METHODS AND COMPOSITIONS FOR MODULATING SPLICING AND TRANSLATION

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 62/838,010, filed April 24, 2019, 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 April 22, 2020, is named 47991-725_601_SL.txt and is 4,882,023 bytes in size.

BACKGROUND

[0003] Alternative splicing events in genes can lead to non-productive or less productive mRNA transcripts. Therapeutic agents that 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

[0003a] In one aspect, provided herein is a pharmaceutical composition comprising a therapeutic agent or a vector encoding the therapeutic agent, and a pharmaceutically acceptable excipient, wherein the therapeutic agent is an antisense oligomer (ASO) complementary to a targeted region of a pre-mRNA in a cell, wherein the pre-mRNA encodes a target peptide sequence and comprises an inefficient translation region, whereby splicing of the inefficient translation region from the pre-mRNA encoding the target peptide sequence is promoted, thereby increasing a level of a first processed mRNA that is devoid of the inefficient translation region and encodes a first protein comprising the target peptide sequence, and thereby increasing the expression of the target peptide sequence in the cell, wherein the first processed mRNA has a higher translation efficiency for producing the first protein comprising the target peptide sequence in the cell as compared to translation efficiency of a second processed mRNA for producing a second protein comprising the target peptide sequence, wherein the second processed mRNA comprises the inefficient translation region, wherein the pre-mRNA encoding the target peptide sequence comprises a first start codon, a second start codon, and a premature

termination codon (PTC) located downstream of the first start codon and upstream of the second start codon, and wherein the inefficient translation region comprises the premature termination codon (PTC) and the second start codon, and wherein the pre-mRNA is a MeCP2 pre-mRNA.

[0003b] In one aspect, provided herein is the pharmaceutical composition as described herein for use in the treatment of a disease or condition in a subject in need thereof.

[0003c] In one aspect, provided herein is a use of the pharmaceutical composition as described herein in manufacturing a medicament for the treatment of a disease or condition in a subject in need thereof, wherein the disease or the condition is selected from the group consisting of: Intellectual Disability; Rett Syndrome, Preserved Speech Variant; Lubs X-linked mental retardation syndrome; Encephalopathy, Neonatal Severe, due to MeCP2 Mutations; Mental Retardation with Psychosis, Pyramidal Signs, and Macroorchidism; Mental Retardation, X-Linked, With Spasticity; Trisomy Xq28; Mental Retardation, X-Linked 16; Epileptic encephalopathy; Mental Retardation, X-Linked, Syndromic 13; Mental Retardation, X-Linked 1; Rett Syndrome, Atypical; Mental Retardation, X-Linked 79; Microcephaly; Ppm-X Syndrome; Rett Syndrome, Zappella Variant; Rett Syndrome; or Autism susceptibility, X-linked 3; optionally wherein the pharmaceutical composition treats Rett Syndrome.

[0003d] In one aspect, provided herein is a use of the pharmaceutical composition as described herein in manufacturing a medicament for the treatment of a disease or condition in a subject in need thereof, wherein the disease or the condition is caused by a deficient amount or activity of a MeCP2 protein.

[0004] Disclosed herein, in some examples, is a method of modulating expression of a target peptide sequence by a cell having a pre-mRNA that encodes the target peptide sequence and comprises an inefficient translation region, the method comprising contacting the cell with a therapeutic agent that binds to a targeted region of the pre-mRNA encoding the target peptide sequence, whereby splicing of the inefficient translation region from the pre-mRNA encoding the target peptide sequence is modulated, thereby modulating a level of a first processed mRNA that is devoid of the inefficient translation region and encodes a first protein comprising the target peptide sequence, and thereby modulating the expression of the target peptide sequence in the cell, wherein the first processed mRNA has a higher translation efficiency for producing the first protein comprising the target peptide sequence in the cell as compared to translation efficiency of a second processed mRNA for producing a second protein comprising the target peptide sequence, and wherein the second processed mRNA comprises the inefficient translation region.

