RNAI CONSTRUCTS FOR INHIBITING SCAP EXPRESSION AND METHODS OF USE THEREOF

FIELD OF THE INVENTION

[0001] The present invention relates to compositions and methods for modulating liver expression of sterol regulatory element binding protein (SREBP) cleavage-activating protein (SCAP). In particular, the present invention relates to nucleic acid-based therapeutics for reducing SCAP expression via RNA interference (RNAi) and methods of using such nucleic acid-based therapeutics to treat or prevent liver disease, such as nonalcoholic fatty liver disease (NAFLD).

CROSS-REFERENCE TO RELATED APPLICATIONS

[0002] This application claims the benefit of U.S. Provisional Patent Application No. 62/854,433, filed on May 30, 2019, which is incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

[0003] Comprising a spectrum of hepatic pathologies, nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in the world, the prevalence of which doubled in the last 20 years and now is estimated to affect approximately 20% of the world population (Sattar et al. (2014) BMJ 349:g4596; Loomba and Sanyal (2013) Nature Reviews Gastroenterology & hepatology 10(11):686-690; Kim and Kim (2017) Clin Gastroenterol Hepatol 15(4):474-485; Petta et al. (2016) Dig Liver Dis 48(3):333-342). NAFLD begins with the accumulation of triglyceride in the liver and is defined by the presence of cytoplasmic lipid droplets in more than 5% of hepatocytes in an individual 1) without a history of significant alcohol consumption and 2) in which the diagnosis of other types of liver disease have been excluded (Zhu et al (2016) World J Gastroenterol 22(36):8226-33; Rinella (2015) JAMA 313(22):2263-73; Yki-Jarvinen (2016) Diabetologia 59(6):1104-11). In some individuals the accumulation of ectopic fat in the liver, called steatosis, triggers inflammation and hepatocellular injury leading to a more advanced stage of disease called, nonalcoholic steatohepatitis (NASH) (Rinella, supra). As of 2015, 75-100 million Americans are predicted

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 OF THE INVENTION

[0007] The present invention 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 embodiment, the present invention 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 embodiments, the antisense strand comprises a region having at least 15 contiguous nucleotides from an antisense sequence listed in Table 1 or Table 2.

[0008] In some embodiments, 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 embodiments, the sense and antisense strands each are about 15 to about 30 nucleotides in length. In some embodiments, the RNAi constructs comprise at least one blunt end. In other embodiments, 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 embodiments, 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 embodiments, 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 invention may comprise one or more modified nucleotides, including nucleotides having modifications to the ribose ring, nucleobase, or phosphodiester backbone. In some embodiments, 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 embodiment, the RNAi constructs comprise one or more 2'-fluoro modified nucleotides, 2'-O-methyl modified nucleotides, or combinations thereof. In some embodiments, all of the nucleotides in the sense and antisense strand of the RNAi construct are modified nucleotides.

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

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

[0011] In some embodiments, the antisense strand and/or the sense strand of the RNAi constructs of the invention may comprise or consist of a sequence from the antisense and sense sequences listed in Tables 1 or 2. In certain embodiments, 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

liver specific removal of SCAP activity dramatically lowers the liver TG content in wildtype, Ob/Ob mice and high fat diet fed hamsters. This is accompanied by reduced VLDL secretion and decreased plasma TG levels after SCAP silencing. However, body weight, insulin and glucose levels remain unchanged (Moon et al. (2012) Cell Metab 15, 240-246). More recently, published findings showed that siRNA silencing of SCAP in mouse and dyslipidemic rhesus monkeys reduced TG levels significantly (Jensen et al. (2016) J Lipid Res 57, 2150-2162; Murphy et al. (2017) Metabolism 71, 202-212). Based upon these published reports, we hypothesized that administration of siSCAP in NASH patients will reduce liver steatosis and prevent further progression of fibrosis.

[0016] RNA interference (RNAi) is the process of introducing exogeneous RNA into a cell leading to specific degradation of the mRNA encoding the targeted protein with a resultant decrease in protein expression. Advances in both the RNAi technology and hepatic delivery and growing positive outcomes with other RNAi-based therapies, suggest RNAi as a compelling means to therapeutically treat NAFLD by directly targeting SCAP. The inhibitory effect of these sequences was confirmed by screening on Hep3B cells. Using C57Bl6 mice, we then demonstrated treatment with SCAP siRNA reduced SCAP expression in mice.

[0017] As used herein, the term "RNAi construct" refers to an agent comprising an RNA molecule that is capable of downregulating expression of a target gene (e.g. SCAP) via an RNA interference mechanism when introduced into a cell. RNA interference is the process by which a nucleic acid molecule induces the cleavage and degradation of a target RNA molecule (e.g. messenger RNA or mRNA molecule) in a sequence-specific manner, e.g. through an RNA induced silencing complex (RISC) pathway. In some embodiments, the RNAi construct comprises a double-stranded RNA molecule comprising two antiparallel strands of contiguous nucleotides that are sufficiently complementary to each other to hybridize to form a duplex region. "Hybridize" or "hybridization" refers to the pairing of complementary polynucleotides, typically via hydrogen bonding (e.g. Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding) between complementary bases in the two polynucleotides. The strand comprising a region having a sequence that is substantially complementary to a target sequence (e.g. target mRNA) is referred to as the "antisense strand." The "sense strand" refers to the strand that includes a region that is substantially complementary to a region of the antisense strand. In some embodiments, the sense strand may comprise a region that has a sequence that is substantially identical to the target sequence.

[0018] In some embodiments, the invention provides an RNAi construct directed to SCAP. In some embodiments, the invention includes an RNAi construct that contains any of the sequences found in Table 1 or 2.

[0019] A double-stranded RNA molecule may include chemical modifications to ribonucleotides, including modifications to the ribose sugar, base, or backbone components of the ribonucleotides, such as those described herein or known in the art. Any such modifications, as used in a double-stranded RNA molecule (e.g. siRNA, shRNA, or the like), are encompassed by the term "double-stranded RNA" for the purposes of this disclosure.

[0020] As used herein, a first sequence is "complementary" to a second sequence if a polynucleotide comprising the first sequence can hybridize to a polynucleotide comprising the second sequence to form a duplex region under certain conditions, such as physiological conditions. Other such conditions can include moderate or stringent hybridization conditions, which are known to those of skill in the art. A first sequence is considered to be fully complementary (100% complementary) to a second sequence if a polynucleotide comprising the first sequence base pairs with a polynucleotide comprising the second sequence over the entire length of one or both nucleotide sequences without any mismatches. A sequence is "substantially complementary" to a target sequence if the sequence is at least about 80%, 85%, 90%, 95%,96%, 97%, 98%, 99% or 100% complementary to a target sequence. Percent complementarity can be calculated by dividing the number of bases in a first sequence that are complementary to bases at corresponding positions in a second or target sequence by the total length of the first sequence. A sequence may also be said to be substantially complementary to another sequence if there are no more than 5, 4, 3, 2, or 1 mismatches over a 30 base pair duplex region when the two sequences are hybridized. Generally, if any nucleotide overhangs, as defined herein, are present, the sequence of such overhangs is not considered in determining the degree of complementarity between two sequences. By way of example, a sense strand of 21 nucleotides in length and an antisense strand of 21 nucleotides in length that hybridize to form a 19 base pair duplex region with a 2 nucleotide overhang at the 3' end of each strand would be considered to be fully complementary as the term is used herein.

[0021] In some embodiments, a region of the antisense strand comprises a sequence that is fully complementary to a region of the target RNA sequence (e.g. SCAP mRNA). In such embodiments, the sense strand may comprise a sequence that is fully complementary to the sequence of the antisense strand. In other such embodiments, the sense strand may comprise a

sequence that is substantially complementary to the sequence of the antisense strand, e.g. having 1, 2, 3, 4, or 5 mismatches in the duplex region formed by the sense and antisense strands. In certain embodiments, it is preferred that any mismatches occur within the terminal regions (e.g. within 6, 5, 4, 3, 2, or 1 nucleotides of the 5' and/or 3' ends of the strands). In one embodiment, any mismatches in the duplex region formed from the sense and antisense strands occur within 6, 5, 4, 3, 2, or 1 nucleotides of the 5' end of the antisense strand.

[0022] In certain embodiments, the sense strand and antisense strand of the double-stranded RNA may be two separate molecules that hybridize to form a duplex region, but are otherwise unconnected. Such double-stranded RNA molecules formed from two separate strands are referred to as "small interfering RNAs" or "short interfering RNAs" (siRNAs). Thus, in some embodiments, the RNAi constructs of the invention comprise a siRNA.

