Compositions and Methods for Inhibiting Gene Expression of LPA

BACKGROUND

Lipoprotein(a) [Lp(a)] is a heterogeneous low density lipoprotein (LDL)-like particle containing a lipid core and apolipoprotein B (apoB-100) with a unique constituent, apolipoprotein (a) (apo(a)), that is attached to apoB-100 through a disulfide bond.

The apo(a) gene (*LPA*) is expressed predominantly in the liver and expression is restricted to human and non-human primates. Lp(a) levels in humans are genetically defined and do not change significantly with diet, exercise, or other lifestyle changes. *LPA* varies in length depending upon the number of Kringle KIV2 domains present and its expression is inversely correlated with the number of domains present. Normal Lp(a) levels range from 0.1-25 mg/dl, with about 25% of the population in the United States of America having Lp(a) levels of 30 mg/dl or higher.

Analysis of Lp(a) levels in multiple studies have implicated high Lp(a) levels as an independent risk factor for cardiovascular disease, stroke, and other related disorders including atherosclerotic stenosis. In addition, genome-wide association analyses have also implicated LPA as a genetic risk factor for diseases such as atherosclerotic stenosis.

When therapeutic lipoprotein apheresis is used to lower both Lp(a) and LDL levels in hyperlipidemic patients, significant reductions of cardiovascular events have been observed. Therefore there exists a need for therapeutics and treatments related to these and other LPA-related diseases.

SUMMARY

In one aspect, the present invention refers to an LPA RNA interference (RNAi) agent comprising a sense strand and an antisense strand, wherein the antisense strand comprises the sequence of any one of SEQ ID NO:1280, SEQ ID NO:1281, SEQ ID NO:1282, SEQ ID NO:1283, SEQ ID NO:1246, SEQ ID NO:1242, SEQ ID NO:1244, SEQ ID NO:1248, SEQ ID NO:1250, SEQ ID NO:1252, or SEQ ID NO:1254, and wherein the sense strand comprises a sequence that is complementary to the sequence of the antisense strand.

In another aspect, the present invention refers to a pharmaceutical composition comprising the LPA RNAi agent of the present invention and a pharmaceutically acceptable excipient.

In yet another aspect, the present invention refers to the use of the LPA RNAi agent of any one of the present invention in the preparation of a medicament for inhibiting LPA gene expression in a subject.

In yet another aspect, the present invention refers to the use of the LPA RNAi agent of any one of the present invention in the preparation of a medicament for treating cardiovascular disease in a subject in need thereof.

Described herein are LPA (also termed apo(a)) RNA interference (RNAi) agents (also termed RNAi trigger, or trigger) and compositions containing LPA RNAi agents for selectively and efficiently inhibiting expression of the *LPA* gene. *LPA* is the name of the gene which encodes apolipoprotein (a) (apo(a)), a key component of the lipoprotein (a) particle (Lp(a)). The LPA RNAi agents described herein can be used in the prevention or treatment or the preparation of a medicament for the prevention or treatment of diseases including, but not limited to: Berger's disease, peripheral artery disease, coronary artery disease, metabolic syndrome, acute coronary syndrome, aortic valve stenosis, aortic valve regurgitation, aortic dissection, retinal artery occlusion, cerebrovascular disease, mesenteric ischemia, superior mesenteric artery occlusion, renal artery stenosis, stable/unstable angina, acute coronary syndrome, heterozygous or homozygous familial hypercholesterolemia, hyperapobetalipoproteinemia, cerebrovascular atherosclerosis, cerebrovascular disease, and venous thrombosis.

Each LPA RNAi agent includes at least a sense strand and an antisense strand. The sense strand and the antisense strand can be partially, substantially, or fully complementary to each other. The length of the RNAi agent sense and antisense strands described herein each can be 17 to 30 nucleotides in length. In some examples, the sense and antisense strands are independently 17 to 26 nucleotides in length. The sense and antisense strands can be either the same length or different lengths. The RNAi agents described herein, upon delivery to a cell expressing the LPA gene, inhibit the expression of the LPA gene *in vitro* or *in vivo*.

