gene suppression. The off-targets caused by siRNAs originate from base-complementarity of the seed regions of the RISC-loaded antisense strand of siRNA with one or more mRNA. The miRNA-like off-target effects in siRNAs have been reported in several studies, and affect expression of multitude of genes depending on sequences of the seed regions and are serious enough to cause up to 30% of the positive hits in siRNA based phenotype screening. Additionally, in the case of miRNAs, they are also reported to silence target genes through compensatory pairings within their 3' end regions (3'-compensatory pairing) when the interactions between seed region and targets become weak, implicating that the miRNA-like off-target effects are likely to be mediated by such mechanism.

[0005] There is thus an ongoing effort to eliminate or reduce miRNA-like off-target effects of siRNAs by modulating siRNA design by judicious application of chemical modifications without compromising the gene silencing efficacy of siRNA gene therapeutics. This invention is directed to that effort.

SUMMARY

[0006] This disclosure describes effective nucleotide or chemical motifs for dsRNA molecules, which are advantageous for inhibition of target gene expression, while having reduced off-target gene silencing effects, as well as RNAi compositions suitable for therapeutic use.

[0007] The inventors have discovered *inter alia* that dsRNA molecules where the antisense strand comprises at least one thermally destabilizing modification of the duplex within the seed region (*i.e.*, at position 2-9 of the 5'-end of the antisense strand, counting from the 5′-end) and the dsRNA molecule has a melting temperature in the range of from about 40^{o}C to about 80^{o}C can be more effective in mediating RNA interference than the parent dsRNA molecule lacking the destabilizing modification.

[0007a] In one aspect, the present invention refers to a double-stranded RNA (dsRNA) molecule capable of inhibiting the expression of a target gene, comprising a sense strand and an antisense strand, each strand having 14 to 40 nucleotides, wherein the antisense strand has sufficient complementarity to the target sequence to mediate RNA interference~~; comprises a~~ wherein only said antisense strand comprises one nucleotide containing a thermally destabilizing modification and said nucleotide containing the thermally destabilizing modification is at one of positions 5, 6, 7, or 8 of the antisense strand, counting from the 5'-end of the antisense strand,~~;~~ and the antisense strand comprises 2, 3, 4, 5, or 6 2'-fluoro modifications.

We claim:

1. A double-stranded RNA (dsRNA) molecule capable of inhibiting the expression of a target gene, comprising a sense strand and an antisense strand, each strand having 14 to 40 nucleotides, wherein the antisense strand has sufficient complementarity to the target sequence to mediate RNA interference~~; comprises a~~ wherein only said antisense strand comprises one nucleotide containing a thermally destabilizing modification and said nucleotide containing the thermally destabilizing modification is at one of positions 5, 6, 7, or 8 of the antisense strand, counting from the 5'-end of the antisense strand~~;~~, and the antisense strand comprises 2, 3, 4, 5, or 6 2'-fluoro modifications.

2. The dsRNA molecule according to claim 1, wherein the dsRNA comprises at least four 2'-fluoro modifications.

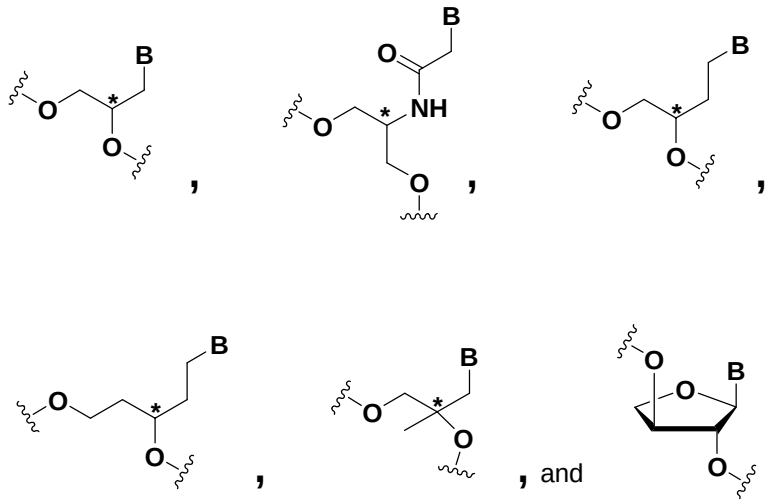
3. The dsRNA molecule according to claim 1, having the following characteristics:
   a) each of the sense and antisense strands comprise at least two 2'-fluoro modifications; and
   b) an ASGPR ligand attached to either end of the sense strand.

