Extrahepatic Delivery

[0001] This application claims benefit of priority to U.S. Provisional Application No. 62/668,072 filed May 7, 2018; U.S. Provisional Application No. 62/738,747 filed September 28, 2018; and U.S. Provisional Application No. 62/773,082 filed November 29, 2018, all of which are herein incorporated by reference in their entirety.

BACKGROUND

[0002] Efficient delivery of an iRNA agent to cells *in vivo* requires specific targeting and substantial protection from the extracellular environment, particularly serum proteins. RNAi-based therapeutics show promising clinical data for treatment of liver-associated disorders. However, siRNA delivery into extra-hepatic tissues remains an obstacle, limiting the use of siRNA-based therapies.

[0003] One of the factors that limit the experimental and therapeutic application of iRNA agents *in vivo* is the ability to deliver intact siRNA efficiently. Particular difficulties have been associated with non-viral gene transfer into the retina *in vivo*. One of the challenges is to overcome the inner limiting membrane, which impedes the transfection of the retina. Additionally, negatively charged sugars of the vitreous have been shown to interact with positive DNA-transfection reagent complexes, promoting their aggregation, which impedes diffusion and cellular uptake.

[0004] Delivery of oligonucleotides to the central nervous system (CNS) poses particular problems due to the blood brain barrier (BBB) that free oligonucleotides cannot cross. One means to deliver oligonucleotides into the CNS is by intrathecal delivery. However, the oligonucleotides need also to be efficiently internalized into target cells of the CNS to achieve the desired therapeutic effect. Previous work has typically used delivery reagents such as liposomes, cationic lipids, and nanoparticles forming complexes to aid the intracellular internalization of oligonucleotides into cells of neuronal origin.

[0005] Thus, there is a continuing need for new and improved methods for delivering siRNA molecules *in vivo*, without the use of tissue delivery reagents, to achieve and enhance the therapeutic potential of iRNA agents.

SUMMARY

[0006] One example of the disclosure provides a double-stranded iRNA agent comprising:

an antisense strand which is complementary to a target gene; a sense strand which is complementary to said antisense strand; and one or more lipophilic moieties conjugated to one or more internal positions on at least one strand, optionally via a linker or carrier.
[0006a] In one aspect, the present disclosure refers to a double-stranded iRNA agent comprising: an antisense strand which is complementary to a target gene; a sense strand which is complementary to said antisense strand; and one or more lipophilic moieties conjugated to one or more internal positions on at least one strand, optionally via a linker or carrier wherein the lipophilic moiety comprises a saturated or unsaturated C_4-C_{18} hydrocarbon chain, and an optional functional group selected from the group consisting of hydroxyl, amine, carboxylic acid, sulfonate, phosphate, thiol, azide, and alkyne; and wherein one or more lipophilic moieties are conjugated to one or more of the following internal positions: positions 4-8 and 13-18 on the sense strand, and positions 6-10 and 15-18 on the antisense strand, counting from the 5'end of each strand.
[0007] In some examples, the lipophilicity of the lipophilic moiety, measured by octanol-water partition coefficient, logK_{ow}, exceeds 0. The lipophilic moiety may possess a logK_{ow} exceeding 1, exceeding 1.5, exceeding 2, exceeding 3, exceeding 4, exceeding 5, or exceeding 10.
[0008] In some examples, the hydrophobicity of the double-stranded iRNA agent, measured by the unbound fraction in the plasma protein binding assay of the double-stranded iRNA agent, exceeds 0.2. In one example, the plasma protein binding assay determined is an electrophoretic mobility shift assay (EMSA) using human serum albumin protein. The hydrophobicity of the double-stranded iRNA agent, measured by fraction of unbound siRNA in the binding assay, exceeds 0.15, exceeds 0.2, exceeds 0.25, exceeds 0.3, exceeds 0.35, exceeds 0.4, exceeds 0.45, or exceeds 0.5 for an enhanced *in vivo* delivery of siRNA.
[0009] In some examples, the lipophilic moiety is an aliphatic, cylic such as alicyclic, or polycyclic such as polyalicyclic compound, such as a steroid (e.g., sterol) or a linear or branched aliphatic hydrocarbon. Exemplary lipophilic moieties are lipid, cholesterol, retinoic acid, cholic acid, adamantane acetic acid, 1-pyrene butyric acid, dihydrotestosterone, 1,3-bis-O(hexadecyl)glycerol, geranyloxyhexyanol, hexadecylglycerol, borneol, menthol, 1,3-propanediol, heptadecyl group, palmitic acid, myristic acid, O3-(oleoyl)lithocholic acid, O3-(oleoyl)cholenic acid, ibuprofen, naproxen, dimethoxytrityl, or phenoxazine.
[0010] Suitable lipophilic moieties also include those containing a saturated or unsaturated C_4-C_{30} hydrocarbon chain (e.g., C_4-C_{30} alkyl or alkenyl), and an optional functional group selected from the group consisting of hydroxyl, amine, carboxylic acid, sulfonate, phosphate, thiol, azide, and alkyne. The functional groups are useful to attach the lipophilic moiety to the iRNA agent. In some examples, the lipophilic moiety contains a saturated or unsaturated C_6-C_{18} hydrocarbon chain (e.g., a linear C_6-C_{18} alkyl or alkenyl). In one example, the lipophilic moiety contains a saturated or unsaturated C_{16} hydrocarbon chain (e.g., a linear C_{16} alkyl or alkenyl).
[0011] In some examples, the lipophilic moiety is a C_6-C_{30} acid (e.g., hexanoic acid, heptanoic acid, octanoic acid, nonanoic acid, decanoic acid, undecanoic acid, dodcanoic acid, tridecanoic acid, tetradecanoic acid, pentadecanoic acid, hexadecanoic acid, heptadecanoic acid, octadecanoic acid, oleic acid, linoleic acid, arachidonic acid, cis-4,7,10,13,16,19-

less than 8, less than 6, less than 4, less than 2, or no 2'-F modifications on the antisense strand.

[0056] In some examples, the double-stranded iRNA agent has one or more 2'-F modifications on any position of the sense strand or antisense strand.

[0057] In some examples, the double-stranded iRNA agent has less than 20%, less than 15%, less than 10%, less than 5% non-natural nucleotide, or substantially no non-natural nucleotide. Examples of non-natural nucleotide include acyclic nucleotides, LNA, HNA, CeNA, 2'-O-methoxyalkyl (*e.g.*, 2'-O-methoxymethyl, 2'-O-methoxyethyl, or 2'-O-2-methoxypropanyl), 2'-O-allyl, 2'-C-allyl, 2'-fluoro, 2'-O-N-methylacetamido (2'-O-NMA), a 2'-O-dimethylaminoethoxyethyl (2'-O-DMAEOE), 2'-O-aminopropyl (2'-O-AP), 2'-ara-F, L-nucleoside modification (such as 2'-modified L-nucleoside, e.g., 2'-deoxy-L-nucleoside), BNA abasic sugar, abasic cyclic and open-chain alkyl.

[0058] In some examples, the double-stranded iRNA agent has greater than 80%, greater than 85%, greater than 90%, greater than 95%, or virtually 100% natural nucleotides. For the purpose of these examples, natural nucleotides can include those having 2'-OH, 2'-deoxy, and 2'-OMe.

[0059] In one example, the double-stranded iRNA agent comprises a sense strand and antisense strand each having a length of 15-30 nucleotides; at least two phosphorothioate internucleotide linkages at the first five nucleotides on the antisense strand (counting from the 5' end); wherein the duplex region is between 19 to 25 base pairs (preferably 19, 20, 21 or 22); wherein the double-stranded iRNA agent has less than 20%, less than 15%, less than 10%, less than 5% non-natural nucleotide, or substantially no non-natural nucleotide.