[0005] Disclosed herein, in some examples, is a method of treating a disease or a condition in a subject in need thereof by modulating expression of a target peptide sequence in a cell of the

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising a therapeutic agent or a vector encoding the therapeutic agent, and a pharmaceutically acceptable excipient,

wherein the therapeutic agent is an antisense oligomer (ASO) complementary to a targeted region of a pre-mRNA in a cell, wherein the pre-mRNA encodes a target peptide sequence and comprises an inefficient translation region, whereby splicing of the inefficient translation region from the pre-mRNA encoding the target peptide sequence is promoted, thereby increasing a level of a first processed mRNA that is devoid of the inefficient translation region and encodes a first protein comprising the target peptide sequence, and thereby increasing the expression of the target peptide sequence in the cell, wherein the first processed mRNA has a higher translation efficiency for producing the first protein comprising the target peptide sequence in the cell as compared to translation efficiency of a second processed mRNA for producing a second protein comprising the target peptide sequence, wherein the second processed mRNA comprises the inefficient translation region, wherein the pre-mRNA encoding the target peptide sequence comprises a first start codon, a second start codon, and a premature termination codon (PTC) located downstream of the first start codon and upstream of the second start codon, and wherein the inefficient translation region comprises the premature termination codon (PTC) and the second start codon, and wherein the pre-mRNA is a MeCP2 pre-mRNA.

2. The pharmaceutical composition of claim 1, wherein the target peptide sequence has at least 100 amino acids.

3. The pharmaceutical composition of claim 1, wherein at least a portion of the targeted region of the pre-mRNA is upstream or downstream of the inefficient translation region.

4. The pharmaceutical composition of claim 1, wherein at least a portion of the targeted region of the pre-mRNA is within the inefficient translation region.

5. The pharmaceutical composition of claim 1, wherein the first processed mRNA and the second processed mRNA are both splicing products of the pre-mRNA in the cell.

6. The pharmaceutical composition of claim 1, wherein the second processed mRNA comprises a sequence of the first processed mRNA, the PTC, and the second start codon.

7. The pharmaceutical composition of claim 6, wherein the first protein comprising the target peptide sequence is translated starting from the first start codon on the first processed

mRNA, and the second protein is translated starting from the second start codon on the second processed mRNA.

8. The pharmaceutical composition of claim 1, wherein exclusion of the inefficient translation region from the pre-mRNA encoding the target peptide sequence is increased.
9. The pharmaceutical composition of claim 1, wherein the therapeutic agent increases the level of the first processed mRNA encoding the target peptide sequence in the cell.
10. The pharmaceutical composition of claim 1, wherein the therapeutic agent increases the level of the target peptide sequence in the cell.
11. The pharmaceutical composition of claim 1, wherein the target peptide sequence is a portion of a full length protein.
12. The pharmaceutical composition of claim 1, wherein the target peptide sequence produced is a fully functional protein.
13. The pharmaceutical composition of claim 1, wherein the target peptide sequence is an N-terminal portion of a full length protein.
14. The pharmaceutical composition of claim 13, wherein the target peptide sequence is encoded by a portion of the pre-mRNA upstream of the inefficient translation region.
15. The pharmaceutical composition of claim 1, wherein the inefficient translation region is in a 3' untranslated region (3' UTR) of the second processed mRNA.
16. The pharmaceutical composition of claim 1, wherein the target peptide sequence is a C-terminal portion of a full length protein.
17. The pharmaceutical composition of claim 16, wherein the target peptide sequence is encoded by a portion of the pre-mRNA downstream of the inefficient translation region.
18. The pharmaceutical composition of claim 1, wherein the pre-mRNA comprises a sequence with at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%, or 100% sequence identity to anyone selected from the group consisting of SEQ ID NOs: 2-27.
19. The pharmaceutical composition of claim 1, wherein the target peptide sequence is translated from a sequence selected from the group consisting of SEQ ID NOs: 32-34.
20. The pharmaceutical composition of claim 1, wherein the targeted region is within a sequence selected from the group consisting of SEQ ID NOs: 28-31.
21. The pharmaceutical composition of claim 1, wherein the first processed mRNA encodes a protein having at least 80% sequence identity to SEQ ID NO: 8204 or 8205.
22. The pharmaceutical composition of claim 1, wherein the therapeutic agent decreases a level of a second processed mRNA encoding a protein having at least 80% sequence identity to SEQ ID NO: 440 or 441.

23. The pharmaceutical composition of claim 1, wherein the therapeutic agent decreases level of e2 isoform of MeCP2 protein and increases level of e1 isoform of MeCP2 protein in the cell.

