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 one of the sequences selected from the group consisting of 5'-mC*mG*G*mCCmUmGAmUTmCAmCmAAmCAmCCmA*G*mC*mU-3' (SEQ ID NO: 13), and 5'-mC*mG*mG*mCmCTmGAmUTmCAmCAmACmACmCmCmA*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.

[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.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009] The invention will be better understood with reference to the detailed description when considered in conjunction with the non-limiting examples and the accompanying drawings, in which:

[0010] **Fig. 1** shows light microscopic images of the U87MG cells (human glioblastoma cell line) transfected with anti-miR-138 oligonucleotides as described in the Example section. In particular, Fig. 1 shows day 7 post-transfection with 40 nanomoles of anti-sense oligos anti-miR-138-1 (SEQ ID NO: 10), anti-miR-138-2 (SEQ ID NO: 12), anti-miR-138-3 (SEQ ID NO: 13), anti-miR-138-4 (SEQ ID NO: 5 or "Oligo-4") and negative controls ("scrambled control" for anti-miR-138-1, anti-miR-138-2, and anti-miR-138-3; "control sequence" for Oligo-4) of U87MG cells. Sequences of anti-sense oligos used in Fig. 1 are provided in Table 1 below. Fig. 1 shows that whilst anti-miR-138 oligonucleotides inhibit the growth of U87MG cells, control anti-sense oligos were not found to inhibit the proliferation of U87MG cells. Thus, showing anti-miR-138 oligonucleotide can prevent the proliferation of malignant gliomas.

[0011] **Fig. 2** shows bar graphs of the results of miRNA quantification of miR-138 levels in U87MG cells (human glioblastoma cell line). RNA was isolated from cells harvested 7 days after transfection with anti-miR-138 oligonucleotides described in Fig. 1. Left panel of Fig. 2 shows the regulation of miR-138 expression by lentiviral antagomir; Right panel of Fig. 2 shows modified anti-miR-138 oligonucleotides inhibit miR-138 expression in U87MG cells. Thus, Fig. 2 shows modified anti-miR138-3 (i.e. AMO_138_23_3 in Table 1) oligonucleotides are as effective as miR-138 lentiviral antagomir in inhibiting the expression of miR-138.

[0012] **Fig. 3** shows regulation of miR-138 expression in both the glioblastoma stem cells (GSCs) and U87MG (human glioblastoma cell line). In particular, Fig. 3 shows that, as compared to two non-targeting controls (i.e. scrambled and anti-miR-106), anti-miR-138-3 (i.e. AMO_138_23_3 in Table 1) oligonucleotides significantly down-regulates the expression of miR-138. ** $p<0.001$.

[0013] **Fig. 4** shows the relative expression level of miR-138 in U87MG cells (human glioblastoma cell line) after transfection with anti-miR-138 oligonucleotides. Fig. 4 shows transfection with modified oligonucleotides anti-miR-138-1 (SEQ ID NO: 10), anti-miR-138-2 (SEQ ID NO: 12), anti-miR-138-3 (SEQ ID NO: 13), anti-miR-138-4 (SEQ ID NO: 5 or "Oligo-4") and negative controls ("scrambled control" for anti-miR-138-1, anti-miR-138-2, and anti-miR-138-3; "control sequence" for Oligo-4). Thus, Fig. 4 shows the oligonucleotides as described in the Example section are capable of down-regulating the expression of miR-138 in human glioblastoma cells.

[0014] **Fig. 5** shows the regulation of TXNIP gene (Thioredoxin-interacting protein gene) expression by anti-miR-138 oligonucleotides. As compared to the two non-targeting controls, anti-miR-138-1 (SEQ ID NO: 10), anti-miR-138-2 (SEQ ID NO: 12), anti-miR-138-3 (SEQ ID NO: 13),

and anti-miR-138-4 (SEQ ID NO: 5 or "Oligo-4" or "138-4") significantly up-regulates TXNIP gene. Fig. 5 shows that, as compared to other oligonucleotides used, anti-miR-138-3 and anti-miR-138-4 oligonucleotides appear to up-regulate more TXNIP.