[0060] In one example, the double-stranded iRNA agent comprises a sense strand and antisense strand each having a length of 15-30 nucleotides; at least two phosphorothioate internucleotide linkages at the first five nucleotides on the antisense strand (counting from the 5' end); wherein the duplex region is between 19 to 25 base pairs (preferably 19, 20, 21 or 22); wherein the double-stranded iRNA agent has greater than 80%, greater than 85%, greater than 95%, or virtually 100% natural nucleotides, such as those having 2'-OH, 2'-deoxy, or 2'-OMe.

[0061] Another example of the disclosure relates to a method of reducing the expression of a target gene in a cell, comprising contacting said cell with a double-stranded iRNA agent comprising an antisense strand which is complementary to a target gene; a sense strand which

is complementary to said antisense strand; and one or more lipophilic moieties conjugated to one or more internal positions on at least one strand, optionally via a linker or carrier.

[0061a] In another aspect, the present disclosure refers to an *in vitro* method of reducing the expression of a target gene in a cell, comprising contacting said cell with a double-stranded iRNA agent as disclosed herein.

[0062] All the above examples relating to the lipophilic moieties and their conjugation to the double-stranded iRNA agent in the first example of the disclosure relating to the double-stranded iRNA agent are suitable in this example of the disclosure relating to a method of reducing the expression of a target gene in a cell.

[0063] In one example, the cell is an extraheptic cell.

[0064] Another example of the disclosure relates to a method of reducing the expression of a target gene in a subject, comprising administering to the subject a double-stranded iRNA agent comprising contacting said cell with a double-stranded iRNA agent comprising an antisense strand which is complementary to a target gene; a sense strand which is complementary to said antisense strand; and one or more lipophilic moieties conjugated to one or more internal positions on at least one strand, optionally via a linker or carrier.

[0065] All the above examples relating to the lipophilic moieties and their conjugation to the double-stranded iRNA agent in the first example of the disclosure relating to the double-stranded iRNA agent are suitable in this example of the disclosure relating to a method of reducing the expression of a target gene in a subject.

[0066] In some examples, the double-stranded iRNA agent is administered extrahepatically.

[0067] In one example, the double-stranded iRNA agent is administered intrathecally. By intrathecal administration of the double-stranded iRNA agent, the method can reduce the expression of a target gene in a brain or spine tissue, for instance, cortex, cerebellum, cervical spine, lumbar spine, and thoracic spine.

[0068] In some examples, exemplary target genes are APP, ATXN2, C9orf72, TARDBP, MAPT(Tau), HTT, SNCA, FUS, ATXN3, ATXN1, SCA1, SCA7, SCA8, MeCP2, PRNP, SOD1, DMPK, and TTR. To reduce the expression of these target genes in the subject, the double-stranded iRNA agent can be administered intravitreally. By intravitreal administration of the double-stranded iRNA agent, the method can reduce the expression of the target gene in an ocular tissue.

[0069] Another aspect of the disclosure relates to a use the double-stranded RNAi agent as disclosed herein in the manufacture of a medicament for treating a subject having a CNS disorder. Another example of the disclosure relates to a method of treating a subject having a CNS disorder, comprising administering to the subject a therapeutically effective amount of a double-stranded RNAi agent, thereby treating the subject. The double-stranded RNAi agent comprises an antisense strand which is complementary to a target gene; a sense strand which

We claim:

1. A double-stranded iRNA agent comprising:

an antisense strand which is complementary to a target gene;

a sense strand which is complementary to said antisense strand; and

one or more lipophilic moieties conjugated to one or more internal positions on at least one strand, optionally via a linker or carrier

wherein the lipophilic moiety comprises a saturated or unsaturated C_4-C_{18} hydrocarbon chain, and an optional functional group selected from the group consisting of hydroxyl, amine, carboxylic acid, sulfonate, phosphate, thiol, azide, and alkyne;

and wherein one or more lipophilic moieties are conjugated to one or more of the following internal positions: positions 4-8 and 13-18 on the sense strand, and positions 6-10 and 15-18 on the antisense strand, counting from the 5'end of each strand.

