MODIFIED ANTIMIR-138 OLIGONUCLEOTIDES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority of Singapore patent application No. 10201404535S, filed 31 July 2014, the contents of it being hereby incorporated by reference in its entirety for all purposes.

FIELD OF THE INVENTION

[0002] The present invention relates to biochemistry in particular biomarkers. In particular, the present invention relates to oligonucleotides that can regulate the expression of biomarker miR-138, pharmaceutical compositions and methods of using the biomarkers thereof.

BACKGROUND OF THE INVENTION

[0003] MicroRNAs (miRNAs) are endogenous small noncoding RNAs that regulate various physiological pathways such as cellular differentiation and proliferation via gene silencing. The microRNAs are thought to contribute to the initiation, progression and eventual metastasis of various proliferative diseases. For example, dysregulation of the expression of a miRNA may lead to aberrant expression of its target proteins, resulting in an altered phenotype in a cell. Impaired miRNA regulatory network is known to be one of the key mechanisms in the pathogenesis of proliferative diseases such as cancer. Thus, there is a need to provide molecules that have the potential to influence the expression of miRNA.

SUMMARY OF THE INVENTION

[0004] In one aspect, there is provided an oligonucleotide comprising at least one locked nucleic acid (LNA), wherein the oligonucleotide is substantially complementary to a nucleotide sequence of miR-138, wherein the oligonucleotide reduces or inhibits the activity of one or more miR-138, wherein only the 5' end and 3' end of the oligonucleotide is modified with phosphorothioate link and the middle portion of the oligonucleotide is not modified with phosphorothioate link, and the oligonucleotide is not: 5'mC*mG*G*mCmCTmGmATmUmCAmCmAAmCmACmCmA*G*mC*mU-3' (SEQ ID NO: 12), wherein m is a 2'-O-methylation, * is a phosphorothioate linkage and underlined nucleotides represent a locked nucleic acid modification.

[0005] In another aspect, there is provided a pharmaceutical composition comprising an effective amount of the oligonucleotide as described herein, or a pharmaceutically-acceptable salt thereof, and a pharmaceutically-acceptable carrier or diluent.

[0006] Also described herein is a method of treating a proliferative disease in a subject in need thereof comprising administering the oligonucleotide as described herein to the subject.

[0007] In another aspect, there is provided a use of an oligonucleotide as described herein in the manufacture of a medicament for treating proliferative disease in a subject in need thereof.

[0008] Also described herein is a method of reducing or inhibiting the activity of miR-138 in a cell comprising contacting the cell with the oligonucleotide as described herein.

Claims

1. An oligonucleotide comprising at least one locked nucleic acid (LNA),
wherein the oligonucleotide is substantially complementary to a nucleotide sequence of miR-138, wherein the oligonucleotide reduces or inhibits the activity of one or more miR-138, wherein only the 5' end and 3' end of the oligonucleotide is modified with phosphorothioate link and the middle portion of the oligonucleotide is not modified with phosphorothioate link, and
the oligonucleotide is not:
5'mC*mG*G*mCmCTmGmATmUmCAmCmAAmCmACmCmA*G*mC*mU-3' (SEQ ID NO: 12), wherein m is a 2'-O-methylation, * is a phosphorothioate linkage and underlined nucleotides represent a locked nucleic acid modification.

2. The oligonucleotide of claim 1, wherein the locked nucleic acid creates a bi-cyclic structure that locks the conformation of the ribose.

3. The oligonucleotide of claim 2, wherein the bi-cyclic structure is created by ribose moiety modification which creates a bridge connecting 2' oxygen to 4' carbon.

4. The oligonucleotide of claim 3, wherein the bridge is an ethylene, methylene or oxy-methylene bridge.

5. The oligonucleotide of any one of claims 1 to 4, wherein the oligonucleotide further comprises a modification that resists degradation by cellular enzymes.

6. The oligonucleotide of claim 5, wherein the modification is a modification with at least one phosphorothioate link.

7. The oligonucleotide of any one of claims 1 to 6, wherein the oligonucleotide is from about 11 to 23 nucleotides in length.

