

COMPOSITIONS AND METHODS FOR MODULATING SPLICING AND PROTEIN EXPRESSION

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 62/888,887, filed August 19, 2019, and U.S. Provisional Application No. 63/049,262, filed July 08, 2020, each of which is incorporated herein by reference in its entirety.

REFERENCE TO SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on August 19, 2020, is named 47991-727_601_SL.txt and is 17,694,293 bytes in size.

BACKGROUND

[0003] Alternative splicing events in genes can lead to non-productive or less productive mRNA transcripts, and therapeutic agents which can target the alternative splicing events in genes can modulate the expression level of functional proteins in patients and/or inhibit aberrant protein expression. Such therapeutic agents can be used to treat a condition or disease caused by protein deficiency.

SUMMARY

[003a] In one aspect, disclosed herein is a composition comprising a non-sense mediated RNA decay alternative exon (NSAE)-modulating agent that interacts with a target motif within a pre-processed mRNA transcript to modulate splicing at an alternative 3' splice site of a pre-processed mRNA transcript in a cell, thereby promoting inclusion of a canonical exon in a processed mRNA transcript that is processed from the pre-processed mRNA transcript, and promoting exclusion of an alternative exon from the pre-processed mRNA transcript in the cell, wherein the NSAE-modulating agent is an antisense oligomer (ASO) or a viral delivery system encoding the ASO, wherein

the processed mRNA transcript encodes a target protein that is SYNGAP1 protein,

wherein the NSAE comprises:

a canonical exon and at least a portion of an intron upstream of the canonical exon and adjacent to the canonical exon;

wherein the NSAE-modulating agent increases a level of the processed mRNA transcript and expression of the target protein in the cell, and

wherein the target motif comprises a sequence with at least 85% sequence identity to a region comprising at least 8 contiguous nucleic acids of a sequence selected from the group consisting of SEQ ID NOs: 166-169 and 980-983.

[003b] In another aspect, disclosed herein is a use of the composition disclosed herein in manufacturing a medicament for treating a disease or condition in a subject in need thereof, wherein the disease or condition is associated with a functional-SYNGAP1 protein deficiency.

[003c] In yet another aspect, disclosed herein is a pharmaceutical composition comprising the composition disclosed herein, and a pharmaceutically acceptable excipient and/or a delivery vehicle.

[003d] In yet another aspect, disclosed herein is the composition disclosed herein for use in therapy.

[0004] In some examples, provided herein is a composition comprising a non-sense mediated RNA decay alternative exon (NSAE)-modulating agent that interacts with a target motif within a pre-processed mRNA transcript to modulate exclusion of an NSAE from a processed mRNA transcript and to modulate inclusion of a canonical exon in the processed mRNA transcript, wherein the target motif is located (i) in an intronic region between two canonical exons, (ii) in one of the two canonical exons, or (iii) in a region spanning both an intron and canonical exon; and wherein the NSAE comprises (i) only a portion of a canonical exon, or (ii) a canonical exon and at least a portion of an intron adjacent to the canonical exon; wherein the NSAE-modulating agent modulates exclusion of an NSAE from the processed mRNA transcript and modulates inclusion of a canonical exon in the processed mRNA transcript.

[0005] In some examples, provided herein is a composition comprising a non-sense mediated RNA decay alternative exon (NSAE)-modulating agent that modulates expression of a target protein in a cell comprising a pre-processed mRNA transcript (pre-mRNA) that encodes the target protein and comprises: an alternative nonsense mediated RNA decay-inducing (NMD) exon comprising an alternative 5' splice site downstream of the 5' splice site of a canonical exon and within the canonical exon, or upstream of the 5' splice site of the canonical exon and within an intron, wherein the NSAE-modulating agent modulates processing of an mRNA transcript from the pre-processed mRNA transcript by modulating splicing of the pre-mRNA at the 5' alternative splice site, wherein the splicing of the pre-mRNA at the 5' alternative splice site modulates the expression of the target protein in a cell.

CLAIMS

1. A composition comprising a non-sense mediated RNA decay alternative exon (NSAE)-modulating agent that interacts with a target motif within a pre-processed mRNA transcript to modulate splicing at an alternative 3' splice site of a pre-processed mRNA transcript in a cell, thereby promoting inclusion of a canonical exon in a processed mRNA transcript that is processed from the pre-processed mRNA transcript, and promoting exclusion of an alternative exon from the pre-processed mRNA transcript in the cell, wherein the NSAE-modulating agent is an antisense oligomer (ASO) or a viral delivery system encoding the ASO, wherein the processed mRNA transcript encodes a target protein that is SYNGAP1 protein,
wherein the NSAE comprises:
a canonical exon and at least a portion of an intron upstream of the canonical exon and adjacent to the canonical exon;
wherein the NSAE-modulating agent increases a level of the processed mRNA transcript and expression of the target protein in the cell, and
wherein the target motif comprises a sequence with at least 85% sequence identity to a region comprising at least 8 contiguous nucleic acids of a sequence selected from the group consisting of SEQ ID NOs: 166-169 and 980-983.
2. The composition of claim 1, wherein the antisense oligomer is at least 95% complementary to the target motif of the pre-mRNA encoding the target protein.
3. The composition of claim 1, wherein the antisense oligomer comprises a backbone modification comprising a phosphorothioate linkage or a phosphorodiamidate linkage.
4. The composition of claim 1, wherein the antisense oligomer comprises a phosphorodiamidate morpholino, a locked nucleic acid, or a peptide nucleic acid.
5. The composition of claim 1, wherein the antisense oligomer comprises a 2'-O-methyl moiety, a 2'-Fluoro moiety, or a 2'-O-methoxyethyl moiety.
6. The composition of claim 1, wherein the antisense oligomer comprises at least one modified sugar moiety.
7. The composition of claim 1, wherein the antisense oligomer consists of 8 to 50 nucleobases.
8. The composition of claim 1, wherein the pre-mRNA comprises a sequence selected from the group consisting of premrna_ENST00000293748.9, premrna_ENST00000418600.7, premrna_ENST00000428982.4, premrna_ENST00000449372.7, premrna_ENST00000470232.1, premrna_ENST00000479510.2, premrna_ENST00000628646.2, premrna_ENST00000629380.3, premrna_ENST00000635885.1, premrna_ENST00000636075.1, premrna_ENST00000636116.1, premrna_ENST00000636146.1, premrna_ENST00000636193.1, premrna_ENST00000636436.1,

