SUMMARY

This disclosure provides effective nucleotide or chemical motifs for dsRNA agents optionally conjugated to at least one ligand, which are advantageous for inhibition of target gene expression, as well as RNAi compositions suitable for therapeutic use.

The inventors surprisingly discovered that introducing one or more motifs of three identical modifications on three consecutive nucleotides at or near the cleavage site of a dsRNA agent that is comprised of modified sense and antisense strands enhances the gene silencing acitivity of the dsRNA agent.

In one aspect, the disclosure relates to a double-stranded RNAi (dsRNA) agent capable of inhibiting the expression of a target gene. The dsRNA agent comprises a sense strand and an antisense strand, each strand having 14 to 30 nucleotides. The dsRNA duplex is represented by formula (III):

sense: $5'\ n_p\text{-}N_a\text{-}(X\ X\ X)_i\text{-}N_b\text{-}Y\ Y\ Y\text{-}N_b\text{-}(Z\ Z\ Z)_j\text{-}N_a\text{-}n_q\ 3'$

antisense: $3'\ n_p'\text{-}N_a'\text{-}(X'X'X')_k\text{-}N_b'\text{-}Y'Y'Y'\text{-}N_b'\text{-}(Z'Z'Z')_l\text{-}N_a'\text{-}n_q'\ 5'$

(III),

In formula (III), i, j, k, and l are each independently 0 or 1; p and q are each independently 0-6; n represents a nucleotide; each N_a and N_a' independently represents an oligonucleotide sequence comprising ~~0~~2-25 nucleotides which are either modified or unmodified or combinations thereof, each sequence comprising at least two differently modified nucleotides; each N_b and N_b' independently represents an oligonucleotide sequence comprising 0-10 nucleotides which are either modified or unmodified or combinations thereof; each n_p and n_q independently represents an overhang nucleotide sequence comprising 0-6 nucleotides; and XXX, YYY, ZZZ, X'X'X', Y'Y'Y', and Z'Z'Z' each independently represent one motif of three identical modifications on three consecutive nucleotides; wherein the modifications on Nb is different than the modification on Y and the modifications on Nb' is different than the modification on Y'. At least one of the Y nucleotides forms a base pair with its complementary Y' nucleotides, and wherein the modification on the Y nucleotide is different than the modification on the Y' nucleotide.

In another aspect, the disclosure relates to a pharmaceutical composition comprising the double-stranded RNAi agent as defined above alone or in combination with a pharmaceutically acceptable carrier or excipient.

In another aspect, the disclosure relates to a double-stranded RNAi agent as defined above for use in inhibiting the expression of a target gene.

In another embodiment, the disclosure further provides a method for delivering the dsRNA to a specific target in a subject by subcutaneous or intravenenuous administration.

In another aspect, the disclosure relates to a dsRNA agent for use in delivering a polynucleotide to a specific target in a subject, the dsRNA agent being represented by formula (III):

sense: $5'\ n_p\text{-}N_a\text{-}(X\ X\ X)_i\text{-}N_b\text{-}Y\ Y\ Y\ \text{-}N_b\text{-}(Z\ Z\ Z)_j\text{-}N_a\text{-}\ n_q\ 3'$

antisense: $3'\ n_p'\text{-}N_a'\text{-}(X'X'X')_k\text{-}N_b'\text{-}Y'Y'Y'\text{-}N_b'\text{-}(Z'Z'Z')_l\text{-}N_a'\text{-}\ n_q'\ 5'$

(III)

wherein:

i, j, k, and l are each independently 0 or 1;

p and q are each independently 0-6;

each N_a and N_a' independently represents an oligonucleotide sequence comprising ~~0~~2-25 nucleotides which are either modified or unmodified or combinations thereof, each sequence comprising at least two differently modified nucleotideseach N_b and N_b' independently represents an oligonucleotide sequence comprising 0-10 nucleotides which are either modified or unmodified or combinations thereof;

each n_p, n_p', n_q and n_q' independently represents an overhang nucleotide sequence comprising 0-6 nucleotide sequence; and

XXX, YYY, ZZZ, X'X'X', Y'Y'Y', and Z'Z'Z' each independently represent one motif of three identical modifications on three consecutive nucleotides; and

wherein the modifications on N_b is different than the modification on Y and the modification on N_b' is different than the modification on Y'.

We claim:

1. A double-stranded RNAi agent capable of inhibiting the expression of a target gene, comprising a sense strand and an antisense strand, each strand having 14 to 30 nucleotides, wherein the duplex is represented by formula (III):

sense: 5' n_p -N_a -(X X X) $_i$-N_b -Y Y Y -N_b -(Z Z Z)$_j$ -N_a - n_q 3'

antisense: 3' n_p'-N_a'-(X'X'X')$_k$-N_b'-Y'Y'Y'-N_b'-(Z'Z'Z')$_l$-N_a'- n_q' 5'

(III)

wherein:

i, j, k, and l are each independently 0 or 1;

p and q are each independently 0-6;

each Na and Na′ independently represents an oligonucleotide sequence comprising 02-25 nucleotides which are either modified or unmodified or combinations thereof, each sequence comprising at least two differently modified nucleotides, each Nb and Nb′ independently represents an oligonucleotide sequence comprising 0-10 nucleotides which are either modified or unmodified or combinations thereof;

each n_p, n_p', n_q and n_q' independently represents an overhang nucleotide sequence comprising 0-6 nucleotides; and

XXX, YYY, ZZZ, X′X′X′, Y′Y′Y′, and Z′Z′Z′ each independently represent one motif of three identical modifications on three consecutive nucleotides; and

wherein the modification on Nb is different than the modification on Y and the modification on Nb' is different than the modification on Y'.

2. The double-stranded RNAi agent of claim 1, wherein i is 1; j is 1; or both i and j are 1.

3. The double-stranded RNAi agent of claim 1, wherein k is 1; l is 1; or both k and l are 1.

4. The double-stranded RNAi agent of claim 1, wherein the YYY motif occurs at or near the cleavage site of the sense strand.

25. A pharmaceutical composition comprising the double-stranded RNAi agent according to any one of the preceding claims alone or in combination with a pharmaceutically acceptable carrier or excipient.

26. A double-stranded RNAi agent according to any one of claims 1 to 24 for use in inhibiting the expression of a target gene.

27. The double-stranded RNAi agent of claim 26, wherein the double-stranded RNAi agent is formulated for administration through subcutaneous or intravenous administration.

28. A dsRNA agent for use in delivering a polynucleotide to a specific target in a subject, the dsRNA agent being represented by formula (III):

sense: 5' n_p -N_a -(X X X) $_i$-N_b -Y Y Y -N_b -(Z Z Z)$_j$ -N_a - n_q 3'

antisense: 3' n_p'-N_a'-(X'X'X')$_k$-N_b'-Y'Y'Y'-N_b'-(Z'Z'Z')$_l$-N_a'- n_q' 5'

(III)

wherein:

i, j, k, and l are each independently 0 or 1;

p and q are each independently 0-6;

each N_a and N_a' independently represents an oligonucleotide sequence comprising ~~0~~2-25 nucleotides which are either modified or unmodified or combinations thereof, each sequence comprising at least two differently modified nucleotideseach N_b and N_b' independently represents an oligonucleotide sequence comprising 0-10 nucleotides which are either modified or unmodified or combinations thereof;

each n_p, n_p', n_q and n_q' independently represents an overhang nucleotide sequence comprising 0-6 nucleotide sequence; and

XXX, YYY, ZZZ, X'X'X', Y'Y'Y', and Z'Z'Z' each independently represent one motif of three identical modifications on three consecutive nucleotides; and