[0023] Where the two substantially complementary strands of a dsRNA are comprised by separate RNA molecules, those molecules need not, but can be covalently connected. Where the two strands are connected covalently by means other than an uninterrupted chain of nucleotides between the 3 '-end of one strand and the 5' -end of the respective other strand forming the duplex structure, the connecting structure is referred to as a "linker." The RNA strands may have the same or a different number of nucleotides. The maximum number of base pairs in the duplex is the number of nucleotides in the shortest strand of the dsRNA minus any overhangs that are present in the duplex. In addition to the duplex structure, an RNAi construct may comprise one or more nucleotide overhangs.

[0024] In other embodiments, the sense strand and the antisense strand that hybridize to form a duplex region may be part of a single RNA molecule, i.e. the sense and antisense strands are part of a self-complementary region of a single RNA molecule. In such cases, a single RNA molecule comprises a duplex region (also referred to as a stem region) and a loop region. The 3' end of the sense strand is connected to the 5' end of the antisense strand by a contiguous sequence of unpaired nucleotides, which will form the loop region. The loop region is typically of a sufficient length to allow the RNA molecule to fold back on itself such that the antisense strand can base pair with the sense strand to form the duplex or stem region. The loop region can comprise from about 3 to about 25, from about 5 to about 15, or from about 8 to about 12 unpaired nucleotides. Such RNA molecules with at least partially self-complementary regions are referred to as "short hairpin RNAs" (shRNAs). In some embodiments, the loop region can comprise at least 1, 2, 3, 4, 5, 10, 20, or 25 unpaired nucleotides. In some embodiments, the

loop region can have 10, 9, 8, 7, 6, 5, 4, 3, 2, or fewer unpaired nucleotides. In certain embodiments, the RNAi constructs of the invention comprise a shRNA. The length of a single, at least partially self-complementary RNA molecule can be from about 35 nucleotides to about 100 nucleotides, from about 45 nucleotides to about 85 nucleotides, or from about 50 to about 60 nucleotides and comprise a duplex region and loop region each having the lengths recited herein.

[0025] In some embodiments, the RNAi constructs of the invention comprise a sense strand and an antisense strand, wherein the antisense strand comprises a region having a sequence that is substantially or fully complementary to a SCAP messenger RNA (mRNA) sequence. As used herein, a "SCAP mRNA sequence" refers to any messenger RNA sequence, including splice variants, encoding a SCAP protein, including SCAP protein variants or isoforms from any species (e.g. mouse, rat, non-human primate, human).

[0026] A SCAP mRNA sequence also includes the transcript sequence expressed as its complementary DNA (cDNA) sequence. A cDNA sequence refers to the sequence of an mRNA transcript expressed as DNA bases (e.g. guanine, adenine, thymine, and cytosine) rather than RNA bases (e.g. guanine, adenine, uracil, and cytosine). Thus, the antisense strand of the RNAi constructs of the invention may comprise a region having a sequence that is substantially or fully complementary to a target SCAP mRNA sequence or SCAP cDNA sequence. A SCAP mRNA or cDNA sequence can include, but is not limited to, any SCAP mRNA or cDNA sequence such as can be derived from the NCBI Reference sequence for human SCAP (NM_012235) or mouse SCAP (NM_001001144).

[0027] A region of the antisense strand can be substantially complementary or fully complementary to at least 15 consecutive nucleotides of the SCAP mRNA sequence. In some embodiments, the target region of the SCAP mRNA sequence to which the antisense strand comprises a region of complementarity can range from about 15 to about 30 consecutive nucleotides, from about 16 to about 28 consecutive nucleotides, from about 18 to about 26 consecutive nucleotides, from about 17 to about 24 consecutive nucleotides, from about 19 to about 25 consecutive nucleotides, from about 19 to about 23 consecutive nucleotides, or from about 19 to about 21 consecutive nucleotides. In certain embodiments, the region of the antisense strand comprising a sequence that is substantially or fully complementary to a SCAP mRNA sequence may, in some embodiments, comprise at least 15 contiguous nucleotides from an antisense sequence listed in Table 1 or Table 2. In other embodiments, the antisense

sequence comprises at least 16, at least 17, at least 18, or at least 19 contiguous nucleotides from an antisense sequence listed in Table 1 or Table 2. In some embodiments, the sense and/or antisense sequence comprises at least 15 nucleotides from a sequence listed in Table 1 or 2 with no more than 1, 2, or 3 nucleotide mismatches.

[0028] The sense strand of the RNAi construct typically comprises a sequence that is sufficiently complementary to the sequence of the antisense strand such that the two strands hybridize under physiological conditions to form a duplex region. A "duplex region" refers to the region in two complementary or substantially complementary polynucleotides that form base pairs with one another, either by Watson-Crick base pairing or other hydrogen bonding interaction, to create a duplex between the two polynucleotides. The duplex region of the RNAi construct should be of sufficient length to allow the RNAi construct to enter the RNA interference pathway, e.g. by engaging the Dicer enzyme and/or the RISC complex. For instance, in some embodiments, the duplex region is about 15 to about 30 base pairs in length. Other lengths for the duplex region within this range are also suitable, such as about 15 to about 28 base pairs, about 15 to about 26 base pairs, about 15 to about 24 base pairs, about 15 to about 22 base pairs, about 17 to about 28 base pairs, about 17 to about 26 base pairs, about 17 to about 24 base pairs, about 17 to about 23 base pairs, about 17 to about 21 base pairs, about 19 to about 25 base pairs, about 19 to about 23 base pairs, or about 19 to about 21 base pairs. In one embodiment, the duplex region is about 17 to about 24 base pairs in length. In another embodiment, the duplex region is about 19 to about 21 base pairs in length.

[0029] In some embodiments, an RNAi construct of the invention contains a duplex region of about 24 to about 30 nucleotides that interacts with a target RNA sequence, e.g., a SCAP target mRNA sequence, to direct the cleavage of the target RNA. Without wishing to be bound by theory, long double stranded RNA introduced into cells can be broken down into siRNA by a Type III endonuclease known as Dicer (Sharp et al. (2001) Genes Dev. 15:485). Dicer, a ribonuclease-III-like enzyme, processes the dsRNA into 19-23 base pair short interfering RNAs with characteristic two base 3' overhangs (Bernstein, et al., (2001) Nature 409:363). The siRNAs are then incorporated into an RNA-induced silencing complex (RISC) where one or more helicases unwind the siRNA duplex, enabling the complementary antisense strand to guide target recognition (Nykanen, et al., (2001) Cell107:309). Upon binding to the appropriate target mRNA, one or more endonucleases within the RISC cleave the target to induce silencing (Elbashir, et al., (2001) Genes Dev. 15: 188).

[0030] For embodiments in which the sense strand and antisense strand are two separate molecules (e.g. RNAi construct comprises a siRNA), the sense strand and antisense strand need not be the same length as the length of the duplex region. For instance, one or both strands maybe longer than the duplex region and have one or more unpaired nucleotides or mismatches flanking the duplex region. Thus, in some embodiments, the RNAi construct comprises at least one nucleotide overhang. As used herein, a "nucleotide overhang" refers to the unpaired nucleotide or nucleotides that extend beyond the duplex region at the terminal ends of the strands. Nucleotide overhangs are typically created when the 3' end of one strand extends beyond the 5' end of the other strand or when the 5' end of one strand extends beyond the 3' end of the other strand. The length of a nucleotide overhang is generally between 1 and 6 nucleotides, 1 and 5 nucleotides, 1 and 4 nucleotides, 1 and 3 nucleotides, 2 and 6 nucleotides, 2 and 5 nucleotides, or 2 and 4 nucleotides. In some embodiments, the nucleotide overhang comprises 1, 2, 3, 4, 5, or 6 nucleotides. In one particular embodiment, the nucleotide overhang comprises 1 to 4 nucleotides. In certain embodiments, the nucleotide overhang comprises 2 nucleotides. The nucleotides in the overhang can be ribonucleotides, deoxyribonucleotides, or modified nucleotides as described herein. In some embodiments, the overhang comprises a 5'-uridine-uridine-3' (5'-UU-3') dinucleotide. In such embodiments, the UU dinucleotide may comprise ribonucleotides or modified nucleotides, e.g. 2'-modified nucleotides. In other embodiments, the overhang comprises a 5'-deoxythymidine-deoxythymidine-3' (5'-dTdT-3') dinucleotide.

[0031] The nucleotide overhang can be at the 5' end or 3' end of one or both strands. For example, in one embodiment, the RNAi construct comprises a nucleotide overhang at the 5' end and the 3' end of the antisense strand. In another embodiment, the RNAi construct comprises a nucleotide overhang at the 5' end and the 3' end of the sense strand. In some embodiments, the RNAi construct comprises a nucleotide overhang at the 5' end of the sense strand and the 5' end of the antisense strand. In other embodiments, the RNAi construct comprises a nucleotide overhang at the 3' end of the sense strand and the 3' end of the antisense strand.