[0015] **Fig. 6** shows the regulation of PANX2 gene (pannexin 2) expression by anti-miR-138 oligonucleotides. As compared to the two non-targeting controls, anti-miR-138-1 (SEQ ID NO: 10), anti-miR-138-2 (SEQ ID NO: 12), anti-miR-138-3 (SEQ ID NO: 13) and anti-miR-138-4 (SEQ ID NO: 5 or "Oligo-4" or "138-4" or AMO_138_11_cap_PS) significantly up-regulates PANX2 gene. Fig. 6 shows that anti-miR138-3 and anti-miR138-4 oligonucleotides appear to up-regulate PANX2 gene better.

[0016] **Fig. 7** shows the regulation of CASP3 gene (caspase-3) expression by anti-miR-138 oligonucleotides. As compared to the two non-targeting controls, anti-miR-138-1 (SEQ ID NO: 10), anti-miR-138-2 (SEQ ID NO: 12), anti-miR-138-3 (SEQ ID NO: 13), and anti-miR-138-4 (SEQ ID NO: 5 or "Oligo-4" or "138-4" or AMO_138_11_cap_PS) significantly up-regulates CASP3 gene. Fig. 7 shows that anti-miR138-2, anti-miR138-3 and anti-miR138-4 oligonucleotides appear to up-regulate CASP3 better than anti-miR138-1.

[0017] **Fig. 8** shows the regulation of MXD1 gene (MAX dimerization protein 1) expression by anti-miR-138 oligonucleotides. As compared to the two non-targeting controls, anti-miR-138-1 (SEQ ID NO: 10), anti-miR-138-2 (SEQ ID NO: 12), anti-miR-138-3 (SEQ ID NO: 13), and anti-miR-138-4 (SEQ ID NO: 5 or "Oligo-4" or "138-4" or AMO_138_11_cap_PS) significantly up-regulates MXD1 gene. Fig. 8 shows that both anti-miR138-3 and anti-miR138-4 oligonucleotides appear to up-regulate MXD1 gene more than anti-miR138-1 and anti-miR138-2.

[0018] **Fig. 9** shows the regulation of cMYC gene (v-myc avian myelocytomatosis viral oncogene homolog gene) expression by anti-miR-138 oligonucleotides. As compared to the two non-targeting controls, anti-miR-138-1 (SEQ ID NO: 10), anti-miR-138-2 (SEQ ID NO: 12), anti-miR-138-3 (SEQ ID NO: 13), and anti-miR-138-4 (SEQ ID NO: 5 or "Oligo-4" or "138-4" or AMO_138_11_cap_PS) significantly downregulates cMYC gene. Fig. 9 shows a trend of better performance of cMYC regulation with anti-miR138-3 oligonucleotide.

[0019] **Fig. 10** shows the regulation of GAAD45A gene (DNA damage-inducible gene 45 alpha) expression by anti-miR-138 oligonucleotides. As compared to the two non-targeting controls, anti-miR-138-1 (SEQ ID NO: 10), anti-miR-138-2 (SEQ ID NO: 12), anti-miR-138-3 (SEQ ID NO: 13), and anti-miR-138-4 (SEQ ID NO: 5 or "Oligo-4" or "138-4" or AMO_138_11_cap_PS) significantly up-regulates GAAD45A gene. Fig. 10 shows a trend of better performance of GAAD45A regulation with anti-miR138-3 and anti-miR138-4 oligonucleotides.

[0020] **Fig. 11** shows the regulation of AURKA gene (aurora kinase A gene) expression by anti-miR-138 oligonucleotides. As compared to the two non-targeting controls, anti-miR-138-1 (SEQ ID

NO: 10), anti-miR-138-2 (SEQ ID NO: 12), anti-miR-138-3 (SEQ ID NO: 13), and anti-miR-138-4 (SEQ ID NO: 5 or "Oligo-4" or "138-4" or AMO_138_11_cap_PS) significantly down-regulates AURKA gene. Fig. 11 shows a trend of better performance of AURKA regulation with anti-miR138-2 and anti-miR138-3 oligonucleotides.

