Antisense Oligonucleotide Discovery Platform And Splice Modulating Drugs For Hemophilia

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BACKGROUND

Aberrant splicing contributes to the etiology of many inherited diseases. Pathogenic variants impact pre-mRNA splicing through a variety of mechanisms. Most notably, variants remodel the *cis*-regulatory landscape of pre-mRNAs by ablation or creation of splice sites, and auxiliary splicing regulatory sequences such as exonic or intronic splicing enhancers (ESE and ISE, respectively) and splicing silencers (ESS and ISS, respectively). Splicing-sensitive variants cripple the integrity of the gene, resulting in the production of a faulty message that is either unstable or encodes an internally deleted protein. Antisense oligonucleotides (ASOs) are a promising therapeutic modality for rescuing pathogenic aberrant splicing patterns as their direct base pairing abilities make them highly customizable and specific to targets. Although challenges such as toxicity, delivery and stability represent barriers to the clinical translation of ASOs, solutions to these challenges exist, as exemplified by the recent FDA approval of multiple ASO drugs.

Generally, ASO's that target splicing mutations are limited to mutations in and around splicing enhancers and exonic mutations are commonly not targeted because of the idea that the mutation causes a significant change in protein function.

TECHNOLOGY DESCRIPTION

UC Santa Cruz researchers have developed a system for rapidly identifying antisense oligonucleotides that can rescue splicing sensitive variants. The system involves a splicing reporter construct that includes an exon of interest. The exon of interest is then mutated using site directed mutagenesis to generate known disease causing mutations. The constructs resulting from the site directed mutagenesis are transfected into cells. Cells with the reporter constructs are then treated with a set of modified ASO's complementary to the exon and flanking introns. The modified ASO's are designed to contiguously tile across the exon and any flanking introns included in the construct. Total RNA is isolated from the cells post transfection and analyzed relative to cells with reporter constructs untreated with the modified ASO's to identify aberrent splicing. Splicing efficiency is quantified by comparing relative fluorescent units between 5'FAM labeled reporter isoforms that either include or exclude the exon of interest. From that, a PSI (percent spliced-in) index is calculated. Pathogenic variants identified by the method can be further

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OTHER INFORMATION

KEYWORDS

RNA, antisense, antisense oligonucleotide, aberrent splicing, ASO, genetic diseases, rare diseases, antisense treatment, Hemophilia A, Factor VIII, F8 gene, exon, intron, mutation

CATEGORIZED AS

- **Biotechnology**
  - Bioinformatics
  - Genomics
  - Health
- **Materials & Chemicals**
  - Biological
- **Medical**
  - Disease: Genetic Diseases and Dysmorphic Syndromes
  - Therapeutics

RELATED CASES

2023-931-0, 2011-892-0

confirmed by in vitro transcription and RNA structures of the variants predicted by SHAPE-MaP sequencing and analysis.

This system was tested in the human Factor VIII (F8) gene, mutations of which result in Hemophilia A. Prior work by theis group identified F8 as a gene with the highest number of variants per exon in the human population as well as the total number of putative splicing-sensitive point variants of those genes analyzed. A total of 97 known HA-causing variants across 11 exons were tested by generating heterologous splicing reporters for each exon (plus 100-250 bp of intronic sequence.) Variants associated with HA caused exon skipping in four exons, particularly in exon 16 where 6 pathogenic variants reduced inclusion of exon 16 in the spliced product. SHAPE-MaP-seq analysis indicated that one of the variants (exon-16$^{c.5543A>G}$) forms a three-way junction structure that occludes the 3' splice site of exon 16, resulting in the aberrant splicing.

ASO's that targeted this structure, termed TWJ-3-15 (Three-Way Junction at the 3'-end of Intron 15) were designed and shown to significantly increase exon 16 inclusion relative to the controls. Importantly, ASO's targeting upstream and downstream regions of the splice site could also increase inclusion of exon 16. These are likely to perturb the influence of inhibitory elements found in the flanking introns. Multiple ASO's designed to target regions upstream and downstream of the splice site performed *better* than those that directly targeted the TWJ-3-15 structure itself, particularly when used in combination.

APPLICATIONS

- Platform technology for the discovery of exonic splicing mutations
- Potential for personalized antisense treatment
- Antisense oligonucleotides that promote proper splicing despite targeting regions upstream and downstream of the splice site
- Antisense oligonucleotides that promote proper splicing *better* than antisense oligonucleotides designed to directly target the splice site, particularly in combination.
- Antisense oligonuclotides useful in the treatment of genetic diseases such as hemophila A

ADVANTAGES

- Technology opens up a wider variety of potential targets for ASO treatments - effectively any disease causing mutation in an exon that does not significantly affect protein function is a potential ASO target.
- Workflow can be used to identify combinations of ASO's relatively far upstream or downstream of mutation.
- Powerful combinations of ASO's that can target Hemophilia A caused by F8 mutations

INTELLECTUAL PROPERTY INFORMATION

Patent Pending

RELATED MATERIALS

- An intronic RNA element modulates Factor VIII exon-16 splicing - 01/11/2024

RELATED TECHNOLOGIES

- Functional Rna Elements As Targets For Amelioration Of Aberrant Pre-Mrna Splicing

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