A sense strand of an LPA RNAi agent comprises a nucleotide sequence having at least 90% identity over a core stretch of at least 17 consecutive nucleotides to a sequence in an *LPA* mRNA. In some examples, the sense strand nucleotide sequence having at least 90% identity to a sequence in the *LPA* mRNA is 17, 18, 19, 20, 21, 22, or 23 nucleotides in length. An antisense strand of an LPA RNAi agent comprises a nucleotide sequence having at least 90% complementary over a core stretch of at least 16 consecutive nucleotides to a sequence in the *LPA* mRNA and the corresponding sense strand. In some examples, the antisense strand

nucleotide sequence having at least 90% complementarity to a sequence in the *LPA* mRNA or the corresponding sense strand is 17, 18, 19, 20, 21, 22, or 23 nucleotides in length.

In some examples, one or more LPA RNAi agents are delivered to target cells or tissues using any oligonucleotide delivery technology known in the art. Nucleic acid delivery methods include, but are not limited to, by encapsulation in liposomes, by iontophoresis, or by incorporation into other vehicles, such as hydrogels, cyclodextrins, biodegradable nanocapsules, and bioadhesive microspheres, proteinaceous vectors or Dynamic Polyconjugates™ (DPCs (see, for example WO 2000/053722, WO 2008/0022309, WO 2011/104169, and WO 2012/083185, each of which is incorporated herein by reference). In some examples, an LPA RNAi agent is conjugated to a targeting group. In some examples, the targeting group can include a cell receptor ligand, such as a galactose cluster, including a galactose cluster comprising an N-acetyl-galactosamine trimer.

In some examples are described pharmaceutical compositions comprising one or more LPA RNAi agents. In some examples, an LPA RNAi agent is optionally combined with one or more additional (i.e., second, third, etc.) therapeutics. An additional therapeutic can be another LPA RNAi agent (e.g., an LPA RNAi agent which targets a different sequence within the LPA target). An additional therapeutic can also be a small molecule drug, antibody, antibody fragment, and/or vaccine. The LPA RNAi agents, with or without the one or more additional therapeutics, can be combined with one or more excipients to form pharmaceutical compositions.

In some examples, compositions for delivering an LPA RNAi agent to a liver cell, particularly hepatocytes, *in vivo* are described, comprising: an LPA RNAi agent conjugated to a targeting group. In some examples, the targeting group is a asialoglycoprotein ligand.

Also described are methods of treating a human subject having a pathological state or at risk of developing a pathological state mediated at least in part by LPA expression, the methods comprising the step(s) of administering to the subject a therapeutically effective amount of an LPA RNAi agent or LPA RNAi agent-containing composition. The method of treating a subject with an LPA RNAi agent or LPA RNAi agent-containing composition can optionally be combined with one or more steps of administering one or more additional (i.e., second) therapeutics or treatments. The LPA RNAi agent and additional therapeutics can be administered in a single composition or they may be administered separately. Examples of additional therapeutics include, but are not limited to, HMg Co-A reductase inhibitors (statins), ezetimibe, PCSK-9 inhibitors, CTEP inhibitors, therapies targeting ANGPTL3, therapies targeting APOC3, and niacin.

Use of the described LPA RNAi agents can be used in methods for therapeutic prevention or treatment of diseases including, but not limited to: Berger's disease, peripheral artery disease, coronary artery disease, metabolic syndrome, acute coronary syndrome, aortic valve stenosis, aortic valve regurgitation, aortic dissection, retinal artery occlusion, cerebrovascular disease, mesenteric ischemia, superior mesenteric artery occlusion, renal artery stenosis, stable/unstable angina, acute coronary syndrome, heterozygous or homozygous familial hypercholesterolemia, hyperapobetalipoproteinemia, cerebrovascular atherosclerosis, cerebrovascular disease, and venous thrombosis. Such methods comprise administration of an LPA RNAi agent as described herein to a subject, e.g., a human or animal subject.

The pharmaceutical compositions can be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration can be made by any way commonly known in the art, such as, but not limited to, topical (e.g., by a transdermal patch), pulmonary (e.g., by inhalation or insufflation of powders or aerosols, including by nebulizer, intratracheal, intranasal), epidermal, transdermal, oral or parenteral. Parenteral administration includes, but is not limited to, intravenous, intraarterial, subcutaneous, intraperitoneal or intramuscular injection or infusion; subdermal (e.g., via an implanted device), intracranial, intraparenchymal, intrathecal, and intraventricular, administration. In some examples, the pharmaceutical compositions described herein are administered by subcutaneous injection.

