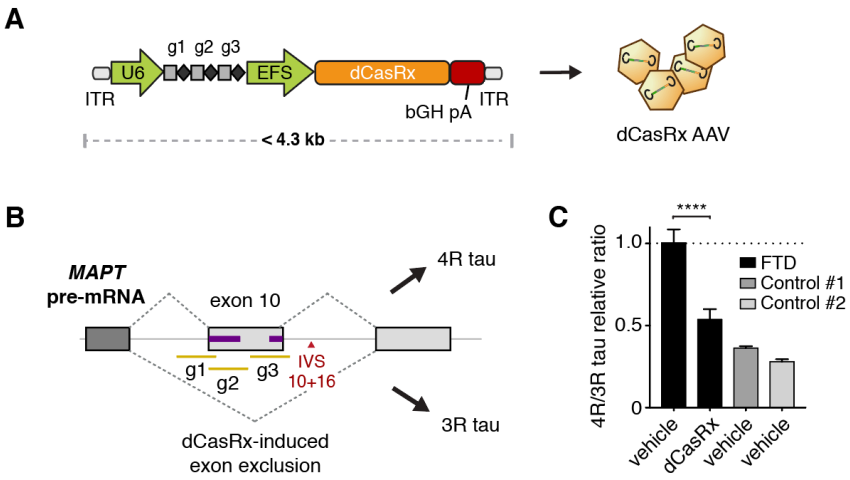

Figure 2: AAV delivery of catalytically inactive dCasRx splice effectors to manipulate alternative splicing. (A) AAV design carrying dCasRx and a three-guide array with total transgene size < 4.3 kb. (B) Frontotemporal dementia (FTD) is associated with SNPs in a putative intronic splice enhancer following exon 10 of the *MAPT* transcript encoding for tau. Alternative splicing of *MAPT* exon 10 results in 4R tau (by inclusion) and 3R tau (by exclusion). (C) AAV-delivered dCasRx can normalize dysregulated tau isoform ratios in FTD patient-derived neurons.

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