[0032] The RNAi constructs may comprise a single nucleotide overhang at one end of the double-stranded RNA molecule and a blunt end at the other. A "blunt end" means that the sense strand and antisense strand are fully base-paired at the end of the molecule and there are no unpaired nucleotides that extend beyond the duplex region. In some embodiments, the RNAi

construct comprises a nucleotide overhang at the 3' end of the sense strand and a blunt end at the 5' end of the sense strand and 3' end of the antisense strand. In other embodiments, the RNAi construct comprises a nucleotide overhang at the 3' end of the antisense strand and a blunt end at the 5' end of the antisense strand and the 3' end of the sense strand. In certain embodiments, the RNAi construct comprises a blunt end at both ends of the double-stranded RNA molecule. In such embodiments, the sense strand and antisense strand have the same length and the duplex region is the same length as the sense and antisense strands (i.e. the molecule is double-stranded over its entire length).

[0033] The sense strand and antisense strand can each independently be about 15 to about 30 nucleotides in length, about 18 to about 28 nucleotides in length, about 19 to about 27 nucleotides in length, about 19 to about 25 nucleotides in length, about 19 to about 23 nucleotides in length, about 21 to about 25 nucleotides in length, or about 21 to about 23 nucleotides in length. In certain embodiments, the sense strand and antisense strand are each about 18, about 19, about 20, about 21, about 22, about 23, about 24, or about 25 nucleotides in length. In some embodiments, the sense strand and antisense strand have the same length but form a duplex region that is shorter than the strands such that the RNAi construct has two nucleotide overhangs. For instance, in one embodiment, the RNAi construct comprises (i) a sense strand and an antisense strand that are each 21 nucleotides in length, (ii) a duplex region that is 19 base pairs in length, and (iii) nucleotide overhangs of 2 unpaired nucleotides at both the 3' end of the sense strand and the 3' end of the antisense strand. In another embodiment, the RNAi construct comprises (i) a sense strand and an antisense strand that are each 23 nucleotides in length, (ii) a duplex region that is 21 base pairs in length, and (iii) nucleotide overhangs of 2 unpaired nucleotides at both the 3' end of the sense strand and the 3' end of the antisense strand. In other embodiments, the sense strand and antisense strand have the same length and form a duplex region over their entire length such that there are no nucleotide overhangs on either end of the double-stranded molecule. In one such embodiment, the RNAi construct is blunt ended and comprises (i) a sense strand and an antisense strand, each of which is 21 nucleotides in length, and (ii) a duplex region that is 21 base pairs in length. In another such embodiment, the RNAi construct is blunt ended and comprises (i) a sense strand and an antisense strand, each of which is 23 nucleotides in length, and (ii) a duplex region that is 23 base pairs in length.

[0034] In other embodiments, the sense strand or the antisense strand is longer than the other strand and the two strands form a duplex region having a length equal to that of the shorter strand such that the RNAi construct comprises at least one nucleotide overhang. For example, in one embodiment, the RNAi construct comprises (i) a sense strand that is 19 nucleotides in length, (ii)an antisense strand that is 21 nucleotides in length, (iii) a duplex region of 19 base pairs in length, and (iv) a single nucleotide overhang of 2 unpaired nucleotides at the 3' end of the antisense strand. In another embodiment, the RNAi construct comprises (i) a sense strand that is 21 nucleotides in length, (ii) an antisense strand that is 23 nucleotides in length, (iii) a duplex region of 21 base pairs in length, and (iv) a single nucleotide overhang of 2 unpaired nucleotides at the 3' end of the antisense strand.

[0035] The antisense strand of the RNAi constructs of the invention can comprise the sequence of any one of the antisense sequences listed in Table 1 or Table 2 or the sequence of nucleotides 1-19 or 1-21 of any of these antisense sequences. Each of the antisense sequences listed in Tables 1 and 2 comprises a sequence of 19 consecutive nucleotides (first 19 nucleotides counting from the 5' end) that is complementary to a SCAP mRNA sequence plus a two nucleotide overhang sequence. Thus, in some embodiments, the antisense strand comprises a sequence of nucleotides, for example nucleotides 1-19 of any one of even numbered sequences of SEQ ID NOs: 2-160, 162-320, 322-462, or 464-604. In some embodiments, the sense strand comprises a sequence of nucleotides, for example nucleotides 1-19 of any one of odd numbered sequences of SEQ ID NOs: 1-159, 161-319, 321-461, or 463-603. In a particular embodiment, the antisense sequence has SEQ ID NO: 82. In a particular embodiment, the antisense sequence has SEQ ID NO: 242. In a particular embodiment, the antisense sequence has SEQ ID NO: 84. In a particular embodiment, the antisense sequence has SEQ ID NO: 244. In a particular embodiment, the antisense sequence has SEQ ID NO: 86. In a particular embodiment, the antisense sequence has SEQ ID NO: 246. In a particular embodiment, the antisense sequence has SEQ ID NO: 88. In a particular embodiment, the antisense sequence has SEQ ID NO: 248. In a particular embodiment, the antisense sequence has SEQ ID NO: 90. In a particular embodiment, the antisense sequence has SEQ ID NO: 250.

Modified nucleotides

[0036] The RNAi constructs of the invention may comprise one or more modified nucleotides. A "modified nucleotide" refers to a nucleotide that has one or more chemical modifications to the nucleoside, nucleobase, pentose ring, or phosphate group. As used herein, modified nucleotides do not encompass ribonucleotides containing adenosine monophosphate, guanosine monophosphate, uridine monophosphate, and cytidine monophosphate, and deoxyribonucleotides containing deoxyadenosine monophosphate, deoxyguanosine monophosphate, deoxythymidine monophosphate, and deoxycytidine monophosphate. However, the RNAi constructs may comprise combinations of modified nucleotides, ribonucleotides, and deoxyribonucleotides. Incorporation of modified nucleotides into one or both strands of double-stranded RNA molecules can improve the in vivo stability of the RNA molecules, e.g., by reducing the molecules' susceptibility to nucleases and other degradation processes. The potency of RNAi constructs for reducing expression of the target gene can also be enhanced by incorporation of modified nucleotides.

[0037] In certain embodiments, the modified nucleotides have a modification of the ribose sugar. These sugar modifications can include modifications at the 2' and/or 5' position of the pentose ring as well as bicyclic sugar modifications. A 2'-modified nucleotide refers to a nucleotide having a pentose ring with a substituent at the 2' position other than H or OH. Such 2' modifications include, but are not limited to, 2'-O-alkyl (e.g. O-C1-C10 or O-C1-C10 substituted alkyl), 2'-O-allyl (O-CH2CH=CH2), 2'-C-allyl, 2'-fluoro, 2'-O-methyl (OCH3), 2'-O-methoxyethyl (O-(CH2)2OCH3), 2'-OCF3, 2'-O(CH2)2SCH3, 2'-O-aminoalkyl, 2'-amino (e.g.NH2), 2'-O-ethylamine, and 2'-azido. Modifications at the 5' position of the pentose ring include, but are not limited to, 5'-methyl (R or S); 5'-vinyl, and 5'-methoxy.

[0038] A "bicyclic sugar modification" refers to a modification of the pentose ring where a bridge connects two atoms of the ring to form a second ring resulting in a bicyclic sugar structure. In some embodiments the bicyclic sugar modification comprises a bridge between the 4' and 2' carbons of the pentose ring. Nucleotides comprising a sugar moiety with a bicyclic sugar modification are referred to herein as bicyclic nucleic acids or BNAs. Exemplary bicyclic sugar modifications include, but are not limited to, α-L-Methyleneoxy (4'-CH2-O-2') bicyclicnucleic acid (BNA); β-D-Methyleneoxy (4'-CH2-O-2') BNA (also referred to as a locked nucleic acid or LNA); Ethyleneoxy (4'-(CH2)2-O-2') BNA; Aminooxy (4'-CH2-O-N(R)- 2') BNA; Oxyamino (4'-CH2-N(R)-O-2') BNA; Methyl(methyleneoxy) (4'-

CH(CH3)-O-2') BNA (also referred to as constrained ethyl or cEt); methylene-thio (4'-CH2-S-2') BNA; methylene-amino (4'-CH2-N(R)-2') BNA; methyl carbocyclic (4'-CH2-CH(CH3)-2') BNA; propylene carbocyclic (4'-(CH2)3-2') BNA; and Methoxy(ethyleneoxy) (4'-CH(CH2OMe)-O-2')BNA (also referred to as constrained MOE or cMOE). These and other sugar-modified nucleotides that can be incorporated into the RNAi constructs of the invention are described in U.S. Patent No. 9,181,551, U.S. Patent Publication No. 2016/0122761, and Deleavey and Damha, Chemistry and Biology, Vol. 19: 937-954, 2012, all of which are hereby incorporated by reference in their entireties.

