to have NAFLD; NASH accounting for approximately 10-30% of NAFLD diagnoses (Rinella, supra; Younossi et al (2016) Hepatology 64(5):1577-1586).

[0004] SCAP (SREBP Cleavage Activating Protein) is the only known post-transcriptional regulator of the transcription factors of the SREBP family. The SREBP (Sterol Response Element Binding Protein) family play important roles in regulating *de novo* lipogenesis and TG accumulation within the liver. SREBPs are synthesized as inactive precursors in the ER. Immediately after synthesis, SCAP forms a complex with SREBPs and escorts transport of the SREBPs to the Golgi vesicles. SREBPs are then further processed to release the active amino terminal of the transcription factor. Active SREBP translocates to the nucleus and binds to SREBP response elements to drive transcriptional activation of the target genes (Brown, M. S., and Goldstein, J. L. (1997) Cell 89, 331-340). Targeted silencing of SCAP is proposed to prevent processing of active SREBP and downstream transcriptional changes.

[0005] The SREBP family of proteins includes three isoforms, SREBP-1a, SREBP-1c and SREBP-2 with distinct but overlapping functions. SREPB-1c is abundant in liver, and primarily activates fatty acid and TG synthesis. Germline deletion of SREBP-1 exhibits a concomitant increase in SREBP-2 levels that compensates for the loss of SREBP-1.

[0006] SREBP-2 drives cholesterol production and LDL processing by activating LDL receptor (LDLR). SREBP-2 also regulates PCSK9, a secreted protein that interacts with LDLR to promote its degradation and reducing cholesterol uptake. Loss of SCAP/SREBP maintains the protein levels of LDLR. SREBP1c is also the only known transcriptional regulator of PNPLA3. PNPLA3 polymorphism rs738409 (I148M) is a major genetic determinant for NASH/NAFLD, present in 50% of the patients. Silencing SCAP activity is proposed to benefit individuals carrying this mutation. Accordingly, novel therapeutics targeting SCAP function represents a novel approach to reducing SCAP levels and treating hepatologic diseases, such as nonalcoholic fatty liver disease.

SUMMARY

[0007] The present disclosure is based, in part, on the design and generation of RNAi constructs that target the SCAP gene and reduce expression of SCAP in liver cells. The sequence specific inhibition of SCAP expression is useful for treating or preventing conditions associated with SCAP expression, such as liver-related diseases, such as, for example, simple

fatty liver (steatosis), nonalcoholic steatohepatitis (NASH), cirrhosis (irreversible, advanced scarring of the liver), or SCAP related obesity. Accordingly, in one example, the present disclosure provides an RNAi construct comprising a sense strand and an antisense strand, wherein the antisense strand comprises a region having a sequence that is complementary to a SCAP mRNA sequence. In certain examples, the antisense strand comprises a region having at least 15 contiguous nucleotides from an antisense sequence listed in Table 1 or Table 2.

[0007a] In one aspect, the present disclosure refers to an RNAi construct comprising a sense strand and an antisense strand, wherein the antisense strand comprises a region having at least 15 contiguous nucleotides differing by no more than 3 nucleotides from an antisense sequence of SEQ ID NO:336 or 478, and wherein the RNAi construct inhibits the expression of SREBP Cleavage Activating Protein (SCAP).

[0007b] In another aspect, the present disclosure refers to a pharmaceutical composition comprising the RNAi construct as disclosued herein and a pharmaceutically acceptable carrier, excipient, or diluent.

[0007c] In another aspect, the present disclosure refers to the use of the RNAi construct as disclosed herein in the manufacture of a medicament for reducing the expression of SCAP in a patient in need thereof.

[0007d] In another aspect, the present disclosure refers to the use of the RNAi construct as disclosed herein or the pharmaceutical composition as disclosed herein in the manufacture of a medicament for treating a subject having a SCAP-associated disease.

