RNA-Editing of C9ORF72 Loss-of-Function for Treatment of Neurodegenerative Diseases

Innovative approach to correct C9ORF72 Loss-of-Function and restore protein levels in Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Dementia (FTD).

Technology

Researchers in the Vogel Lab at NYU are developing nuclease-resistant antisense oligonucleotides (ASOs) that recruit adenosine deaminase acting on RNA (ADAR) for site-specific adenosine-to-inosine editing of C9ORF72 transcripts to alleviate the loss-of-function phenotype. C9ORF72-based repeat expansion is a major cause of neurodegenerative diseases, such as ALS and FTD. The repeat expansion produces both a gain-of-function and a loss-of-function phenotype. The oligonucleotides hybridize to the C9ORF72 5′ untranslated region (5′UTR) and direct editing at specific positions to disrupt two inhibitory elements and subsequently increase C9ORF72 levels. The disruption of the inhibitory elements in dual-luciferase reporter assays led to a >8-fold increase in C9ORF72 levels. qRT-PCR confirmed that these changes in protein output occurred without alterations in transcript levels. In addition, ADAR-based editing in cell line models changed >40% of the target sites. In summary, these findings demonstrate that targeted ADAR-mediated editing of the C9ORF72 5′UTR can specifically enhance Variant 2 translation without affecting transcript abundance or genomic sequence, establishing a promising therapeutic strategy that can provide the critical complement to approaches targeting the gain-of-function phenotype to treat C9ORF72-linked neurodegenerative diseases, including ALS and FTD.

Background

ALS and FTD are increasingly common, devastating neurodegenerative diseases without a cure. ALS affects over 30,000 adults in the US, causes progressive paralysis, and has a median survival of 2–5 years from symptom onset. FTD, the leading cause of dementia in adults under 65, affects more than 50,000 people in the US and leads to severe behavioral, language, and executive dysfunction, with a median survival of 6–8 years. The C9ORF72 hexanucleotide repeat expansion (HRE) is the most common genetic cause of both disorders, accounting for ~40–50% of familial ALS, ~5–10% of sporadic ALS, and ~25% of familial FTD. The C9ORF72 HRE, located in the intron between exons 1a and 1b, can expand from 6–25 to >1,000 repeats. The C9ORF72 HRE causes a gain-of-function phenotype due to the large amounts of RNA repeats and subsequent dipeptide repeats, and a loss-of-function phenotype due to loss of functional C9ORF72 protein. Specifically, the C9ORF72 HRE is thought to reduce C9ORF72 transcript levels by ~50% due to promoter DNA hypermethylation, decreasing levels of functional C9ORF72 protein. In model systems, reduced C9ORF72 protein leads to axon growth defects, cilia abnormalities, impaired autophagy, increased glutamate receptors, lymphadenopathy, and splenomegaly. Reduced C9ORF72 levels also aggravate the gain-of-function phenotype. Emerging C9ORF72-targeted therapies largely focus on gain-of-function mechanisms by degrading RNA repeats with ASOs to reduce toxic RNA foci and dipeptide-repeat proteins, but

Technology ID

VOG01-02

Category

Life Sciences/Neuroscience

Life

Sciences/Therapeutics/Neurodeg

Diseases

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they do not correct the loss of functional C9ORF72 – creating a critical, unmet need. The Vogel Lab discovered a novel approach to address this need. The Lab discovered that two elements in the 5′ untranslated region of C9ORF72's transcript variant 2 (whose sequence is unaffected by the HRE) repress translation of the gene. Disrupting the repressive elements increases translation of C9ORF72 and therefore provides a path to rescue the loss-of-function phenotype. This approach directly targets the loss-of-function phenotype and restores C9ORF72 expression to normal physiological levels without disrupting other gene regulatory circuits.

Development Status

The Vogel Lab has shown in dual-luciferase reporter assays that disrupting the inhibitory elements increases C9ORF72 levels >8-fold. The Vogel Lab has also shown that ASO-guided ADAR-mediated RNA editing of the respective nucleotides situated in the inhibitory elements is possible, in principle. Editing efficiency is >40% currently, using exogenous ADAR and a reporter. In the next 6 months, the group will work on demonstrating that RNA editing of the inhibitory elements has the same positive effect on C9ORF72 levels, first using reporter constructs and, once successful, the endogenous gene. The work will be performed first in standard human cell lines and then in patient-derived induced pluripotent stem cells.

Applications

Treatment of C9ORF72-linked neurodegenerative diseases, including Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Dementia (FTD).

Advantages

- **Restores loss-of-function without increasing transcript levels:** Directly enhances translation of C9ORF72 Variant 2 while leaving mRNA abundance unchanged.
- **Leverages endogenous ADAR for targeted RNA editing:** Uses recruitment of native ADAR rather than exogenous enzymes or nucleases, potentially improving safety, immunogenicity, and delivery.
- **Transcript and site-specific tuning of protein expression:** Targets discrete regulatory elements, enabling fine control of translation and avoiding broad disruption of C9ORF72 regulation.
- **Complementary to existing C9ORF72 therapies:** Addresses the loss-of-function component of C9ORF72 pathology, making it potentially synergistic with ASOs or other strategies.
- **Enables tissue-specific therapies:** Can be targeted into specific cell types.
- **Reversible modulation of C9ORF72 expression rather than permanent genomic alteration.**

Intellectual Property

NYU has filed a US provisional patent application covering the composition of the oligonucleotide to guide site-specific 5′ UTR editing of the C9ORF72 and methods of using the editing approach to treat neurodegenerative diseases such as ALS and FTD.