8. The oligonucleotide of claim 7, wherein the oligonucleotide contains at least two locked nucleic acid(s).

9. The oligonucleotide of any one of claims 1 to 8, wherein the non-locked nucleic acid is comprised at least two times as 2 to 4 contiguous nucleic acids.

10. The oligonucleotide of any one of claim 1 to 9, wherein the oligonucleotide does not contain a stretch of nucleic acid with more than one, or two or three contiguous locked nucleic acid.

11. The oligonucleotide of any one of claims 1 to 9, wherein the oligonucleotide has at least one phosphorothioate link.

12. The oligonucleotide of claim 11, wherein the phosphorothioate link is comprised independently of each other at least at 1, of the 3' end and/or 5' end of the oligonucleotide.

13. The oligonucleotide of any one of claims 1 to 12, wherein the oligonucleotide further comprises a modification that offers stability against base hydrolysis and nucleases.

14. The oligonucleotide of claim 13, wherein the modification comprises adding 2'-*O*-deoxy, 2'-*O*-methyl, 2'-*O*-alkyl, 2'-halo, or 2'-fluoro to the 2-hydroxyl group of the ribose moiety of a non-locked nucleic acid.

15. The oligonucleotide of any one of claims 1 to 14, comprising the sequence 5'-CGGCCUGAUTCACAACACCAGCU-3' (SEQ ID NO: 2; antimRNA-138) and 5'-CGGCCUGAUUCACAACACCAGCU-3' (SEQ ID NO: 3; antimRNA-138), wherein SEQ ID NO: 2 and SEQ ID NO: 3, independently, comprises at least one locked nucleic acid.

16. The oligonucleotide of claim 15, wherein the locked nucleic acids are at positions 3, 5, 8, 10, 12, 15, 17, 19 and 21 of SEQ ID NO: 2, wherein position 1 of SEQ ID NO: 2 is the 5' end terminus and position 23 of SEQ ID NO: 2 is the 3' end terminus.

17. The oligonucleotide of claim 16, wherein the phosphorothioate links are independently selected from positions 1 to 5 or 19 to 23 of SEQ ID NO: 2.

18. The oligonucleotide of claim 17, wherein the phosphorothioate links are at positions 1, 2, 3, 20, 21, and 23 of SEQ ID NO: 2.

19. The oligonucleotide of claim 18, wherein the modification is a 2'-O-methylation at positions 1, 2, 4, 6, 7, 9, 11, 13, 14, 16, 18, 20, 22, 23 of SEQ ID NO: 2.

20. The oligonucleotide of any one of claims 1 to 19, comprising one or more of the sequence selected from the group consisting of 5'-mC*mG*G*mCCmUmGA mUTmCAmCmAAmC AmCCmA*G*mC*mU-3' (SEQ ID NO: 13) and 5'-mC*mG*mG*mCmCTmGAmUTmCAmCAmACmACmCmA*mG*mC*mU-3' (SEQ ID NO: 10), wherein m is a 2'-*O*-methylation, * is a phosphorothioate linkage and underlined nucleotides represent a locked nucleic acid modification.

21. The oligonucleotide of any one of claims 1 to 9, comprising the sequence 5'-A*C*AACACCAG*C*-3' (SEQ ID NO: 5), wherein * is a phosphorothioate linkage and underlined nucleotides represent a locked nucleic acid modification.

22. A pharmaceutical composition comprising an effective amount of the oligonucleotide of any one of claims 1 to 21, or a pharmaceutically-acceptable salt thereof, and a pharmaceutically-acceptable carrier or diluent.

23. Use of an oligonucleotide as claimed in any one of claims 1 to 21 in the manufacture of a medicament for treating a proliferative disease in a subject in need thereof.

24. The use of claim 23, wherein the proliferative disease is a benign tumor or cancer.

25. The use of claim 24, wherein the cancer is selected from the group consisting of malignant glioma and breast cancer.