4. The dsRNA molecule according to claim 1, wherein the antisense strand has the following characteristics:
   a) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2 (counting from the 5' end); and
   b) it has a length of 18 to 35 nucleotides.

5. The dsRNA molecule according to claim 1, wherein the sense strand has at least one of the following characteristics:
   a) an ASGPR ligand attached to either end of the sense strand;
   b) at least two 2'-fluoro modifications; and
   c) the sense strand and the antisense strand show sufficient complementarity to form a double stranded region spanning at least 19 nucleotide positions and wherein the

thermally destabilizing modification of the duplex is located within said double-stranded region.

6. The dsRNA molecule according to claim 1, wherein the thermally destabilizing modification of the duplex is selected from the group consisting of



wherein B is nucleobase and * is *R*, *S* or racemic.

7. The dsRNA molecule of claim 1, wherein the thermally destabilizing modification of the duplex is located at position 5, 6 or 7 of the antisense strand, counting from the 5′-end of the antisense strand.

8. The dsRNA molecule of claim 1, wherein the thermally destabilizing modification is located in position 6 of the antisense strand, counting from the 5’-end of the antisense strand.

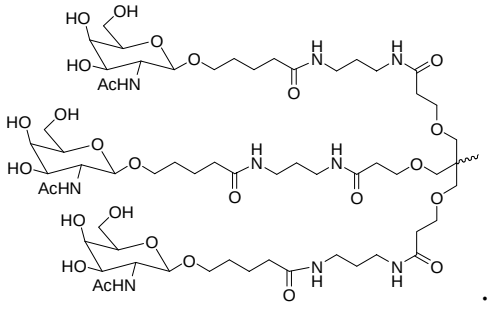
9. The dsRNA molecule according to 1, wherein the thermally destabilizing modification is located in position 7 of the antisense strand, counting from the 5’-end of the antisense strand.

10. The dsRNA molecule according to claim 1, wherein the sense strand further comprises a ligand.

11. The dsRNA molecule according to claim 10, wherein the ligand is an ASGPR ligand.

12. The dsRNA molecule according to claim 11, wherein the ASGPR ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker.

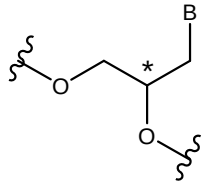
13. The dsRNA molecule of claim 12, wherein the ASGPR ligand is:



14. The dsRNA molecule of claim 1, wherein the dsRNA further has at least one of the following characteristics: (i) the antisense comprises 1, 2, 3 or 4 phosphorothioate internucleotide linkages; (ii) the sense strand is conjugated with a ligand; (iii) the sense strand comprises 2, 3, 4 or 5 2'-fluoro modifications; (iv) the sense strand comprises 1, 2, 3 or 4 phosphorothioate internucleotide linkages; (v) the dsRNA comprises at least four 2'-fluoro modifications; (vi) the dsRNA comprises a duplex region of 12-40 nucleotide pairs in length; and (vii) a blunt end at 5'end of the antisense strand.

15. The dsRNA molecule according to any one of the preceding claims wherein the sense strand has 21 nucleotides, and the antisense strand has 23 nucleotides.

16. The dsRNA molecule according to any one of the preceding claims, wherein there are no 2'-fluoro modifications at positions 3-9 of the antisense strand, counting from the 5'-end of the antisense strand

17. The dsRNA molecule of claim 6, wherein the thermally destabilizing modification of the duplex is 

, wherein the * stereocenter has (S)-stereochemisty (S-GNA).

18. The dsRNA molecule of claim 1, wherein the antisense strand comprises:
    (i) 2'-fluoro modifications only at positions 2, 14 and 16, counting from the 5'-end of the antisense strand; or
    (ii) 2'-fluoro modifications only at positions 2, 6, 14 and 16, counting from the 5'-end of the antisense strand; or
    (iii) 2'-fluoro modifications only at positions 2, 6, 8, 9, 14 and 16, counting from the 5'-end of the antisense strand.

19. A pharmaceutical composition comprising the dsRNA molecule according to any one of the preceding claims alone or in combination with a pharmaceutically acceptable carrier or excipient.

20. The dsRNA molecule of any one of claims 1 to 18 for use in therapy.