(SD2018-386) Directed Pseudouridylation of Cellular RNA Via Delivery of Crispr/Cas and esgRNA Guide Combinations

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BACKGROUND

Resent strategies aimed to target and manipulate RNA in living cells mainly rely on the use of antisense oligonucleotides (ASO) or engineered RNA binding proteins (RBP). Although ASO therapies have been shown great promise in eliminating pathogenic transcripts or modulating RBP binding, they are synthetic in construction and thus cannot be encoded within DNA. This complicates potential gene therapy strategies, which would rely on regular administration of ASOs throughout the lifetime of the patient. Furthermore, they are incapable of modulating the genetic sequence of RNA. Although engineered RBPs such as PUF proteins can be designed to recognize target transcripts and fused to RNA modifying effectors to allow for specific recognition and manipulation, these constructs require extensive protein engineering for each target and may prove to be laborious and costly.

TECHNOLOGY DESCRIPTION

Researchers from UC San Diego have developed a Cas directed pseudouridylation technology, largely built upon the existing RCas9 system, which uses a single-guide RNA to provide a simple and rapidly programmable system for modification of specific RNA molecules in live cells. Due to its overall design simplicity as well as its highly encodable nature, this platform promises high utility and versatility when compared to other similar RNA modifying methods.

More specifically, the researchers have generated a technology to perform programmable RNA pseudouridylation at single-nucleotide resolution using RNA-targeting CRISPR/Cas: engineered single guide RNA (esgRNA) combinations. This approach provides a means to reversibly alter genetic information in a temporal manner, unlike traditional CRISPR/Cas9 driven genomic engineering which relies on permanently altering DNA sequence.

This invention stems from taking a nuclease-dead version of DNA/RNA-targeting Cas (e.g. Sp/Sau/Cje dCas9 or dCas13a/b/d) and generating recombinant proteins with effector enzymes capable of performing ribonucleotide base modification to alter how sequence of the RNA molecule is recognized by cellular machinery. Specifically, constructs have been made that express RNA-targeting Cas (for example dCas9 or dCas13 fused to the pseudouridylation domain of human DKC1, PUS1, PUS3 or PUS7) using either a short

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OTHER INFORMATION

KEYWORDS

RNA pseudouridylation, cellular dynamics, Hurler's syndrome, Cystic fibrosis, Duchenne muscular dystrophy, Cas directed pseudouridylation

CATEGORIZED AS

- Biotechnology
  - Genomics
- Medical
  - Disease: Genetic Diseases and Dysmorphic Syndromes
  - Research Tools
  - Therapeutics

RELATED CASES

2018-386-0

peptide linker or an ‘XTEN’ linker peptide for spatial separation. With RNA-targeting Cas as a surrogate RNA-binding motif, researchers may now use esgRNAs to direct pseudouridylation to RNA sites that they wish to modify.

Current systems used to directly pseudouridylate RNA rely on recruitment of endogenous pseudouridylation machinery by exogenously expressed guide RNAs, and have not yet been demonstrated to be effective in mammalian systems. Because Cas proteins bind with picomolar affinity to guide RNA scaffolds/direct repeat hairpins, and because our system uses dual guide architecture to increase both target affinity and specificity, the UCSD technology can potentially direct RNA pseudouridylation with higher efficiency and specificity, leading to fewer off-target editing events.

APPLICATIONS

1) Research tool. Characterize the effects of directed cellular RNA pseudouridylation on RNA processing and cellular dynamics.

2) Therapeutic for diseases. Viral (AAV) or other delivery approaches to treat: premature termination codon/nonsense-mediated decay (NMD) RNA diseases such as Hurler’s syndrome, Cystic fibrosis, Duchenne muscular dystrophy. This also could include diseases associated with deficiencies in RNA pseudouridylation.

ADVANTAGES

STATE OF DEVELOPMENT

INTELLECTUAL PROPERTY INFO

RELATED MATERIALS

PATENT STATUS

Country	Type	Number	Dated	Case
Patent Cooperation Treaty	Published Application	2020/047489 A1	03/05/2020	2018-386

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