[0021] **Fig. 12** shows the regulation of BLCAP gene (bladder cancer associated protein gene) expression by anti-miR-138 oligonucleotides. As compared to the two non-targeting controls, anti-miR-138-1 (SEQ ID NO: 10), anti-miR-138-2 (SEQ ID NO: 12), anti-miR-138-3 (SEQ ID NO: 13), and anti-miR-138-4 (SEQ ID NO: 5 or "Oligo-4" or "138-4" or AMO_138_11_cap_PS) significantly up-regulates BLCAP gene. Fig. 12 shows a trend of better performance of BLCAP regulation with anti-miR138-1, anti-miR138-3 and anti-miR138-4 oligonucleotides.

[0022] **Fig. 13** shows the indirect regulation of BCL2 gene (B-cell CLL/lymphoma 2 gene) expression by anti-miR-138 oligonucleotides. As compared to the two non-targeting controls, anti-miR-138-1 (SEQ ID NO: 10), anti-miR-138-2 (SEQ ID NO: 12), anti-miR-138-3 (SEQ ID NO: 13) and anti-miR-138-4 (SEQ ID NO: 5 or "Oligo-4" or "138-4" or AMO_138_11_cap_PS) significantly down-regulates BCL2 gene. Fig. 13 shows a trend of better performance of BCL2 regulation with anti-miR138-3 and anti-miR138-4 oligonucleotides.

[0023] **Fig. 14** shows the indirect regulation of HIF1A gene (hypoxia inducible factor 1 alpha gene) expression by anti-miR-138 oligonucleotides. As compared to the two non-targeting controls, anti-miR-138-1 (SEQ ID NO: 10), anti-miR-138-2 (SEQ ID NO: 12), anti-miR-138-3 (SEQ ID NO: 13) and anti-miR-138-4 (SEQ ID NO: 5 or "Oligo-4" or "138-4" or AMO_138_11_cap_PS) significantly down-regulates HIF1A gene. Fig. 14 shows a trend of better performance of HIF1A regulation with anti-miR138-1, anti-miR138-2 and anti-miR138-3 oligonucleotides.

[0024] **Fig. 15** shows the indirect regulation of LASP1 gene (LIM and SH3 domain protein gene) expression by anti-miR-138 oligonucleotides. As compared to the two non-targeting controls, anti-miR-138-1 (SEQ ID NO: 10), anti-miR-138-2 (SEQ ID NO: 12), anti-miR-138-3 (SEQ ID NO: 13) and anti-miR-138-4 (SEQ ID NO: 5 or "Oligo-4" or "138-4" or AMO_138_11_cap_PS) significantly down-regulates LASP1 gene. Fig. 15 shows a trend of better performance of LASP1 regulation with anti-miR138-1, and anti-miR138-3 oligonucleotides.

[0025] **Fig. 16** shows the regulation of various genes expression by anti-miR-138-3 oligonucleotide. Genes significantly regulated by anti-miR-138-3 oligonucleotides are MXD1, CASP3, BLCAP, TXNIP, TUSC2, PANX2, GADD45α, HIF1α, LASP1, cMYC, BCL2, and AURKA. Regulation of miR-138 expression with anti-miR-138-3 oligonucleotide (i.e. SEQ ID NO: 13) is compared with scrambled control. * $P<0.05$ and ** $P<0.001$. Thus, Fig. 16 shows anti-miR-138-3 oligonucleotide up-regulates or down-regulates miR-138 target level.

Claims

1. An oligonucleotide comprising one of the sequences selected from the group consisting of
5'-mC*mG*G*mCCmUmGAmUTmCAmCmAAmCAmCCmA*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.

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. A pharmaceutical composition comprising an effective amount of the oligonucleotide of any one of claims 1 to 4, or a pharmaceutically-acceptable salt thereof, and a pharmaceutically-acceptable carrier or diluent.

6. Use of an oligonucleotide as claimed in any one of claims 1 to 4 in the manufacture of a medicament for treating a proliferative disease in a subject in need thereof.

7. The use of claim 6, wherein the proliferative disease is a benign tumor or cancer.

8. The use of claim 7, wherein the cancer is selected from the group consisting of malignant glioma and breast cancer.