[0039] In some embodiments, the RNAi constructs comprise one or more 2'-fluoro modified nucleotides, 2'-O-methyl modified nucleotides, 2'-O-methoxyethyl modified nucleotides, 2'-O-allyl modified nucleotides, bicyclic nucleic acids (BNAs), glycol nucleic acids, or combinations thereof. In certain embodiments, the RNAi constructs comprise one or more 2'-fluoro modified nucleotides, 2'-O-methyl modified nucleotides, 2'-O-methoxyethyl modified nucleotides, or combinations thereof. In one particular embodiment, the RNAi constructs comprise one or more 2'-fluoro modified nucleotides, 2'-O-methyl modified nucleotides or combinations thereof.

[0040] Both the sense and antisense strands of the RNAi constructs can comprise one or multiple modified nucleotides. For instance, in some embodiments, the sense strand comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more modified nucleotides. In certain embodiments, all nucleotides in the sense strand are modified nucleotides. In some embodiments, the antisense strand comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more modified nucleotides. In other embodiments, all nucleotides in the antisense strand are modified nucleotides. In certain other embodiments, all nucleotides in the sense strand and all nucleotides in the antisense strand are modified nucleotides. In these and other embodiments, the modified nucleotides can be 2'-fluoro modified nucleotides, 2'-O-methyl modified nucleotides, or combinations thereof.

[0041] In some embodiments, all pyrimidine nucleotides preceding an adenosine nucleotide in the sense strand, antisense strand, or both strands are modified nucleotides. For example, where the sequence 5'-CA-3' or 5'-UA-3' appears in either strand, the cytidine and uridine nucleotides are modified nucleotides, preferably 2'-O-methyl modified nucleotides. In certain embodiments, all pyrimidine nucleotides in the sense strand are modified nucleotides (e.g. 2'-O-methyl modified nucleotides), and the 5' nucleotide in all occurrences of the sequence 5'-CA-3' or 5'-UA-3' in the antisense strand are modified nucleotides (e.g. 2'-O-methyl

modified nucleotides). In other embodiments, all nucleotides in the duplex region are modified nucleotides. In such embodiments, the modified nucleotides are preferably 2'-O-methyl modified nucleotides, 2'-fluoro modified nucleotides or combinations thereof.

[0042] In embodiments in which the RNAi construct comprises a nucleotide overhang, the nucleotides in the overhang can be ribonucleotides, deoxyribonucleotides, or modified nucleotides. In one embodiment, the nucleotides in the overhang are deoxyribonucleotides, e.g., deoxythymidine. In another embodiment, the nucleotides in the overhang are modified nucleotides. For instance, in some embodiments, the nucleotides in the overhang are 2'-O-methyl modified nucleotides, 2'-fluoro modified nucleotides, 2'-methoxyethyl modified nucleotides, or combinations thereof.

[0043] The RNAi constructs of the invention may also comprise one or more modified internucleotide linkages. As used herein, the term "modified internucleotide linkage" refers to an internucleotide linkage other than the natural 3' to 5' phosphodiester linkage. In some embodiments, the modified internucleotide linkage is a phosphorous-containing internucleotide linkage, such as a phosphotriester, aminoalkyl phosphotriester, an alkylphosphonate (e.g.methylphosphonate, 3' -alkylene phosphonate), a phosphinate, a phosphoramidate (e.g. 3'-aminophosphoramidate and aminoalkylphosphoramidate), a phosphorothioate (P=S), a chiralphosphorothioate, a phosphorodithioate, a thionophosphoramidate, a thionoalkylphosphonate, athionoalkylphosphotriester, and a boranophosphate. In one embodiment, a modified internucleotide linkage is a 2' to 5' phosphodiester linkage. In other embodiments, the modified internucleotide linkage is a non-phosphorous-containing internucleotide linkage and thus can be referred to as a modified internucleoside linkage. Such non-phosphorous-containing linkages include, but are not limited to, morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane linkages (-O-Si(H)2-O-); sulfide, sulfoxide and sulfone linkages; formacetyl and thioformacetyl linkages; alkene containing backbones; sulfamate backbones; methylenemethylimino (-CH2-N(CH3)-O-CH2-) and methylenehydrazino linkages; sulfonate and sulfonamide linkages; amide linkages; and others having mixed N, O, S and CH2 component parts. In one embodiment, the modified internucleoside linkage is a peptide-based linkage (e.g. aminoethylglycine) to create a peptide nucleic acid or PNA, such as those described in U.S. Patent Nos. 5,539,082; 5,714,331; and 5,719,262. Other suitable modified internucleotide and internucleoside linkages that may be employed in the RNAi constructs of

the invention are described in U.S. Patent No. 6,693,187, U.S. Patent No. 9,181,551, U.S. Patent Publication No. 2016/0122761, and Deleavey and Damha, Chemistry and Biology, Vol. 19: 937-954, 2012, all of which are hereby incorporated by reference in their entireties.

[0044] In certain embodiments, the RNAi constructs comprise one or more phosphorothioate internucleotide linkages. The phosphorothioate internucleotide linkages may be present in the sense strand, antisense strand, or both strands of the RNAi constructs. For instance, in some embodiments, the sense strand comprises 1, 2, 3, 4, 5, 6, 7, 8, or more phosphorothioate internucleotide linkages. In other embodiments, the antisense strand comprises 1, 2, 3, 4, 5, 6, 7,8, or more phosphorothioate internucleotide linkages. In still other embodiments, both strands comprise 1, 2, 3, 4, 5, 6, 7, 8, or more phosphorothioate internucleotide linkages. The RNAi constructs can comprise one or more phosphorothioate internucleotide linkages at the 3'-end, the 5'-end, or both the 3'- and 5'-ends of the sense strand, the antisense strand, or both strands. For instance, in certain embodiments, the RNAi construct comprises about 1 to about 6 or more (e.g., about 1, 2, 3, 4, 5, 6 or more) consecutive phosphorothioate internucleotide linkages at the 3'-end of the sense strand, the antisense strand, or both strands. In other embodiments, the RNAi construct comprises about 1 to about 6 or more (e.g., about 1, 2, 3, 4, 5, 6 or more) consecutive phosphorothioate internucleotide linkages at the 5'-end of the sense strand, the antisense strand, or both strands. In one embodiment, the RNAi construct comprises a single phosphorothioate internucleotide linkage at the 3' end of the sense strand and a single phosphorothioate internucleotide linkage at the 3' end of the antisense strand. In another embodiment, the RNAi construct comprises two consecutive phosphorothioate internucleotide linkages at the 3' end of the antisense strand (i.e. a phosphorothioate internucleotide linkage at the first and second internucleotide linkages at the 3' end of the antisense strand). In another embodiment, the RNAi construct comprises two consecutive phosphorothioate internucleotide linkages at both the 3' and 5' ends of the antisense strand. In yet another embodiment, 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. In still another embodiment, 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 both the 3' and 5' ends of the sense strand (i.e. a phosphorothioate internucleotide linkage at the first and second internucleotide linkages at both

the 5' and 3' ends of the antisense strand and a phosphorothioate internucleotide linkage at the first and second internucleotide linkages at both the 5' and 3' ends of the sense strand). In any of the embodiments in which one or both strands comprises one or more phosphorothioate internucleotide linkages, the remaining internucleotide linkages within the strands can be the natural 3' to 5' phosphodiester linkages. For instance, in some embodiments, each internucleotide linkage of the sense and antisense strands is selected from phosphodiester and phosphorothioate, wherein at least one internucleotide linkage is a phosphorothioate.

[0045] In embodiments in which the RNAi construct comprises a nucleotide overhang, two or more of the unpaired nucleotides in the overhang can be connected by a phosphorothioate internucleotide linkage. In certain embodiments, all the unpaired nucleotides in a nucleotide overhang at the 3' end of the antisense strand and/or the sense strand are connected by phosphorothioate internucleotide linkages. In other embodiments, all the unpaired nucleotides in a nucleotide overhang at the 5' end of the antisense strand and/or the sense strand are connected by phosphorothioate internucleotide linkages. In still other embodiments, all the unpaired nucleotides in any nucleotide overhang are connected by phosphorothioate internucleotide linkages.

[0046] In certain embodiments, the modified nucleotides incorporated into one or both of the strands of the RNAi constructs of the invention have a modification of the nucleobase (also referred to herein as "base"). A "modified nucleobase" or "modified base" refers to a base other than the naturally occurring purine bases adenine (A) and guanine (G) and pyrimidine bases thymine (T), cytosine (C), and uracil (U). Modified nucleobases can be synthetic or naturally occurring modifications and include, but are not limited to, universal bases, 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine (X), hypoxanthine (I), 2-aminoadenine, 6-methyladenine, 6-methylguanine, and other alkyl derivatives of adenine and guanine, 2-propyland other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo, particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-daazaadenine and 3-deazaguanine and 3-deazaadenine, and abasic residues (apurinic/apyrimidinic residues which lack the purine or pyrimidine base, lacking a nucleobase at position 1 of the ribose sugar), and

inverted nucleotides (nucleotides having 3'-3' linkage, and can be inverted nucleotides of any of the above, including inverted abasic nucleotides and inverted deoxynucleotides).