[0007e] In another aspect, the present disclosure refers to an RNAi construct comprising a sense strand and an antisense strand, wherein the antisense strand comprises a region having at least 15 contiguous nucleotides differing by no more than 3 nucleotides from an antisense sequence of SEQ ID NO:336 or 478, for use in treating a SCAP-associated disease, the use comprising that the RNAi construct as disclosed herein or the pharmaceutical composition as disclosed herein is to be administered.

[0008] In some examples, the sense strand of the RNAi constructs described herein comprises a sequence that is sufficiently complementary to the sequence of the antisense strand to form a duplex region of about 15 to about 30 base pairs in length. In these and other examples, the sense and antisense strands each are about 15 to about 30 nucleotides in length. In some examples, the RNAi constructs comprise

at least one blunt end. In other examples, the RNAi constructs comprise at least one nucleotide overhang. Such nucleotide overhangs may comprise at least 1 to 6 unpaired nucleotides and can be located at the 3' end of the sense strand, the 3' end of the antisense strand, or the 3' end of both the sense and antisense strand. In certain examples, the RNAi constructs comprise an overhang of two unpaired nucleotides at the 3' end of the sense strand and the 3' end of the antisense strand. In other examples, the RNAi constructs comprise an overhang of two unpaired nucleotides at the 3' end of the antisense strand and a blunt end of the 3' end of the sense strand/5' end of the antisense strand.

[0009] The RNAi constructs of the disclosure may comprise one or more modified nucleotides, including nucleotides having modifications to the ribose ring, nucleobase, or phosphodiester backbone. In some examples, the RNAi constructs comprise one or more 2'-modified nucleotides. Such 2'-modified nucleotides can include 2'-fluoro modified nucleotides, 2'-O-methyl modified nucleotides, 2'-O-methoxyethyl modified nucleotides, 2'-O-allyl modified nucleotides, bicyclic nucleic acids (BNA), glycol nucleic acids (GNAs), inverted bases (e.g. inverted adenosine) or combinations thereof In one particular example, the RNAi constructs comprise one or more 2'-fluoro modified nucleotides, 2'-O-methyl modified nucleotides, or combinations thereof. In some examples, all of the nucleotides in the sense and antisense strand of the RNAi construct are modified nucleotides.

[0010] In some examples, the RNAi constructs comprise at least one backbone modification, such as a modified internucleotide or internucleoside linkage. In certain

examples, the RNAi constructs described herein comprise at least one phosphorothioate internucleotide linkage. In particular examples, the phosphorothioate internucleotide linkages may be positioned at the 3' or 5' ends of the sense and/or antisense strands.

[0011] In some examples, the antisense strand and/or the sense strand of the RNAi constructs of the disclosure may comprise or consist of a sequence from the antisense and sense sequences listed in Tables 1 or 2. In certain examples, the RNAi construct may be any one of the duplex compounds listed in any one of Tables 1 to 2.

BRIEF DESCRIPTION OF THE FIGURES

[0012] Figure 1A-F shows effect of SCAP siRNA molecules in vivo in mice using Amylin (AMLN) model; (A) shows expression of liver SCAP mRNA; (B) shows terminal liver weight / body weight ratio; (C) shows liver triglycerides; (D) shows serum PCSK9 levels; (E) shows liver fibrosis pathology readout; (F) shows aSMA staining as a marker of hepatic stellate cell activation.

[0013] Figure 2A-F shows effect of SCAP siRNA molecules in vivo in mice using ALIOS model; (A) shows expression of liver SCAP mRNA; (B) shows terminal liver weight/ body weight ratio; (C) shows liver triglycerides; (D) shows serum PCSK9 levels; (E) shows liver fibrosis pathology readout; (F) shows aSMA staining as a marker of hepatic stellate cell activation.