2. The double-stranded iRNA agent of claim 1, wherein one or more lipophilic moieties are conjugated to one or more of the following internal positions: positions 5, 6, 7, 15, and 17 on the sense strand, and positions 15 and 17 on the antisense strand, counting from the 5'-end of each strand.

3. The double-stranded iRNA agent of claim 1 or 2, wherein said sense and antisense strands are each 19 to 25 nucleotides in length.

4. The double-stranded iRNA agent of claim 1, wherein the lipophilic moiety contains a saturated or unsaturated C_6-C_{18} hydrocarbon chain.

5. The double-stranded iRNA agent of claim 4, wherein the lipophilic moiety contains a saturated or unsaturated C_{16} hydrocarbon chain.

6. The double-stranded iRNA agent of any one of claims 1-5, wherein the lipophilic moiety is conjugated via a carrier that replaces one or more nucleotide(s) in the internal position(s).

7. The double-stranded iRNA agent of claim 6, wherein the carrier is a cyclic group selected from the group consisting of pyrrolidinyl, pyrazolinyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, piperidinyl, piperazinyl, [1,3]dioxolanyl, oxazolidinyl, isoxazolidinyl, morpholinyl, thiazolidinyl, isothiazolidinyl, quinoxalinyl, pyridazinonyl, tetrahydrofuranyl, and decalinyl; or is an acyclic moiety based on a serinol backbone or a diethanolamine backbone.

8. The double-stranded iRNA agent of any one of claims 1-7, wherein the lipophilic moiety is conjugated to the double-stranded iRNA agent via a linker containing an ether, thioether, urea, carbonate, amine, amide, maleimide-thioether, disulfide, phosphodiester, sulfonamide linkage, a product of a click reaction, or carbamate.

9. The double-stranded iRNA agent of any one of claims 1-8, wherein said iRNA agent comprises a single-stranded overhang on at least one of the termini.

10. The double-stranded iRNA agent of any one of claims 1-9, wherein the lipophilic moiety is conjugated to a nucleobase, sugar moiety, or internucleosidic linkage.

11. The double-stranded iRNA agent of any one of claims 1-10, wherein the lipophilic moiety or targeting ligand is conjugated via a bio-clevable linker selected from the group consisting of DNA, RNA, disulfide, amide, functionalized monosaccharides or oligosaccharides of galactosamine, glucosamine, glucose, galactose, mannose, and combinations thereof.

12. The double stranded iRNA agent of any one of claims 1-11, wherein the 3' end of the sense strand is protected via an end cap which is a cyclic group having an amine, said cyclic group being selected from the group consisting of pyrrolidinyl, pyrazolinyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, piperidinyl, piperazinyl, [1,3]dioxolanyl, oxazolidinyl, isoxazolidinyl, morpholinyl, thiazolidinyl, isothiazolidinyl, quinoxalinyl, pyridazinonyl, tetrahydrofuranyl, and decalinyl.

13. The double-stranded iRNA agent of any one of claims 1-12 for use in therapy.

14. An *in vitro* method of reducing the expression of a target gene in a cell, comprising contacting said cell with a double-stranded iRNA agent of any one of claims 1-12.

15. Use of the double-stranded RNAi agent of any one of claims 1-12 in the manufacture of a medicament for treating a subject having a CNS disorder.

16. The use of claim 15, wherein the CNS disorder is selected from the group of alzheimer, amyotrophic lateral schlerosis (ALS), frontotemporal dementia, huntington, Parkinson, spinocerebellar, prion, and lafora.