Title: NUCLEIC ACID COMPOUNDS FOR INHIBITING SIRT2 GENE EXPRESSION AND USES THEREOF

Abstract: The present disclosure provides meroduplex ribonucleic acid molecules (mdRNA) capable of decreasing or silencing SIRT2 gene expression. An mdRNA of this disclosure comprises at least three strands that combine to form at least two non-overlapping double-stranded regions separated by a nick or gap wherein one strand is complementary to a SIRT2 mRNA. In addition, the meroduplex may have at least one uridine substituted with a 5-methyluridine, a nucleoside replaced with a locked nucleic acid, or optionally other modifications, and any combination thereof. Also provided are methods of decreasing expression of a SIRT2 gene in a cell or in a subject to treat a SIRT2-related disease.
AMENDED CLAIMS
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1. A meroduplex ribonucleic acid (mdRNA) molecule that down
regulates the expression of a human silent mating type information regulation 2
homolog (SIRT2) mRNA, the mdRNA molecule comprising a first strand of 15 to 40
nucleotides in length that is complementary to a portion of the human SIRT2 mRNA
as set forth in SEQ ID NOS:1158 or 1159, and a second strand and a third strand that
is each complementary to non-overlapping regions of the first strand, wherein the
second strand and third strand can anneal with the first strand to form at least two
double-stranded regions spaced apart by a nick or a gap.

2. The mdRNA molecule of claim 1 wherein the first strand is 15 to 25
nucleotides in length or 26 to 40 nucleotides in length.

3. The mdRNA molecule of claim 1 wherein the gap comprises from 1 to
10 unpaired nucleotides.

4. The mdRNA molecule of claim 1 wherein the mdRNA molecule
comprises at least one 5-methyluridine, 2-thioribothymidine, or 2'-O-methyl-5-
methyluridine.

5. The mdRNA molecule of claim 1 wherein the mdRNA molecule
comprises at least one locked nucleic acid (LNA) molecule, deoxy nucleotide, G
clamp, 2'-sugar modification, modified internucleoside linkage, or any combination
thereof.

6. The mdRNA molecule of claim 1 wherein the mdRNA contains an
overhang of one to four nucleotides on at least one 3'-end that is not part of the gap or
has a blunt end at one or both ends of the mdRNA.

7. An mdRNA molecule that down regulates the expression of a human
SIRT2 mRNA, the mdRNA molecule comprising a first strand of 15 to 40 nucleotides
in length that is complementary to a portion of the human SIRT2 mRNA as set forth
in SEQ ID NOS:1158 or 1159, and a second strand and a third strand that is each
complementary to non-overlapping regions of the first strand, wherein the second
strand and third strand can anneal with the first strand to form at least two
double-stranded regions spaced apart by a nick or a gap, and wherein at least one
pyrimidine of the mdRNA molecule is a pyrimidine nucleoside according to Formula I or II:

wherein:

- $R^1$ and $R^2$ are each independently a $-H$, $-OH$, $-OCH_3$, $-OCH_2OCH_2CH_3$, $-OCH_2CH_2OCH_3$, halogen, substituted or unsubstituted $C_{1-10}$ alkyl, alkoxy, alkoxyalkyl, hydroxyalkyl, carboxyalkyl, alkylsulfonylamino, aminoalkyl, dialkylamino, alkylaminoalkyl, dialkylaminoalkyl, haloalkyl, trifluoromethyl, cycloalkyl, (cycloalkyl)alkyl, substituted or unsubstituted $C_{2-10}$ alkenyl, substituted or unsubstituted $-O$-allyl, $-O$-CH$_2$CH=CH$_2$, $-O$-CH=CHCH$_3$, substituted or unsubstituted $C_{2-10}$ alkynyl, carbamoyl, carbamyl, carboxy, carbonylamino, substituted or unsubstituted aryl, substituted or unsubstituted aralkyl, $-NH_2$, $-NO_2$, $-C\equiv$, or heterocyclo group,

- $R^3$ and $R^4$ are each independently a hydroxyl, a protected hydroxyl, a phosphate, or an internucleoside linking group, and
- $R^5$ and $R^6$ are each independently $O$ or $S$.