[0047] In some embodiments, the modified base is a universal base. A "universal base" refers to a base analog that indiscriminately forms base pairs with all of the natural bases in RNA and DNA without altering the double helical structure of the resulting duplex region. Universal bases are known to those of skill in the art and include, but are not limited to, inosine, C-phenyl, C-naphthyl and other aromatic derivatives, azole carboxamides, and nitroazole derivatives, such as3-nitropyrrole, 4-nitroindole, 5-nitroindole, and 6-nitroindole.

[0048] Other suitable modified bases that can be incorporated into the RNAi constructs of the invention include those described in Herdewijn, Antisense Nucleic Acid Drug Dev., Vol. 10:297-310, 2000 and Peacock et al., J. Org. Chern., Vol. 76: 7295-7300, 2011, both of which are hereby incorporated by reference in their entireties. The skilled person is well aware that guanine, cytosine, adenine, thymine, and uracil may be replaced by other nucleobases, such as the modified nucleobases described above, without substantially altering the base pairing properties of a polynucleotide comprising a nucleotide bearing such replacement nucleobase.

[0049] In some embodiments of the RNAi constructs of the invention, the 5' end of the sense strand, antisense strand, or both the antisense and sense strands comprises a phosphate moiety. As used herein, the term "phosphate moiety" refers to a terminal phosphate group that includes unmodified phosphates (-O-P=O)(OH)OH) as well as modified phosphates. Modified phosphates include phosphates in which one or more of the O and OH groups is replaced with H, O, S, N(R) or alkyl where R is H, an amino protecting group or unsubstituted or substituted alkyl. Exemplary phosphate moieties include, but are not limited to, 5'-monophosphate; 5'diphosphate; 5'-triphosphate; 5'-guanosine cap (7-methylated or non-methylated); 5'-adenosinecap or any other modified or unmodified nucleotide cap structure; 5'-monothiophosphate (phosphorothioate); 5'-monodithiophosphate (phosphorodithioate); 5'-alpha-thiotriphosphate; 5'-gamma-thiotriphosphate, 5'-phosphoramidates; 5'-vinylphosphates; 5'-alkylphosphonates (e.g.,alkyl= methyl, ethyl, isopropyl, propyl, etc.); and 5'-alkyletherphosphonates (e.g., alkylether =methoxymethyl, ethoxymethyl, etc.).

[0050] The modified nucleotides that can be incorporated into the RNAi constructs of the invention may have more than one chemical modification described herein. For instance, the modified nucleotide may have a modification to the ribose sugar as well as a modification

to the nucleobase. By way of example, a modified nucleotide may comprise a 2' sugar modification(e.g. 2'-fluoro or 2'-methyl) and comprise a modified base (e.g. 5-methyl cytosine or pseudouracil). In other embodiments, the modified nucleotide may comprise a sugar modification in combination with a modification to the 5' phosphate that would create a modified internucleotide or internucleoside linkage when the modified nucleotide was incorporated into a polynucleotide. For instance, in some embodiments, the modified nucleotide may comprise a sugar modification, such as a 2'-fluoro modification, a 2'-O-methyl modification, or a bicyclic sugar modification, as well as a 5' phosphorothioate group. Accordingly, in some embodiments, one or both strands of the RNAi constructs of the invention comprise a combination of 2' modified nucleotides or BNAs and phosphorothioate internucleotide linkages. In certain embodiments, both the sense and antisense strands of the RNAi constructs of the invention comprise a combination of 2'-fluoro modified nucleotides, 2'-O-methyl modified nucleotides, and phosphorothioate internucleotide linkages. Exemplary RNAi constructs comprising modified nucleotides and internucleotide linkages are shown in Table 2.

Function of RNAi constructs

[0051] Preferably, the RNAi constructs of the invention reduce or inhibit the expression of SCAP in cells, particularly liver cells. Accordingly, in one embodiment, the present invention provides a method of reducing SCAP expression in a cell by contacting the cell with any RNAi construct described herein. The cell may be in vitro or in vivo. SCAP expression can be assessed by measuring the amount or level of SCAP mRNA, SCAP protein, or another biomarker linked to SCAP expression. The reduction of SCAP expression in cells or animals treated with an RNAi construct of the invention can be determined relative to the SCAP expression in cells or animals not treated with the RNAi construct or treated with a control RNAi construct. For instance, in some embodiments, reduction of SCAP expression is assessed by (a) measuring the amount or level of SCAP mRNA in liver cells treated with an RNAi construct of the invention, (b) measuring the amount or level of SCAP mRNA in liver cells treated with a control RNAi construct (e.g., RNAi construct directed to an RNA molecule not expressed in liver cells or an RNAi construct having a nonsense or scrambled sequence) or no construct, and (c) comparing the measured SCAP mRNA levels from treated cells in (a) to the measured SCAP mRNA levels from control cells in (b). The SCAP mRNA levels in the treated cells and controls cells can be normalized to RNA levels for a control gene (e.g. 18S ribosomal

RNA) prior to comparison. SCAP mRNA levels can be measured by a variety of methods, including Northern blot analysis, nuclease protection assays, fluorescence in situ hybridization (FISH), reverse-transcriptase (RT)-PCR, real-time RT-PCR, quantitative PCR, and the like.

[0052] In other embodiments, reduction of SCAP expression is assessed by (a) measuring the amount or level of SCAP protein in liver cells treated with an RNAi construct of the invention,(b) measuring the amount or level of SCAP protein in liver cells treated with a control RNAi construct (e.g. RNAi construct directed to an RNA molecule not expressed in liver cells or an RNAi construct having a nonsense or scrambled sequence) or no construct, and (c) comparing the measured SCAP protein levels from treated cells in (a) to the measured SCAP protein levels from control cells in (b). Methods of measuring SCAP protein levels are known to those of skill in the art, and include Western Blots, immunoassays (e.g. ELISA), and flow cytometry. Example 3 describes an exemplary method for measuring SCAP mRNA using RNA FISH. Any method capable of measuring SCAP mRNA or protein can be used to assess the efficacy of the RNAi constructs of the invention.

[0053] In some embodiments, the methods to assess SCAP expression levels are performed in vitro in cells that natively express SCAP (e.g. liver cells) or cells that have been engineered to express SCAP. In certain embodiments, the methods are performed in vitro in liver cells. Suitable liver cells include, but are not limited to, primary hepatocytes (e.g. human, non-human primate, or rodent hepatocytes), HepAD38 cells, HuH-6 cells, HuH-7 cells, HuH-5-2 cells, BNLCL2 cells, Hep3B cells, or HepG2 cells.

[0054] In other embodiments, the methods to assess SCAP expression levels are performed in vivo. The RNAi constructs and any control RNAi constructs can be administered to an animal (e.g. rodent or non-human primate) and SCAP mRNA or protein levels assessed in liver tissue harvested from the animal following treatment. Alternatively or additionally, a biomarker or functional phenotype associated with SCAP expression can be assessed in the treated animals.

[0055] In certain embodiments, expression of SCAP is reduced in liver cells by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, or at least 50% by an RNAi construct of the invention. In some embodiments, expression of SCAP is reduced in liver cells by at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, or at least 85% by an RNAi construct of the invention. In other embodiments, the expression of SCAP is reduced in liver cells by about 90% or more, e.g., 91%, 92%, 93%, 94%,

95%, 96%,97%, 98%, 99%, or more by an RNAi construct of the invention. The percent reduction of SCAP expression can be measured by any of the methods described herein as well as others known in the art. For instance, in certain embodiments, the RNAi constructs of the invention inhibit at least 45% of SCAP expression, as described in Examples 2 and 4, in Hep3B cells (contains wild type SCAP) in vitro. In related embodiments, the RNAi constructs of the invention inhibit at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, or at least 75% of SCAP expression in Hep3B cells in vitro, as described in Examples 2 and 4. In other embodiments, the RNAi constructs of the invention inhibit at least 80%, at least 85%, at least 90%, at least 92%, at least 94%, at least 96%, or at least 98% of SCAP expression in Hep3B cells in vitro, as described in Examples 2 and 4. In certain embodiments, the RNAi constructs of the invention inhibit at least 45% of SCAP expression in C57Bl6 mouse livers, as described in the Examples. In related embodiments, the RNAi constructs of the invention inhibit at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, or at least 75% of SCAP expression in C57Bl6 mouse livers, as described in the Examples. In other embodiments, the RNAi constructs of the invention inhibit at least 80%, at least 85%, at least 90%, at least 92%, at least 94%, at least 96%, or at least 98% of SCAP expression in C57Bl6 mouse livers, as described in the Examples. Reduction of SCAP can be measured using a variety of techniques including RNA FISH or droplet digital PCR, as described in Examples 2 and 4, or in vivo studies, as described in Examples 3, 5, 6, 7, and 8.