DETAILED DESCRIPTION OF THE INVENTION

[0014] The present invention is directed to compositions and methods for regulating the expression of the SREBP Cleavage Activating Protein (SCAP) gene. In some embodiments, the gene may be within a cell or subject, such as a mammal (e.g. a human). In some embodiments, compositions of the invention comprise RNAi constructs that target a SCAP mRNA and reduce SCAP expression in a cell or mammal. Such RNAi constructs are useful for treating or preventing various forms of liver-related diseases, such as, for example, simple fatty liver (steatosis), nonalcoholic steatohepatitis (NASH), cirrhosis (irreversible, advanced scarring of the liver), or SCAP related obesity.

[0015] NASH/NAFLD patient population exhibit increased expression and transcriptional activity of SREBP1c and its target genes (Higuchi et al. (2008) Hepatol Res 38, 1122-1129). Using mouse genetics and siRNA mediated silencing, studies have shown that

What is Claimed

1. An RNAi construct comprising a sense strand and an antisense strand, wherein the antisense strand comprises a region having at least 15 contiguous nucleotides differing by no more than 3 nucleotides from an antisense sequence of SEQ ID NO:336 or 478, and wherein the RNAi construct inhibits the expression of SREBP Cleavage Activating Protein (SCAP).

2. The RNAi construct of claim 1, wherein the antisense strand comprises a region that is complementary to a SCAP mRNA sequence.

3. The RNAi construct any of the above claims, wherein the sense strand comprises a region having at least 15 contiguous nucleotides differing by no more than 3 nucleotides from an antisense sequence of SEQ ID NO:335 or 477.

4. The RNAi construct any of the above claims, wherein the sense strand comprises a sequence that is sufficiently complementary to the sequence of the antisense strand to form a duplex region of 15 to 30 base pairs in length.

5. The RNAi construct of claim 4, wherein the duplex region is 17 to 24 base pairs in length.

6. The RNAi construct of claim 4, wherein the duplex region is 19 to 21 base pairs in length.

7. The RNAi construct of claim 6, wherein the duplex region is 19 base pairs in length.

8. The RNAi construct of any one of claims 4 to 7, wherein the sense strand and the antisense strand are each 15 to 30 nucleotides in length.

9. The RNAi construct of claim 8, wherein the sense strand and the antisense strand are each 19 to 27 nucleotides in length.

10. The RNAi construct of claim 8, wherein the sense strand and the antisense strand are each 21 to 25 nucleotides in length.

11. The RNAi construct of claim 8, wherein the sense strand and the antisense strand are each 21 to 23 nucleotides in length.

12. The RNAi construct of any one of claims 1 to 11, wherein the RNAi construct comprises at least one blunt end.

13. The RNAi construct of any one of claims 1 to 11, wherein the RNAi construct comprises at least one nucleotide overhang of 1 to 4 unpaired nucleotides.

14. The RNAi construct of claim 13, wherein the nucleotide overhang has 2 unpaired nucleotides.

15. The RNAi construct of claim 13 or 14, wherein the RNAi construct comprises a nucleotide overhang at the 3' end of the sense strand, the 3' end of the antisense strand, or the 3' end of both the sense strand and the antisense strand.

16. The RNAi construct of any one of claims 13 to 15**,** wherein the nucleotide overhang comprises a 5'-UU-3' dinucleotide or a 5'-dTdT-3' dinucleotide.

17. The RNAi construct of any one of claims 1 to 16, wherein the RNAi construct comprises at least one modified nucleotide.

18. The RNAi construct of claim 17, wherein the modified nucleotide is a 2'-modified nucleotide.

19. The RNAi construct of claim 17, wherein the modified nucleotide is a 2'-fluoro modified nucleotide, a 2'-O-methyl modified nucleotide, a 2'-O-methoxyethyl modified nucleotide, a 2'-O-allyl modified nucleotide, a bicyclic nucleic acid (BNA), a glycol nucleic acid, an inverted base or combinations thereof.