8. The mdRNA molecule of claim 7 wherein the first strand is 15 to 25 nucleotides in length or 26 to 40 nucleotides in length.

9. The mdRNA molecule of claim 7 wherein the gap comprises from 1 to 10 unpaired nucleotides.

10. The mdRNA molecule of claim 7 wherein at least one nucleoside is according to Formula I and in which $R^1$ is methyl and $R^2$ is $-OH$ or $-O$-methyl.

11. The mdRNA molecule of claim 7 wherein at least one $R^2$ is selected from the group consisting of $2'$-O-(C$_1$-C$_9$) alkyl, $2'$-O-methyl, $2'$-OCH$_2$OCH$_2$CH$_3$, $2'$-OCH$_2$CH$_2$OCH$_3$, $2'$-O-allyl, and fluoro.
12. The mRNA molecule of claim 7 wherein the mRNA molecule comprises at least one 5-methyluridine, 2-thioribothymidine, or 2'-O-methyl-5-methyluridine.

13. The mRNA molecule of claim 7 wherein the mRNA molecule comprises at least one locked nucleic acid (LNA) molecule, deoxy nucleotide, G clamp, 2'-sugar modification, modified internucleoside linkage, or any combination thereof.

14. The mRNA molecule of claim 7 wherein contains an overhang of one to four nucleotides on at least one 3'-end that is not a part of the gap or the dsRNA molecule has a blunt end on one or both ends of the mRNA molecule.

15. An mRNA molecule that down regulates the expression of a human SIRT2 mRNA, the mRNA molecule comprising a first strand of 15 to 40 nucleotides in length that is complementary to a portion of the human SIRT2 mRNA as set forth in SEQ ID NO:1158 or 1159, and a second strand and a third strand that is each complementary to non-overlapping regions of the first strand, wherein the second strand and third strand can anneal with the first strand to form at least two double-stranded regions spaced apart by a nick or a gap, and wherein the double-stranded regions have a combined length of about 15 base pairs to about 40 base pairs.

16. The mRNA molecule of claim 15 wherein the first strand is 15 to 25 nucleotides in length or 26 to 40 nucleotides in length.

17. The mRNA molecule of claim 15 wherein the gap comprises from 1 to 10 unpaired nucleotides.

18. The mRNA molecule of claim 15 wherein the mRNA molecule comprises at least one 5-methyluridine, 2-thioribothymidine, or 2'-O-methyl-5-methyluridine.

19. The mRNA molecule of claim 15 wherein the first strand is 19 to 23 nucleotides in length and is complementary to a human SIRT2 nucleic acid sequence as set forth in any one of SEQ ID NO:1160-1535.
20. The mdRNA molecule of claim 15 wherein the first strand is 25 to 29 nucleotides in length and is complementary to a human SIRT2 nucleic acid sequence as set forth in any one of SEQ ID Nos: 1150-1535.

21. A method for reducing the expression of a human SIRT2 gene, comprising administering an mdRNA molecule according to any one of claims 1-20 to a cell expressing the human SIRT2 gene, wherein the mdRNA molecule reduces the expression of the human SIRT2 gene in the cell.

22. The method according to claim 21 wherein the cell is a human cell.

23. Use of an mdRNA as defined in any one of the preceding claims for the manufacture of a medicament for use in the therapy of a hyperproliferative or inflammatory disease.

24. A double-stranded ribonucleic acid (dsRNA) molecule that down regulates the expression of a human silent mating type information regulation 2 homolog (SIRT2) mRNA, the dsRNA molecule comprising a first strand of 26 to 40 nucleotides in length that is complementary to a portion of the human SIRT2 mRNA as set forth in SEQ ID Nos: 1158 or 1159, and a second strand that is complementary to the first strand, and wherein upon annealing of the first strand and the second strand the dsRNA has a 3' overhang and a blunt end.