[0056] In some embodiments, an IC50 value is calculated to assess the potency of an RNAi construct of the invention for inhibiting SCAP expression in liver cells. An "IC50 value" is the dose/concentration required to achieve 50% inhibition of a biological or biochemical function. The IC50 value of any particular substance or antagonist can be determined by constructing a dose-response curve and examining the effect of different concentrations of the substance or antagonist on expression levels or functional activity in any assay. IC50 values can be calculated for a given antagonist or substance by determining the concentration needed to inhibit half of the maximum biological response or native expression levels. Thus, the IC50 value for any RNAi construct can be calculated by determining the concentration of the RNAi construct needed to inhibit half of the native SCAP expression level in liver cells (e.g. SCAP expression level in control liver cells) in any assay, such as the immunoassay or RNA FISH assay or droplet digital PCR assays described in the Examples. The RNAi constructs of the invention may inhibit SCAP expression in liver cells (e.g. Hep3B cells) with an IC50 of less

than about 100 nM. For example, the RNAi constructs inhibit SCAP expression in liver cells with an IC50 of about 0.001 nM to about 100 nM, about 0.001 nM to about 20 nM, about 0.001 nM to about 10 nM, about 0.001 nM to about 5 nM, about 0.001 nM to about 1 nM, about 0.1 nM to about 10 nM, about 0.1 nM to about 5 nM, or about 0.1 nM to about 1 nM. In certain embodiments, the RNAi construct inhibits SCAP expression in liver cells (e.g. Hep3B cells) with an IC50 of about 1 nM to about 10 nM. In certain embodiments, the RNAi construct inhibits SCAP expression in liver cells (e.g. Hep3B cells) with an IC50 of about 0.1 nM to about 5 nM. The RNAi constructs of the invention may inhibit SCAP expression in liver cells (e.g. Hep3B cells) with an IC50 of less than about 20 nM. For example, the RNAi constructs inhibit SCAP expression in liver cells with an IC50 of about 0.001 nM to about 20 nM, about 0.001 nM to about 10 nM, about 0.001 nM to about 5 nM, about 0.001 nM to about 1 nM, about 0.1 nM to about 10 nM, about 0.1 nM to about 5 nM, or about 0.1 nM to about 1 nM. In certain embodiments, the RNAi construct inhibits SCAP expression in liver cells (e.g. Hep3B cells) with an IC50 of about 1 nM to about 10 nM.

[0057] In some embodiments, the RNAi constructs of the invention can have an extended period of SCAP silencing in vivo, such as in ob/ob mice described in Example 8. In some embodiments, an RNAi construct of the invention can silence at least 50%, at least 70%, or at least 80% of SCAP expression at 20 days following administration of the construct in ob/ob mice, as described in Example 8. In some embodiments, an RNAi construct of the invention can silence at least 50%, at least 60%, or at least 70% of SCAP expression at 30 days following administration of the construct in ob/ob mice, as described in Example 8.

[0058] The RNAi constructs of the invention can readily be made using techniques known in the art, for example, using conventional nucleic acid solid phase synthesis. The polynucleotides of the RNAi constructs can be assembled on a suitable nucleic acid synthesizer utilizing standard nucleotide or nucleoside precursors (e.g. phosphoramidites). Automated nucleic acid synthesizers are sold commercially by several vendors, including DNA/RNA synthesizers from Applied Biosystems (Foster City, CA), MerMade synthesizers from BioAutomation (Irving, TX), and OligoPilot synthesizers from GE Healthcare Life Sciences (Pittsburgh, PA).

[0059] The 2' silyl protecting group can be used in conjunction with acid labile dimethoxytrityl (DMT) at the 5' position of ribonucleosides to synthesize oligonucleotides via phosphoramidite chemistry. Final deprotection conditions are known not to significantly

degrade RNA products. All syntheses can be conducted in any automated or manual synthesizer on large, medium, or small scale. The syntheses may also be carried out in multiple well plates, columns, or glass slides.

[0060] The 2'-O-silyl group can be removed via exposure to fluoride ions, which can include any source of fluoride ion, e.g., those salts containing fluoride ion paired with inorganic counterions, e.g., cesium fluoride and potassium fluoride or those salts containing fluoride ion paired with an organic counterion, e.g., a tetraalkylammonium fluoride. A crown ether catalyst can be utilized in combination with the inorganic fluoride in the deprotection reaction. Preferred fluoride ion source are tetrabutylammonium fluoride or aminohydrofluorides (e.g., combining aqueous HF with triethylamine in a dipolar aprotic solvent, e.g., dimethylformamide).

[0061] The choice of protecting groups for use on the phosphite triesters and phosphotriesters can alter the stability of the triesters towards fluoride. Methyl protection of the phosphotriester or phosphitetriester can stabilize the linkage against fluoride ions and improve process yields.

[0062] Since ribonucleosides have a reactive 2' hydroxyl substituent, it can be desirable to protect the reactive 2' position in RNA with a protecting group that is orthogonal to a 5'-O-dimethoxytrityl protecting group, e.g., one stable to treatment with acid. Silyl protecting groups meet this criterion and can be readily removed in a final fluoride deprotection step that can result in minimal RNA degradation.

[0063] Tetrazole catalysts can be used in the standard phosphoramidite coupling reaction. Preferred catalysts include, e.g., tetrazole, S-ethyl-tetrazole, benzylthiotetrazole, pnitrophenyltetrazole.

[0064] As can be appreciated by the skilled artisan, further methods of synthesizing the RNAi constructs described herein will be evident to those of ordinary skill in the art. Additionally, the various synthetic steps may be performed in an alternate sequence or order to give the desired compounds. Other synthetic chemistry transformations, protecting groups (e.g., for hydroxyl, amino, etc. present on the bases) and protecting group methodologies (protection and deprotection) useful in synthesizing the RNAi constructs described herein are known in the art and include, for example, those such as described in R. Larock, Comprehensive Organic Transformations, VCH Publishers (1989); T. W. Greene and P. G. M.

Wuts, Protective Groups in Organic Synthesis, 2d. Ed., John Wiley and Sons (1991); L. Fieser and M. Fieser, Fieser and Fieser's Reagents for Organic Synthesis, John Wiley and Sons (1994); and L. Paquette, ed., Encyclopedia of Reagents for Organic Synthesis, John Wiley and Sons (1995), and subsequent editions thereof Custom synthesis of RNAi constructs is also available from several commercial vendors, including Dharmacon, Inc. (Lafayette, CO), AxoLabs GmbH (Kulmbach, Germany),and Ambion, Inc. (Foster City, CA).

[0065] The RNAi constructs of the invention may comprise a ligand. As used herein, a "ligand" refers to any compound or molecule that is capable of interacting with another compound or molecule, directly or indirectly. The interaction of a ligand with another compound or molecule may elicit a biological response (e.g. initiate a signal transduction cascade, induce receptor mediated endocytosis) or may just be a physical association. The ligand can modify one or more properties of the double-stranded RNA molecule to which is attached, such as the pharmacodynamic, pharmacokinetic, binding, absorption, cellular distribution, cellular uptake, charge and/or clearance properties of the RNA molecule.

[0066] The ligand may comprise a serum protein (e.g., human serum albumin, low-density lipoprotein, globulin), a cholesterol moiety, a vitamin (biotin, vitamin E, vitamin B12), a folate moiety, a steroid, a bile acid (e.g. cholic acid), a fatty acid (e.g., palmitic acid, myristic acid), a carbohydrate (e.g., a dextran, pullulan, chitin, chitosan, inulin, cyclodextrin or hyaluronic acid), a glycoside, a phospholipid, or antibody or binding fragment thereof (e.g. antibody or binding fragment that targets the RNAi construct to a specific cell type, such as liver). Other examples of ligands include dyes, intercalating agents (e.g. acridines), cross-linkers (e.g. psoralene, mitomycin C), porphyrins (TPPC4, texaphyrin, Sapphyrin), polycyclic aromatic hydrocarbons(e.g., phenazine, dihydrophenazine), artificial endonucleases, lipophilic molecules,e.g, adamantane acetic acid, 1-pyrene butyric acid, dihydrotestosterone, 1,3-BisO(hexadecyl)glycerol, geranyloxyhexyl group, hexadecylglycerol, borneol, menthol, 1,3-propanediol, heptadecyl group, 03-(oleoyl)lithocholic acid, 03-(oleoyl)cholenic acid,dimethoxytrityl, or phenoxazine), peptides (e.g., antennapedia peptide, Tat peptide, RGD peptides), alkylating agents, polymers, such as polyethylene glycol (PEG)(e.g., PEG-40K),poly amino acids, and polyamines (e.g. spermine, spermidine).

[0067] In certain embodiments, the ligands have endosomolytic properties. The endosomolytic ligands promote the lysis of the endosome and/or transport of the RNAi construct of the invention, or its components, from the endosome to the cytoplasm of the cell.

