TARGETED AUGMENTATION OF NUCLEAR GENE OUTPUT

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 62/059,847, filed October 3, 2014, which application is incorporated herein by reference.

BACKGROUND

[0002] Some genetic diseases are caused by haploinsufficiency, in which there is only one functional copy of a gene and that single copy does not produce enough of the gene product. For example, this can be caused by hemizygous deletions, in which one copy of the gene is lost. Other genetic diseases are caused by mutations which alter the gene product, so that it possesses only partial function.

SUMMARY

[0003] As described herein, antisense oligomers (ASOs) can be used to increase production of proteins, or functional RNAs in the case of non-protein coding genes, by promoting constitutive splicing (employing the wild-type sequence) at an intron splice site of an intron-containing gene to increase expression of the gene product. The ASOs described for use in these methods promote constitutive splicing and do not correct aberrant splicing resulting from mutation, or promote constitutive splicing and do not modulate alternative splicing. The methods described herein may therefore be used to treat a condition resulting from reduced expression or insufficient activity of a gene product.

[0003a] In one aspect, there is provided use of an antisense oligomer (ASO) in the manufacture of a medicament to increase the expression of a target protein or functional RNA by cells of a subject, wherein the cells are to be contacted with the antisense oligomer (ASO),
wherein the cells have a retained-intron-containing pre-mRNA (RIC pre-mRNA), wherein the RIC pre-mRNA comprising a retained intron, an exon flanking the 5' splice site, an exon flanking the 3' splice site, and wherein the RIC pre-mRNA encodes the target protein or functional RNA; wherein the antisense oligomer binds to a targeted portion of the RIC pre-mRNA, whereby the retained intron is constitutively spliced from the RIC pre-mRNA encoding the target protein or functional RNA, thereby increasing a level of mRNA encoding the target protein or functional RNA, and increasing the expression of the target protein or functional RNA in the cells of the subject.

[0004] Described here are methods of increasing expression in cells of a target protein encoded by a pre-mRNA that comprises at least one retained intron (an RIC pre-mRNA); a

[0005] retained intron is one that remains present when one or more of the other introns have been spliced out (removed). Expression of the target protein depends on complete splicing (removal) of all introns in the pre-mRNA in the nucleus to generate mature mRNA that is subsequently exported to the cytoplasm and translated into the target protein. Inefficient splicing (removal) of an intron results in a retained intron-containing (RIC) pre-mRNA that accumulates primarily in the nucleus, and if exported to the cytoplasm is degraded, such that RIC pre-mRNA is not translated into the target protein. Treatment with an antisense oligomer (ASO) described by the method herein can promote the splicing of a retained intron from pre-mRNA transcripts (pre-mRNA species comprising one or more introns) and result in an increase in mRNA, which is translated to provide higher levels of target protein.

[0006] In embodiments, the method is a method of increasing expression of a target protein or functional RNA by cells having a retained-intron-containing pre-mRNA (RIC pre-mRNA), the

CLAIMS

1. Use of an antisense oligomer (ASO) in the manufacture of a medicament to increase the expression of a target protein or functional RNA by cells of a subject, wherein the cells are to be contacted with the antisense oligomer (ASO),

wherein the cells have a retained-intron-containing pre-mRNA (RIC pre-mRNA), wherein the RIC pre-mRNA comprising a retained intron, an exon flanking the 5' splice site, an exon flanking the 3' splice site, and wherein the RIC pre-mRNA encodes the target protein or functional RNA; wherein the antisense oligomer binds to a targeted portion of the RIC pre-mRNA, whereby the retained intron is constitutively spliced from the RIC pre-mRNA encoding the target protein or functional RNA, thereby increasing a level of mRNA encoding the target protein or functional RNA, and increasing the expression of the target protein or functional RNA in the cells of the subject.

2. The use according to claim 1, wherein the subject has a condition caused by a deficient amount or activity of the target protein or a deficient amount or activity of the target functional RNA.

3. The use according to claim 2, wherein the deficient amount or activity of the target protein or the target functional RNA is caused by haploinsufficiency of the target protein or the target functional RNA.

4. The use according to claim 2, wherein the subject has a condition caused by an autosomal recessive disorder resulting from a deficiency in the amount or function of the target protein, wherein the subject has

a.a first mutant allele from which

i. the target protein is produced at a reduced level compared to production from a wild-type allele,

ii. the target protein is produced in a form having reduced function compared to an equivalent wild-type protein, or

iii. the target protein is not produced, and

b.a second mutant allele from which

i. the target protein is produced at a reduced level compared to production from a wild-type allele,

ii. the target protein is produced in a form having reduced function compared to an equivalent wild-type protein, or

iii. the target protein is not produced, and

wherein the RIC pre-mRNA is transcribed from the first allele and/or the second allele.