25. The dsRNA molecule of claim 24 wherein the first strand is from 27 to 35 nucleotides in length.

26. The dsRNA molecule of claim 24 wherein the dsRNA molecule comprises at least one 5-methyluridine, 2-thioribothymidine, or 2'-O-methyl-5-methyluridine.

27. The dsRNA molecule of claim 24 wherein the dsRNA molecule comprises at least one locked nucleic acid (LNA) molecule, deoxy nucleotide, G clamp, 2'-sugar modification, modified internucleoside linkage, or any combination thereof.

28. The dsRNA molecule of claim 24 wherein the 3'-overhang has from one to four nucleotides and is on the first strand.
29. The dsRNA molecule of claim 24 wherein the dsRNA molecule has a 5'-terminal end comprising a hydroxyl or a phosphate.

30. A dsRNA molecule that down regulates the expression of a human SIRT2 mRNA, the dsRNA molecule comprising a first strand of 26 to 40 nucleotides in length that is complementary to a portion of the human SIRT2 mRNA as set forth in SEQ ID NOS:1158 or 1159, and wherein upon annealing of the first strand and the second strand the dsRNA has a 3' overhang and a blunt end, and wherein at least one pyrimidine of the dsRNA molecule comprises a pyrimidine nucleoside according to Formula I or II:

![Diagram of nucleosides](image)

wherein:

R¹ and R² are each independently a -H, -OH, -OCH₃, -OCH₂OCH₂CH₃, -OCH₂CH₂OCH₃, halogen, substituted or unsubstituted C₁-C₁₀ alkyl, alkoxy, alkoxyalkyl, hydroxyalkyl, carboxyalkyl, alkylsulfonlamino, aminoalkyl, dialkylamino, alkylaminoalkyl, dialkylaminoalkyl, haloalkyl, trifluoromethyl, cycloalkyl, (cycloalkyl)alkyl, substituted or unsubstituted C₂-C₁₀ alkenyl, substituted or unsubstituted C₂-C₁₀ alkenyl, substituted or unsubstituted -O-allyl, -O-CH₂CH=CH₂, -O-CH=CHCH₃, substituted or unsubstituted C₂-C₁₀ alkynyl, carbamoyl, carbamyl, carboxy, carbonylamino, substituted or unsubstituted aryl, substituted or unsubstituted aralkyl, -NH₂, -NO₂, -C≡N, or heterocyclo group,

R³ and R⁴ are each independently a hydroxyl, a protected hydroxyl, a phosphate, or an internucleoside linking group, and

R⁵ and R⁸ are each independently O or S.

31. The dsRNA molecule of claim 30 wherein the first strand is from 27 to 35 nucleotides in length.
32. The dsRNA molecule of claim 30 wherein at least one nucleoside is according to Formula I and in which R¹ is methyl and R² is –OH or –O-methyl.

33. The dsRNA molecule of claim 30 wherein at least one R² is selected from the group consisting of 2′-O-(C₁-C₅) alkyl, 2′-O-methyl, 2′-OCH₂OCH₂CH₃, 2′-OCH₂CH₂OCH₃, 2′-O-allyl, and 2′-fluoro.

34. The dsRNA molecule of claim 30 wherein the dsRNA molecule comprises at least one 5-methyluridine, 2-thioribothymidine, or 2′-O-methyl-5-methyluridine.

35. The dsRNA molecule of claim 30 wherein the dsRNA molecule comprises at least one LNA, deoxy nucleotide, G clamp, 2′-sugar modification, modified internucleoside linkage, or any combination thereof.

36. The dsRNA molecule of claim 30, wherein the 3′-overhang has from one to four nucleotides and is on the first strand.

37. A method for reducing the expression of a human SIRT2 gene, comprising administering a dsRNA molecule according to any one of claims 24-36 to a cell expressing the human SIRT2 gene, wherein the dsRNA molecule reduces the expression of the human SIRT2 gene in the cell.

38. The method according to claim 37 wherein the cell is a human cell.

39. Use of a dsRNA molecule as defined in any one of claims 24-38 for the manufacture of a medicament for use in the therapy of a hyperproliferative or inflammatory disease.