24. The pharmaceutical composition of claim 1, wherein the targeted region of the pre-mRNA
(a) comprises at least 10 contiguous nucleobases of SEQ ID NO: 238 or 239;
(b) is within a sequence between a pair of genomic sites selected from the group consisting of GRCh38/hg38: chrX 154092307 and GRCh38/hg38: chrX 154092184;
(c) is at least 50 nucleotides downstream of genomic site GRCh38/hg38: chrX 154092184;
(d) is at most 1500 nucleotides downstream of genomic site GRCh38/hg38: chrX 154092184;
(e) is at least 50 nucleotides upstream of genomic site GRCh38/hg38: chrX 154092307; or
(f) is at most 1500 nucleotides upstream of genomic site GRCh38/hg38: chrX 154092307.

25. The pharmaceutical composition of claim 1, wherein the ASO has a sequence with at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%, or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 36-129 and 4548-4611.

26. The pharmaceutical composition of claim 1, wherein the ASO is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, complementary to the targeted region of the pre-mRNA encoding the target peptide sequence.

27. The pharmaceutical composition of claim 1, comprising the vector encoding the therapeutic agent.

28. The pharmaceutical composition of claim 27, wherein the vector is a viral vector.

29. The pharmaceutical composition of claim 1, wherein the ASO comprises a backbone modification comprising a phosphorothioate linkage or a phosphorodiamidate linkage.

30. The pharmaceutical composition of claim 1, wherein the ASO comprises a phosphorodiamidate morpholino, a locked nucleic acid, a peptide nucleic acid, a 2’-O-methyl, a 2’-Fluoro, or a 2’-O-methoxyethyl moiety.

31. The pharmaceutical composition of claim 1, wherein the ASO comprises at least one modified sugar moiety.

32. The pharmaceutical composition of claim 31, wherein each sugar moiety is a modified sugar moiety.

33. The pharmaceutical composition of claim 1, wherein the therapeutic agent is an antisense oligomer, and wherein the therapeutic agent consists of from 8 to 50 nucleobases, 8 to 40 nucleobases, 8 to 35 nucleobases, 8 to 30 nucleobases, 8 to 25 nucleobases, 8 to 20 nucleobases, 8 to 15 nucleobases, 9 to 50 nucleobases, 9 to 40 nucleobases, 9 to 35

nucleobases, 9 to 30 nucleobases, 9 to 25 nucleobases, 9 to 20 nucleobases, 9 to 15 nucleobases, 10 to 50 nucleobases, 10 to 40 nucleobases, 10 to 35 nucleobases, 10 to 30 nucleobases, 10 to 25 nucleobases, 10 to 20 nucleobases, 10 to 15 nucleobases, 11 to 50 nucleobases, 11 to 40 nucleobases, 11 to 35 nucleobases, 11 to 30 nucleobases, 11 to 25 nucleobases, 11 to 20 nucleobases, 11 to 15 nucleobases, 12 to 50 nucleobases, 12 to 40 nucleobases, 12 to 35 nucleobases, 12 to 30 nucleobases, 12 to 25 nucleobases, 12 to 20 nucleobases, or 12 to 15 nucleobases.

34. The pharmaceutical composition of claim 1, wherein the target peptide sequence is a portion of a MECP2 protein.

35. The pharmaceutical composition of claim 1 for use in the treatment of a disease or condition in a subject in need thereof.

36. Use of the pharmaceutical composition of claim 1 in manufacturing a medicament for the treatment of a disease or condition in a subject in need thereof, wherein the disease or the condition is selected from the group consisting of: Intellectual Disability; Rett Syndrome, Preserved Speech Variant; Lubs X-linked mental retardation syndrome; Encephalopathy, Neonatal Severe, due to MeCP2 Mutations; Mental Retardation with Psychosis, Pyramidal Signs, and Macroorchidism; Mental Retardation, X-Linked, With Spasticity; Trisomy Xq28; Mental Retardation, X-Linked 16; Epileptic encephalopathy; Mental Retardation, X-Linked, Syndromic 13; Mental Retardation, X-Linked 1; Rett Syndrome, Atypical; Mental Retardation, X-Linked 79; Microcephaly; Ppm-X Syndrome; Rett Syndrome, Zappella Variant; Rett Syndrome; or Autism susceptibility, X-linked 3; optionally wherein the pharmaceutical composition treats Rett Syndrome..

37. Use of the pharmaceutical composition of claim 1 in manufacturing a medicament for the treatment of a disease or condition in a subject in need thereof, wherein the disease or the condition is caused by a deficient amount or activity of MeCP2 protein.

38. The use according to claim 36 or 37, wherein the pharmaceutical 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.