The endosomolytic ligand may be a polycationic peptide or peptidomimetic which shows pH dependent membrane activity and fusogenicity. In one embodiment, the endosomolytic ligand assumes its active conformation at endosomal pH. The "active" conformation is that conformation in which the endosomolytic ligand promotes lysis of the endosome and/or transport of the RNAi construct of the invention, or its components, from the endosome to the cytoplasm of the cell. Exemplary endosomolytic ligands include the GALA peptide (Subbarao et al., Biochemistry, Vol. 26: 2964-2972, 1987), the EALA peptide (Vogel et al., J. Am. Chern. Soc.,Vol. 118: 1581-1586, 1996), and their derivatives (Turk et al., Biochem. Biophys. Acta, Vol.1559: 56-68, 2002). In one embodiment, the endosomolytic component may contain a chemical group (e.g., an amino acid) which will undergo a change in charge or protonation in response to a change in pH. The endosomolytic component may be linear or branched.

[0068] In some embodiments, the ligand comprises a lipid or other hydrophobic molecule. In one embodiment, the ligand comprises a cholesterol moiety or other steroid. Cholesterol conjugated oligonucleotides have been reported to be more active than their unconjugated counterparts (Manoharan, Antisense Nucleic Acid Drug Development, Vol. 12: 103-228, 2002). Ligands comprising cholesterol moieties and other lipids for conjugation to nucleic acid molecules have also been described in U.S. Patent Nos. 7,851,615; 7,745,608; and 7,833,992, all of which are hereby incorporated by reference in their entireties. In another embodiment, the ligand comprises a folate moiety. Polynucleotides conjugated to folate moieties can be taken up by cells via a receptor-mediated endocytosis pathway. Such folate-polynucleotide conjugates are described in U.S. Patent No.8, 188,247, which is hereby incorporated by reference in its entirety.

[0069] Given that SCAP is expressed in liver cells (e.g. hepatocytes), in certain embodiments, it is desirable to specifically deliver the RNAi construct to those liver cells. In some embodiments, RNAi constructs can be specifically targeted to the liver by employing ligands that bind to or interact with proteins expressed on the surface of liver cells. For example, in certain embodiments, the ligands may comprise antigen binding proteins (e.g. antibodies or binding fragments thereof (e.g. Fab, scFv)) that specifically bind to a receptor expressed on hepatocytes.

[0070] In certain embodiments, the ligand comprises a carbohydrate. A "carbohydrate" refers to a compound made up of one or more monosaccharide units having at least 6 carbon atoms (which can be linear, branched or cyclic) with an oxygen, nitrogen or sulfur atom bonded

to each carbon atom. Carbohydrates include, but are not limited to, the sugars (e.g., monosaccharides, disaccharides, trisaccharides, tetrasaccharides, and oligosaccharides containing from about 4, 5, 6, 7, 8, or 9 monosaccharide units), and polysaccharides, such as starches, glycogen, cellulose and polysaccharide gums. In some embodiments, the carbohydrate incorporated into the ligand is a monosaccharide selected from a pentose, hexose, or heptose and di- and tri-saccharides including such monosaccharide units. In other embodiments, the carbohydrate incorporated into the ligand is an amino sugar, such as galactosamine, glucosamine, N-acetylgalactosamine, and N-acetylglucosamine.

[0071] In some embodiments, the ligand comprises a hexose or hexosamine. The hexose may be selected from glucose, galactose, mannose, fucose, or fructose. The hexosamine may be selected from fructosamine, galactosamine, glucosamine, or mannosamine. In certain embodiments, the ligand comprises glucose, galactose, galactosamine, or glucosamine. In one embodiment, the ligand comprises glucose, glucosamine, or N-acetylglucosamine. In another embodiment, the ligand comprises galactose, galactosamine, or N-acetyl-galactosamine. In particular embodiments, the ligand comprises N-acetyl-galactosamine. Ligands comprising glucose, galactose, and N-acetyl-galactosamine (GalNAc) are particularly effective in targeting compounds to liver cells. See, e.g., D'Souza and Devarajan, J. Control Release, Vol. 203: 126-139, 2015. Examples of GalNAc- or galactose-containing ligands that can be incorporated into the RNAi constructs of the invention are described in U.S. Patent Nos. 7,491,805; 8,106,022; and 8,877,917; U.S. Patent Publication No. 20030130186; and WIPO Publication No. WO2013166155, all of which are hereby incorporated by reference in their entireties.

[0072] In certain embodiments, the ligand comprises a multivalent carbohydrate moiety. As used herein, a "multivalent carbohydrate moiety" refers to a moiety comprising two or more carbohydrate units capable of independently binding or interacting with other molecules. For example, a multivalent carbohydrate moiety comprises two or more binding domains comprised of carbohydrates that can bind to two or more different molecules or two or more different sites on the same molecule. The valency of the carbohydrate moiety denotes the number of individual binding domains within the carbohydrate moiety. For instance, the terms "monovalent," "bivalent," "trivalent," and "tetravalent" with reference to the carbohydrate moiety refer to carbohydrate moieties with one, two, three, and four binding domains, respectively. The multivalent carbohydrate moiety may comprise a multivalent lactose moiety, a multivalent galactose moiety, a multivalent glucose moiety, a multivalent N-

acetyl-galactosamine moiety, a multivalent N-acetyl-glucosamine moiety, a multivalent mannose moiety, or a multivalent fucose moiety. In some embodiments, the ligand comprises a multivalent galactose moiety. In other embodiments, the ligand comprises a multivalent N-acetyl-galactosamine moiety. In these and other embodiments, the multivalent carbohydrate moiety is bivalent, trivalent, or tetravalent. In such embodiments, the multivalent carbohydrate moiety can be bi-antennary or tri-antennary. In one particular embodiment, the multivalent N-acetyl-galactosamine moiety is trivalent or tetravalent. In another particular embodiment, the multivalent galactose moiety is trivalent or tetravalent. Exemplary trivalent and tetravalent GalNAc-containing ligands for incorporation into the RNAi constructs of the invention are described in detail below.

[0073] The ligand can be attached or conjugated to the RNA molecule of the RNAi construct directly or indirectly. For instance, in some embodiments, the ligand is covalently attached directly to the sense or antisense strand of the RNAi construct. In other embodiments, the ligand is covalently attached via a linker to the sense or antisense strand of the RNAi construct. The ligand can be attached to nucleobases, sugar moieties, or internucleotide linkages of polynucleotides (e.g. sense strand or antisense strand) of the RNAi constructs of the invention. Conjugation or attachment to purine nucleobases or derivatives thereof can occur at any position including, endocyclic and exocyclic atoms. In certain embodiments, the 2-, 6-, 7-, or 8-positionsof a purine nucleobase are attached to a ligand. Conjugation or attachment to pyrimidine nucleobases or derivatives thereof can also occur at any position. In some embodiments, the 2-,5-, and 6-positions of a pyrimidine nucleobase can be attached to a ligand. Conjugation or attachment to sugar moieties of nucleotides can occur at any carbon atom. Example carbon atoms of a sugar moiety that can be attached to a ligand include the 2', 3', and 5' carbon atoms. The 1' position can also be attached to a ligand, such as in a basic residue. Internucleotide linkages can also support ligand attachments. For phosphorus-containing linkages (e.g., phosphodiester, phosphorothioate, phosphorodithiotate, phosphoroamidate, and the like), the ligand can be attached directly to the phosphorus atom or to an O, N, or S atom bound to the phosphorus atom. For amine- or amide-containing internucleoside linkages (e.g., PNA), the ligand can be attached to the nitrogen atom of the amine or amide or to an adjacent carbon atom.

[0074] In certain embodiments, the ligand may be attached to the 3' or 5' end of either the sense or antisense strand. In certain embodiments, the ligand is covalently attached to the

5' end of the sense strand. In other embodiments, the ligand is covalently attached to the 3' end of the sense strand. For example, in some embodiments, the ligand is attached to the 3'-terminal nucleotide of the sense strand. In certain such embodiments, the ligand is attached at the 3'-position of the 3'-terminal nucleotide of the sense strand. In alternative embodiments, the ligand is attached near the 3' end of the sense strand, but before one or more terminal nucleotides (i.e. before 1, 2, 3, or 4 terminal nucleotides). In some embodiments, the ligand is attached at the 2'-position of the sugar of the 3'-terminal nucleotide of the sense strand.