20. The RNAi construct of claim 19, wherein the modified nucleotide is a 2'-O-methyl modified nucleotide, a 2'-O-methoxyethyl modified nucleotide, a 2'-fluoro modified nucleotide, or combinations thereof.

21. The RNAi construct of claim 17, wherein all of the nucleotides in the sense and antisense strands are modified nucleotides.

22. The RNAi construct of claim 21, wherein the modified nucleotides are 2'-O-methyl modified nucleotides, 2'-fluoro modified nucleotides, or combinations thereof.

23. The RNAi construct of any one of claims 1 to 22, wherein the RNAi construct comprises at least one phosphorothioate internucleotide linkage.

24. The RNAi construct of claim 23, wherein the RNAi construct comprises two consecutive phosphorothioate internucleotide linkages at the 3' end of the antisense strand.

25. The RNAi construct of claim 23, wherein the RNAi construct comprises two consecutive phosphorothioate internucleotide linkages at both the 3' and 5' ends of the antisense strand and two consecutive phosphorothioate internucleotide linkages at the 5' end of the sense strand.

26. The RNAi construct of any one of claims 1 to 25, wherein the antisense strand comprises a sequence selected from the antisense sequences of SEQ ID NO:336 and 478.

27. The RNAi construct of claim 26, wherein the sense strand comprises a sequence selected from the sense sequences of SEQ ID NO:335 and 477.

28. The RNAi construct of any one of claims 1 to 27, wherein the RNAi construct is any one of the duplex compounds of D-1087 or D-2087.

29. The RNAi construct of any one of claims 1 to 28, wherein the RNAi construct reduces the expression level of SCAP in liver cells following incubation with the RNAi construct as compared to the SCAP expression level in liver cells that have been incubated with a control RNAi construct.

30. The RNAi construct of claim 29, wherein the liver cells are Hep3B cells.

31. The RNAi construct of any one of claims 1 to 30, wherein the RNAi construct inhibits at least 10% of SCAP expression at 5 μM in Hep3B cells *in vitro.*

32. The RNAi construct of any one of claims 1 to 30, wherein the RNAi construct inhibits SCAP expression in Hep3B cells with an IC50 of less than about 1 nM.

33. A pharmaceutical composition comprising the RNAi construct of any one of claims 1 to 32 and a pharmaceutically acceptable carrier, excipient, or diluent.

34. Use of the RNAi construct of any one of claims 1 to 32 in the manufacture of a medicament for reducing the expression of SCAP in a patient in need thereof.

35. The use of claim 34, wherein the expression level of SCAP in hepatocytes is reduced in the patient following that the RNAi construct is to be administered as compared to the SCAP expression level in a patient not receiving the RNAi construct.

36. Use of the RNAi construct of any one of claims 1 to 32 or the pharmaceutical composition of claim 33 in the manufacture of a medicament for treating a subject having a SCAP-associated disease.

37. The use of claim 36, wherein that the RNAi construct of any one of claims 1 to 32 or the pharmaceutical composition of claim 33 results in a reduction of expression of a SCAP gene by at least about 10%.

38. The use of claim 36, wherein said disease is selected from the group consisting of fatty liver (steatosis), nonalcoholic steatohepatitis (NASH), cirrhosis of the liver, accumulation of fat in the liver, inflammation of the liver, hepatocellular necrosis, hepatocellular carcinoma, liver fibrosis, obesity, myocardial infarction, heart failure, coronary artery disease, hypercholesterolemia, or nonalcoholic fatty liver disease (NAFLD).

39. An RNAi construct comprising a sense strand and an antisense strand, wherein the antisense strand comprises a region having at least 15 contiguous nucleotides differing by no more than 3 nucleotides from an antisense sequence of SEQ ID NO:336 or 478, for use in treating a SCAP-associated disease, the use comprising that the RNAi construct of any one of claims 1 to 32 or the pharmaceutical composition of claim 33 is to be administered.