premrna_ENST00000636443.1, premrna_ENST00000636640.1, premrna_ENST00000636731.1, premrna_ENST00000636905.1, premrna_ENST00000637052.1, premrna_ENST00000637194.1, premrna_ENST00000637490.1, premrna_ENST00000637587.1, premrna_ENST00000637671.1, premrna_ENST00000637721.1, premrna_ENST00000637911.1, premrna_ENST00000638127.1, premrna_ENST00000638142.2, premrna_ENST00000644458.1, premrna_ENST00000645250.1, and premrna_ENST00000646630.1.

9. The composition of claim 1, wherein the target motif of the pre-mRNA comprises a sequence with at least 90%, 95%, 97%, or 100% sequence identity to a region comprising at least 8 contiguous nucleic acids of a sequence selected from the group consisting of SEQ ID NOs: 166-169 and 980-983, and complements thereof.

10. The composition of claim 1, wherein the target motif of the *SYNGAP1* pre-mRNA comprises a sequence that is complementary to at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 contiguous nucleic acids of a sequence selected from the group consisting of SEQ ID NOs: 1-165 and 4300-4352, and a complement thereof.

11. The composition of claim 1, wherein the NSAE-modulating agent comprises a sequence that comprises has at least 80% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1-165 and 4300-4352.

12. Use of the composition of any one of claims 1 to 11 in manufacturing a medicament for treating a disease or condition in a subject in need thereof, wherein the disease or condition is associated with a functional-SYNGAP1 protein deficiency.

13. The use according to claim 20, wherein the disease or condition is autosomal dominant mental retardation, epileptic encephalopathy, or autism.

14. The use according to claim 12, wherein the level of processed mRNA encoding the target protein in the cell contacted with the NSAE-modulating agent is increased 1.1 to 10-fold, 1.5 to 10-fold, 2 to 10-fold, 3 to 10-fold, 4 to 10-fold, 1.1 to 5-fold, 1.1 to 6-fold, 1.1 to 7-fold, 1.1 to 8-fold, 1.1 to 9-fold, 2 to 5-fold, 2 to 6-fold, 2 to 7-fold, 2 to 8-fold, 2 to 9-fold, 3 to 6-fold, 3 to 7-fold, 3 to 8-fold, 3 to 9-fold, 4 to 7-fold, 4 to 8-fold, 4 to 9-fold, at least 1.1-fold, at least 1.5-fold, at least 2-fold, at least 2.5-fold, at least 3-fold, at least 3.5-fold, at least 4-fold, at least 5-fold, or at least 10-fold, compared to the level of processed mRNA encoding the target protein in a control cell that is not contacted with the NSAE-modulating agent.

15. The use according to claim 12, wherein the level of the target protein in the cell contacted with the NSAE-modulating agent is increased 1.1 to 10-fold, 1.5 to 10-fold, 2 to 10-fold, 3 to 10-fold, 4 to 10-fold, 1.1 to 5-fold, 1.1 to 6-fold, 1.1 to 7-fold, 1.1 to 8-fold, 1.1 to 9-fold, 2 to 5-fold, 2 to 6-fold, 2 to 7-fold, 2 to 8-fold, 2 to 9-fold, 3 to 6-fold, 3 to 7-fold, 3 to 8-fold, 3 to 9-fold, 4 to 7-fold, 4 to 8-fold, 4 to 9-fold, at least

1.1-fold, at least 1.5-fold, at least 2-fold, at least 2.5-fold, at least 3-fold, at least 3.5-fold, at least 4-fold, at least 5-fold, or at least 10-fold, compared to the level of the target protein in a control cell that is not contacted with the NSAE-modulating agent.

16. The use according to claim 12, wherein the subject is a human.

17. The use according to claim 12, wherein the composition is to be administered to the subject by intracerebroventricular injection, intraperitoneal injection, intramuscular injection, intrathecal injection, subcutaneous injection, oral administration, synovial injection, intravitreal administration, subretinal injection, topical application, implantation, or intravenous injection.

18. A pharmaceutical composition comprising the composition of any one of claims 1 to 11, and a pharmaceutically acceptable excipient and/or a delivery vehicle.

19. The composition of any one of claims 1 to 11 for use in therapy.