The described LPA RNAi agents and/or compositions can be used in methods for therapeutic treatment of diseases, including but not limited to: Berger's disease, peripheral artery disease, coronary artery disease, metabolic syndrome, acute coronary syndrome, aortic valve stenosis, aortic valve regurgitation, aortic dissection, retinal artery occlusion, cerebrovascular disease, mesenteric ischemia, superior mesenteric artery occlusion, renal artery stenosis, stable/unstable angina, acute coronary syndrome, heterozygous or homozygous familial hypercholesterolemia, hyperapobetalipoproteinemia, cerebrovascular atherosclerosis, cerebrovascular disease, and venous thrombosis. Such methods comprise administration of an LPA RNAi agent as described herein to a subject, e.g., a human or animal subject.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

Claims:

1. An LPA RNA interference (RNAi) agent comprising a sense strand and an antisense strand, wherein the antisense strand comprises the sequence of any one of SEQ ID NO:1280, SEQ ID NO:1281, SEQ ID NO:1282, SEQ ID NO:1283, SEQ ID NO:1246, SEQ ID NO:1242, SEQ ID NO:1244, SEQ ID NO:1248, SEQ ID NO:1250, SEQ ID NO:1252, or SEQ ID NO:1254, and wherein the sense strand comprises a sequence that is complementary to the sequence of the antisense strand.
2. The LPA RNAi agent of claim 1, wherein the sense strand and antisense strand are each 19 to 26 nucleotides in length.
3. The LPA RNAi agent of claim 2, wherein the sense strand and antisense strand are each 26 nucleotides in length.
4. The LPA RNAi agent of claim 2, wherein the sense strand and antisense strand are each 21 nucleotides in length.
5. The LPA RNAi agent of any one of claims 1 to 4, wherein the LPA RNAi agent comprises at least one overhang.
6. The LPA RNAi agent of claim 5, wherein the LPA RNAi agent comprises an overhang at the 3' end of the antisense strand.
7. The LPA RNAi agent of claim 5, wherein the LPA RNAi agent comprises an overhang at the 3' end of the antisense strand and an overhang at the 3' end of the sense strand.
8. The LPA RNAi agent of any one of claims 1 to 4, wherein the LPA RNAi agent comprises at least one blunt end.
9. The LPA RNAi agent of claim 8, wherein the LPA RNAi agent comprises one blunt end and one frayed end.
10. The LPA RNAi agent of claim 8, wherein the LPA RNAi agent comprises two blunt ends.
11. The LPA RNAi agent of any one of claims 1 to 10, wherein the sense strand, the antisense strand, or both the sense and antisense strand comprise one or more modified nucleotides.
12. The LPA RNAi agent of claim 11, wherein the one or more modified nucleotides are independently selected from a 2'-modified nucleotide, a locked nucleotide, an abasic

nucleotide, an inverted deoxynucleotide, a morpholino nucleotide, a 2',3'-seco nucleotide mimic, or a nucleotide containing a non-natural base.

13. The LPA RNAi agent of claim 12, wherein the 2'-modified nucleotide is a 2'-O-methyl nucleotide, a 2'-deoxy-2'-fluoro nucleotide, a 2'-deoxynucleotide, a 2'-methoxyethyl nucleotide, a 2'-amino nucleotide, or a 2'-alkyl nucleotide.

14. The LPA RNAi agent of any one of claims 1 to 13, wherein the LPA RNAi agent comprises one or more phosphorothioate internucleoside linkages.

15. The LPA RNAi agent of claim 14, wherein both the sense and antisense strand independently comprise 1, 2, 3, or 4 phosphorothioate internucleoside linkages.

16. The LPA RNAi agent of any one of claims 1 to 15, wherein the LPA RNAi agent further comprises a targeting group.

17. The LPA RNAi agent of claim 16, wherein the targeting group comprises an asialoglycoprotein receptor ligand.

18. The LPA RNAi agent of claim 17, wherein the asialoglycoprotein receptor ligand comprises galactose, galactosamine, N-acetyl-galactosamine, or a galactose derivative.

19. The LPA RNAi agent of claim 16, wherein the targeting group is (Chol-TEG), (TEG-Chol), (C11-PEG3-NAG3), (C6-PEG4-NAG3), (NAG3), (NAG4), (NAG3-AA2), (NAG3-Palm), (NAG13), (NAG18), (NAG24), (NAG25), (NAG25)s, (NAG26), (NAG27), (NAG28), (NAG29), (NAG30) (NAG30)s, (NAG31), (NAG13), (NAG31s), (NAG32), (NAG33), (NAG34), (NAG35), (NAG36), or (NAG37).

20. The LPA RNAi agent of any one of claims 16 to 19, wherein the targeting group is conjugated to the 3' end of the sense strand.