5. The use according to claim 1, wherein the targeted portion of the RIC pre-mRNA is in the retained intron within:

(a) the region +6 relative to the 5' splice site of the retained intron to -16 relative to the 3' splice site of the retained intron;

(b) the region +6 to +100 relative to the 5' splice site of the retained intron;

(c) the region -16 to -100 relative to the 3' splice site of the retained intron;

(d) the region +2e to -4e in the exon flanking the 5' splice site of the retained intron; or

(e) the region +2e to -4e in the exon flanking the 3' splice site of the retained intron.

6. The use according to claim 1, wherein the antisense oligomer does not increase the amount of the target protein or the functional RNA by modulating alternative splicing of pre-mRNA transcribed from a gene encoding the functional RNA or target protein.

7. The use according to claim 1, wherein the antisense oligomer does not increase the amount of the target protein or the functional RNA by modulating aberrant splicing resulting from mutation of the gene encoding the target protein or the functional RNA.

8. The use according to claim 1, wherein the RIC pre-mRNA was produced by partial splicing of a full-length pre-mRNA or partial splicing of a wild-type pre-mRNA.

9. The use according to claim 1, wherein the mRNA encoding the target protein or functional RNA is a full-length mature mRNA, or a wild-type mature mRNA.

10. The use according to claim 1, wherein the target protein produced is full-length protein, or wild-type protein.

11. The use according to claim 1, wherein a total amount of the mRNA encoding the target protein or the target functional RNA produced in the cell contacted with the antisense

oligomer is increased at least 1.1-fold compared to a total amount of the mRNA encoding the target protein or the target functional RNA produced in a control cell.

12. The use according to claim 1, wherein a total amount of the target protein produced by the cell contacted with the antisense oligomer is increased at least 1.1-fold compared to a total amount of the target protein produced by a control cell.

13. The use according to claim 1, wherein the antisense oligomer comprises

a backbone modification comprising a phosphorothioate linkage or a phosphorodiamidate linkage; or

a modified sugar moiety or a sugar analog, optionally comprising a phosphorodiamidate morpholino, a locked nucleic acid, a peptide nucleic acid, a 2'-O-methyl moiety, a 2'-Fluoro moiety, or a 2'-O-methoxyethyl moiety.

14. The use according to claim 1, wherein the antisense oligomer consists of from 8 to 50 nucleobases.

15. The use according to claim 1, wherein the antisense oligomer comprises a sequence with at least 80% complementary to the targeted region of the RIC pre-mRNA that encodes the target protein or the target functional RNA.

16. The use according to claim 1, wherein the antisense oligomer binds to a region of an RIC pre-mRNA comprising a sequence selected from the group consisting of SEQ ID NOS: 1-102 and 375-384.

17. The use according to claim 1, wherein the antisense oligomer comprises a sequence selected from the group consisting of SEQ ID NOs: 103-374 and 385-390.

18. The use according to claim 2, wherein the condition is a disease or disorder, optionally the disease or disorder is selected from: thrombotic thrombocytopenic purpura, tuberous sclerosis complex, polycystic kidney disease, familial dysautonomia, retinitis pigmentosa type 10, retinitis pigmentosa type 11, cystic fibrosis, retinoblastoma, familial adenomatous polyposis, protein S deficiency, beta thalassemia, and sickle cell disease.

19. The use according to claim 18, wherein the target protein or the target functional RNA and the RIC pre-mRNA are encoded by a gene selected from the group consisting of *ADAMTS13, TSC1, PKD1, IKBKAP, IMPDH1, PRPF31, CFTR, RB1, HBG1, HBG2,* and *HBB.*

20. The use according to claim 1, wherein nucleotides that are -3e to -1e of the exon flanking the

5' splice site and +1 to +6 of the retained intron are identical to nucleotides at corresponding positions of a corresponding wild-type sequence.

21. The use according to claim 1, wherein nucleotides that are -15 to -1 of the retained intron and +1e of the exon flanking the 3' splice site are identical to nucleotides at corresponding positions of a corresponding wild-type sequence.

22. The use according to claim 1, wherein the cells are to be contacted with the antisense oligomer *ex vivo*.

23. The use according to claim 1, wherein the antisense oligomer is to be administered to the subject by intravitreal injection, intrathecal injection, intraperitoneal injection, subcutaneous injection, or intravenous injection.

24. The use according to claim 1, wherein the antisense oligomer is further formulated as a pharmaceutical composition.

25. The use according to claim 24, wherein the pharmaceutical composition further comprises a pharmaceutically acceptable excipient or carrier.