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Chemically Modified Cas13 Guides Enhance CRISPR-Cas13 RNA-Targeting Effects

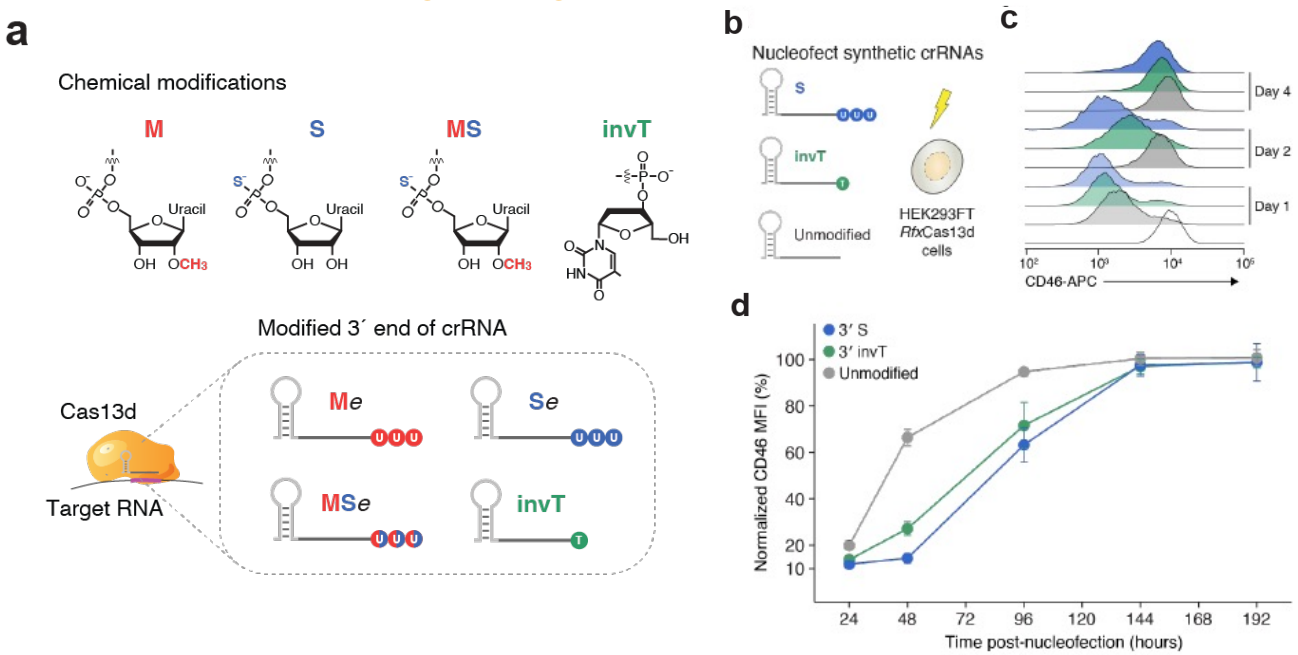

Figure 1. Chemically-modified CRISPR RNAs (crRNAs) improve Cas13 knockdown efficacy in human cells. (a) Overview of chemical modifications incorporated during synthesis of crRNAs: M, 3′-O-methyl base; S, phosphorothioate bond; MS, 3′-O-methyl base and phosphorothioate bond; invT, inverted thymidine. (b) Experimental design to measure the temporal dynamics of transient CD46 knockdown by nucleofecting the synthetic crRNAs in the HEK293FT-TetO-RfxCas13d-NLS cells. Synthetic crRNAs were unmodified, chemically modified with a phosphorothioate bond (S) on three uridines at the 3′ end, or chemically modified with an inverted thymidine at the 3′ end (invT). (c) Representative CD46 histograms at 1, 2 and 4 days after synthetic crRNA nucleofection. (d) Relative CD46 protein expression upon nucleofection with the synthetic crRNAs in panel b, normalized to cells nucleofected with non-targeting NT crRNAs. Points represent mean values ± s.d. , n = 3 biological replicate nucleofections.

BACKGROUND

RNA-targeting CRISPR-Cas13 proteins have emerged as a powerful platform to transiently modulate gene expression outcomes. Many regions of the human genome that were not previously editable by DNA-targeting CRISPR enzymes, along with organisms like RNA viruses (e.g., Coronavirus or Influenza), are now viable targets using RNA-targeting CRISPR-Cas13 proteins. However, one of the main challenges for transcriptome manipulations is to achieve efficient and precise delivery of CRISPR systems for robust RNA manipulation without modifying host DNA sequence. Delivery of CRISPR enzymes and CRISPR RNAs (crRNAs) in human cells can be challenging due to rapid crRNA degradation, yielding only transient knockdown. A current unmet need is development of crRNAs with increased efficiency that extend transient Cas13-mediated effects. Although recent work has shown that *in vitro* transcribed crRNAs and recombinant Cas13 proteins can be used in zebrafish, the potential stability and knockdown efficiency of chemically-modified Cas13 crRNAs in human cells remain unclear.

DESCRIPTION

Scientists in the lab of Dr. Neville Sanjana at the New York Genome Center have identified synthetic crRNAs that improve targeting efficiency and half-life of the CRISPR-Cas13 complex in human cells. Without a continuous source of crRNA expression, Cas13 effects are short lived due to endogenous RNA nucleases and regeneration of the cellular steady-state by continuous target RNA expression. They have shown that co-delivery of modified crRNAs and recombinant Cas13 enzyme in ribonucleoprotein (RNP) complexes enables transient gene expression modulation in primary CD4+ and CD8+ T-cells. Given the recent development of Cas13-based research tools, diagnostics and therapeutics, chemically-modified crRNAs can further enhance CRISPR-Cas13 RNA editing for diverse biotechnology applications. This technology can be used to degrade viral RNA in RNA viruses (such as SARS-CoV-2) and to alter immune cells (such as T cell reprogramming for immunotherapy).

BENEFITS

- Chemically modified crRNAs stabilize the CRISPR-Cas13 complex and extend Cas13-based transcriptome engineering to multiple days (e.g. RNA knockdown, editing, labeling etc.)
- Robust RNA target knockdown with increased efficacy over existing methods
- New methods for non-viral Cas13 delivery into human primary cells
- Efficient and precise delivery of CRISPR systems for robust RNA manipulation without modifying host DNA sequence

APPLICATIONS

- Development of therapeutics that target known disease-causing variants without altering a patient's DNA
- Development of Cas13-based therapeutics against RNA viruses such as coronavirus
- Development of next generation biosensors that discriminate between closely related RNA species

PATENT INFORMATION

US Provisional Patent Application #: 63/148,966

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