21. The LPA RNAi agent of any one of claims 16 to 19, wherein the targeting group is conjugated to the 5' end of the sense strand.

22. The LPA RNAi agent of any one of claims 1 to 21, wherein:

a) the antisense strand comprises the sequence of SEQ ID NO:1246 and the sense strand comprises the sequence of SEQ ID NO:1247;

b) the antisense strand comprises the sequence of SEQ ID NO:1242 and the sense strand comprises the sequence of SEQ ID NO:1243;

c) the antisense strand comprises the sequence of SEQ ID NO:1244 and the sense

strand comprises the sequence of SEQ ID NO:1245;

d) the antisense strand comprises the sequence of SEQ ID NO:1248 and the sense strand comprises the sequence of SEQ ID NO:1249;

e) the antisense strand comprises the sequence of SEQ ID NO:1250 and the sense strand comprises the sequence of SEQ ID NO:1251;

f) the antisense strand comprises the sequence of SEQ ID NO:1252 and the sense strand comprises the sequence of SEQ ID NO:1253;

g) the antisense strand comprises the sequence of SEQ ID NO:1254 and the sense strand comprises the sequence of SEQ ID NO:1255;

h) the antisense strand comprises the sequence of SEQ ID NO:1280 and the sense strand comprises the sequence of SEQ ID NO:1260;

i) the antisense strand comprises the sequence of SEQ ID NO:1281 and the sense strand comprises the sequence of SEQ ID NO:1258;

j) the antisense strand comprises the sequence of SEQ ID NO:1280 and the sense strand comprises the sequence of SEQ ID NO:1284;

k) the antisense strand comprises the sequence of SEQ ID NO:1281 and the sense strand comprises the sequence of SEQ ID NO:1285;

l) the antisense strand comprises the sequence of SEQ ID NO:1282 and the sense strand comprises the sequence of SEQ ID NO:1259; or

m) the antisense strand comprises the sequence of SEQ ID NO:1283 and the sense strand comprises the sequence of SEQ ID NO:1249.

23. The LPA RNAi agent of any one of claims 1 to 21, wherein:

a) the antisense strand comprises the sequence of SEQ ID NO:156. and the sense strand comprises the sequence of SEQ ID NO:310;

b) the antisense strand comprises the sequence of SEQ ID NO:164. and the sense strand comprises the sequence of SEQ ID NO:357;

c) the antisense strand comprises the sequence of SEQ ID NO:188 and the sense strand comprises the sequence of SEQ ID NO:384;

d) the antisense strand comprises the sequence of SEQ ID NO:164 and the sense strand comprises the sequence of SEQ ID NO:376; or

e) the antisense strand comprises the sequence of SEQ ID NO:164 and the sense strand comprises the sequence of SEQ ID NO:384.

24. The LPA RNAi agent of any one of claims 1 to 23, wherein:

a) the antisense strand comprises the sequence of modified nucleotides according to SEQ ID NO: 790 and the sense strand comprises the sequence of modified nucleotides according to SEQ ID NO: 1189;

b) the antisense strand comprises the sequence of modified nucleotides according to SEQ ID NO: 637 and the sense strand comprises the sequence of modified nucleotides according to SEQ ID NO: 1132;

c) the antisense strand comprises the sequence of modified nucleotides according to SEQ ID NO: 709 and the sense strand comprises the sequence of modified nucleotides according to SEQ ID NO: 1135;

d) the antisense strand comprises the sequence of modified nucleotides according to SEQ ID NO: 787 and the sense strand comprises the sequence of modified nucleotides according to SEQ ID NO: 1191; or

e) the antisense strand comprises the sequence of modified nucleotides according to SEQ ID NO: 788 and the sense strand comprises the sequence of modified nucleotides according to SEQ ID NO: 1189.

25. The LPA RNAi agent of claim 24, wherein the LPA RNAi agent further comprises a targeting group of (NAG25) or (NAG25)s conjugated to the 5' end of the sense strand.

26. A pharmaceutical composition comprising the LPA RNAi agent of any one of claims 1 to 25 and a pharmaceutically acceptable excipient.

27. Use of the LPA RNAi agent of any one of claims 1 to 25 in the preparation of a medicament for inhibiting LPA gene expression in a subject.

28. The use of claim 27, wherein the subject has or is at risk of having cardiovascular disease.

29. Use of the LPA RNAi agent of any one of claims 1 to 25 in the preparation of a medicament for treating cardiovascular disease in a subject in need thereof.