SUMMARY

This ~~invention~~ 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 ~~invention~~ 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 0-25 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.

Each n_p and n_q independently represents an overhang nucleotide sequence comprising 0-6 nucleotides; each n and n' represents an overhang nucleotide; and p and q are each independently 0-6.

In another aspect, the disclosure ~~invention~~ relates to a 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 sense strand contains at least two motifs of three identical modifications on three consecutive nucleotides, where at least one of the motifs occurs at or near the cleavage site within the strand and at least one of the motifs occurs at another portion of the strand that is separated from the motif at the cleavage site by at least one nucleotide. The antisense strand contains at least one motif of three identical modifications on three consecutive nucleotides, where at least one of the motifs occurs at or near the cleavage site within the strand and at least one of the motifs occurs at another portion of the strand that is separated from the motif at or near cleavage site by at least one nucleotide. The modification in the motif occurring at or near the cleavage site in the sense strand is different than the modification in the motif occurring at or near the cleavage site in the antisense strand.

In another aspect, the invention relates to a 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 sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides, where at least one of the motifs occurs at or near the cleavage site in the strand. The antisense strand contains at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at or near the cleavage site.

In another aspect, the disclosure relates to 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 sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides, one of said motifs occurring at or near the cleavage site in the strand; and wherein the antisense strand contains at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides, one of said motifs occuring at or near the cleavage site.

In another ~~aspect~~embodiment, the disclosure ~~invention~~ relates to a 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 sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides at positions 9,10,11 from the 5'end. The antisense strand contains at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at positions 11,12,13 from the 5'end.

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 ~~aspect~~embodiment, the disclosure ~~invention~~ 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 0-25 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'.

In another aspect, the disclosure relates to a dsRNA agent as defined above for use in delivering a polynucleotide to a specific target of a subject, the dsRNA agent being formulated for subcutaneous administration.

DETAILED DESCRIPTION

A superior result may be obtained by introducing one or more motifs of three identical modifications on three consecutive nucleotides into a sense strand and/or antisense strand of a dsRNA agent, particularly at or near the cleavage site. The sense strand and antisense strand of the dsRNA agent may otherwise be completely modified. The introduction of these motifs interrupts the modification pattern, if present, of the sense and/or antisense strand. The dsRNA agent optionally conjugates with a GalNAc derivative ligand, for instance on the sense strand. The resulting dsRNA agents present superior gene silencing activity.

The inventors surprisingly discovered that having one or more motifs of three identical modifications on three consecutive nucleotides at or near the cleavage site of at least one strand of a dsRNA agent superiorly enhanced the gene silencing acitivity of the dsRNA agent.

Accordingly, the invention provides 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 of the dsRNA agent can range from 12-30 nucleotides in length. For example, each strand can be between 14-30 nucleotides in length, 17-30 nucleotides in length, 25-30 nucleotides in length, 27-30 nucleotides in length, 17-23 nucleotides in length, 17-21 nucleotides in length, 17-19 nucleotides in length, 19-25 nucleotides in length, 19-23 nucleotides in length, 19-21 nucleotides in length, 21-25 nucleotides in length, or 21-23 nucleotides in length.

The sense strand and antisense strand typically form a duplex dsRNA. The duplex region of a dsRNA agent may be 12-30 nucleotide pairs in length. For example, the duplex region can be between 14-30 nucleotide pairs in length, 17-30 nucleotide pairs in length, 25-30 nucleotides in length, 27-30 nucleotide pairs in length, 17 - 23 nucleotide pairs in length, 17-21 nucleotide pairs in length, 17-19 nucleotide pairs in length, 19-25 nucleotide pairs in length, 19-23 nucleotide pairs in length, 19- 21 nucleotide pairs in

40. The double-stranded RNAi agent of claim 33, further comprising a ligand attached to the 3' end of the sense strand.

41. 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.

42. A ~~method for inhibiting the expression of a target gene comprising the step of administering the~~ double-stranded RNAi agent according to any one of the preceding claims for use in inhibiting the expression of a target gene~~, in an amount sufficient to inhibit expression of the target gene~~.

43. The double-stranded RNAi agent ~~method~~ of claim 42, wherein the double-stranded RNAi agent is formulated for administration ~~administered~~ through subcutaneous or intravenous administration.

44. A ~~method for delivering polynucleotide to specific target in a subject by administering said~~ 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 0-25 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'.

45. The dsRNA agent ~~method~~ of claim 44, wherein said dsRNA agent is formulated for administration ~~administering step is carried out~~ by an administration means comprising intramuscular, intrabronchial, intrapleural, intraperitoneal, intraarterial, lymphatic, intravenous, subcutaneous, cerebrospinal, or combinations thereof.

46. A ~~method for delivering a polynucleotide to a specific target of a subject, the method comprising: delivering the~~ dsRNA agent according to claim 1 for use in delivering a polynucleotide to a specific target of a subject, the dsRNA agent being formulated for ~~by~~ subcutaneous administration ~~into the subject, such that the polynucleotide is delivered into specific target of the subject~~.