[0075] In certain embodiments, the ligand is attached to the sense or antisense strand via a linker. A "linker" is an atom or group of atoms that covalently joins a ligand to a polynucleotide component of the RNAi construct. The linker may be from about 1 to about 30 atoms in length, from about 2 to about 28 atoms in length, from about 3 to about 26 atoms in length, from about 4to about 24 atoms in length, from about 6 to about 20 atoms in length, from about 7 to about 20atoms in length, from about 8 to about 20 atoms in length, from about 8 to about 18 atoms in length, from about 10 to about 18 atoms in length, and from about 12 to about 18 atoms in length. In some embodiments, the linker may comprise a bifunctional linking moiety, which generally comprises an alkyl moiety with two functional groups. One of the functional groups is selected to bind to the compound of interest (e.g. sense or antisense strand of the RNAi construct) and the other is selected to bind essentially any selected group, such as a ligand as described herein. In certain embodiments, the linker comprises a chain structure or an oligomer of repeating units, such as ethylene glycol or amino acid units. Examples of functional groups that are typically employed in a bifunctional linking moiety include, but are not limited to, electrophiles for reacting with nucleophilic groups and nucleophiles for reacting with electrophilic groups. In some embodiments, bifunctional linking moieties include amino, hydroxyl, carboxylic acid, thiol, unsaturations (e.g., double or triple bonds), and the like.

[0076] Linkers that may be used to attach a ligand to the sense or antisense strand in the RNAi constructs of the invention include, but are not limited to, pyrrolidine, 8-amino-3,6-di oxaoctanoic acid, succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate, 6-aminohexanoic acid, substituted C1-C10 alkyl, substituted or unsubstituted C2-C10 alkenyl or substituted or unsubstituted C2-C10 alkynyl. Preferred substituent groups for such linkers include, but are not limited to, hydroxyl, amino, alkoxy, carboxy, benzyl, phenyl, nitro, thiol, thioalkoxy, halogen, alkyl, aryl, alkenyl and alkynyl.

[0077] In certain embodiments, the linkers are cleavable. A cleavable linker is one which is sufficiently stable outside the cell, but which upon entry into a target cell is cleaved to release the two parts the linker is holding together. In some embodiments, the cleavable linker is cleaved at least 10 times, 20 times, 30 times, 40 times, 50 times, 60 times, 70 times, 80 times, 90 times, or more, or at least 100 times faster in the target cell or under a first reference condition (which can, e.g., be selected to mimic or represent intracellular conditions) than in the blood of a subject, or under a second reference condition (which can, e.g., be selected to mimic or represent conditions found in the blood or serum).

[0078] Cleavable linkers are susceptible to cleavage agents, e.g., pH, redox potential or the presence of degradative molecules. Generally, cleavage agents are more prevalent or found at higher levels or activities inside cells than in serum or blood. Examples of such degradative agents include: redox agents which are selected for particular substrates or which have no substrate specificity, including, e.g., oxidative or reductive enzymes or reductive agents such as mercaptans, present in cells, that can degrade a redox cleavable linker by reduction; esterases; endosomes or agents that can create an acidic environment, e.g., those that result in a pH of five or lower; enzymes that can hydrolyze or degrade an acid cleavable linker by acting as a general acid, peptidases (which can be substrate specific), and phosphatases.

[0079] A cleavable linker may comprise a moiety that is susceptible to pH. The pH of human serum is 7.4, while the average intracellular pH is slightly lower, ranging from about 7.1-7.3. Endosomes have a more acidic pH, in the range of 5.5-6.0, and lysosomes have an even more acidic pH at around 5.0. Some linkers will have a cleavable group that is cleaved at a preferred pH, thereby releasing the RNA molecule from the ligand inside the cell, or into the desired compartment of the cell.

[0080] A linker can include a cleavable group that is cleavable by a particular enzyme. The type of cleavable group incorporated into a linker can depend on the cell to be targeted. For example, liver-targeting ligands can be linked to RNA molecules through a linker that includes an ester group. Liver cells are rich in esterases, and therefore the linker will be cleaved more efficiently in liver cells than in cell types that are not esterase-rich. Other types of cells rich in esterases include cells of the lung, renal cortex, and testis. Linkers that contain peptide bonds can be used when targeting cells rich in peptidases, such as liver cells and synoviocytes.

[0081] In general, the suitability of a candidate cleavable linker can be evaluated by testing the ability of a degradative agent (or condition) to cleave the candidate linker. It will also be desirable to also test the candidate cleavable linker for the ability to resist cleavage in the blood or when in contact with other non-target tissue. Thus, one can determine the relative susceptibility to cleavage between a first and a second condition, where the first is selected to be indicative of cleavage in a target cell and the second is selected to be indicative of cleavage in other tissues or biological fluids, e.g., blood or serum. The evaluations can be carried out in cell free systems, in cells, in cell culture, in organ or tissue culture, or in whole animals. It may be useful to make initial evaluations in cell-free or culture conditions and to confirm by further evaluations in whole animals. In some embodiments, useful candidate linkers are cleaved at least 2, 4, 10, 20, 50, 70, or 100 times faster in the cell (or under in vitro conditions selected to mimic intracellular conditions) as compared to blood or serum (or under in vitro conditions selected to mimic extracellular conditions).

[0082] In other embodiments, redox cleavable linkers are utilized. Redox cleavable linkers are cleaved upon reduction or oxidation. An example of reductively cleavable group is a disulfide linking group (-S-S-). To determine if a candidate cleavable linker is a suitable "reductively cleavable linker," or for example is suitable for use with a particular RNAi construct and particular ligand, one can use one or more methods described herein. For example, a candidate linker can be evaluated by incubation with dithiothreitol (DTT), or other reducing agent known in the art, which mimics the rate of cleavage that would be observed in a cell, e.g., a target cell. The candidate linkers can also be evaluated under conditions which are selected to mimic blood or serum conditions. In a specific embodiment, candidate linkers are cleaved by at most 10% in the blood. In other embodiments, useful candidate linkers are degraded at least 2, 4, 10, 20, 50,70, or 100 times faster in the cell (or under in vitro conditions selected to mimic intracellular conditions) as compared to blood (or under in vitro conditions selected to mimic extracellular conditions).

[0083] In yet other embodiments, phosphate-based cleavable linkers are cleaved by agents that degrade or hydrolyze the phosphate group. An example of an agent that hydrolyzes phosphate groups in cells are enzymes, such as phosphatases in cells. Examples of phosphate-based cleavable groups are -O-P(O)(ORk)-O-, -O-P(S)(ORk)-O-, -O-P(S)(SRk)-O-, -S-P(O)(ORk)-O-, -O-P(O)(ORk)-S-, -S-P(O)(ORk)-S-, -O-P(S)(ORk)-S-, -S-P(S)(ORk)-O-, -O-P(O)(Rk)-O-, -O-P(S)(Rk)-O-, -S-P(O)(Rk)-O-, -S-P(S)(Rk)-O-, -S-P(O)(Rk)-S-, -O-

P(S)(Rk)-S-. Specific embodiments include -O-P(O)(OH)-O-, -O-P(S)(OH)-O-, -O-P(S)(SH)-O-, -S-P(O)(OH)-O-, -O-P(O)(OH)-S-, -S-P(O)(OH)-S-, -O-P(S)(OH)-S-, -SP(S)(OH)-O-, -O-P(O)(H)-O-, -O-P(S)(H)-O-, -S-P(O)(H)-O-, -S-P(S)(H)-O-, -S-P(O)(H)-S-, -O-P(S)(H)-S-. Another specific embodiment is -O-P(O)(OH)-O-. These candidate linkers can be evaluated using methods analogous to those described above.

[0084] In other embodiments, the linkers may comprise acid cleavable groups, which are groups that are cleaved under acidic conditions. In some embodiments, acid cleavable groups are cleaved in an acidic environment with a pH of about 6.5 or lower (e.g., about 6.0, 5.5, 5.0, or lower), or by agents, such as enzymes that can act as a general acid. In a cell, specific low pH organelles, such as endosomes and lysosomes, can provide a cleaving environment for acid cleavable groups. Examples of acid cleavable linking groups include, but are not limited to, hydrazones, esters, and esters of amino acids. Acid cleavable groups can have the general formula -C=NN-, C(O)O, or -OC(O). A specific embodiment is when the carbon attached to the oxygen of the ester (the alkoxy group) is an aryl group, substituted alkyl group, or tertiaryalkyl group such as dimethyl, pentyl or t-butyl. These candidates can be evaluated using methods analogous to those described above.

[0085] In other embodiments, the linkers may comprise ester-based cleavable groups, which are cleaved by enzymes, such as esterases and amidases in cells. Examples of ester-based cleavable groups include, but are not limited to, esters of alkylene, alkenylene and alkynylene groups. Ester cleavable groups have the general formula -C(O)O-, or -OC(O) -. These candidate linkers can be evaluated using methods analogous to those described above.

[0086] In further embodiments, the linkers may comprise peptide-based cleavable groups, which are cleaved by enzymes, such as peptidases and proteases in cells. Peptide-based cleavable groups are peptide bonds formed between amino acids to yield oligopeptides (e.g., dipeptides, tripeptides etc.) and polypeptides. Peptide-based cleavable groups do not include the amide group (-C(O)NH-). The amide group can be formed between any alkylene, alkenylene or alkynelene. A peptide bond is a special type of amide bond formed between amino acids to yield peptides and proteins. The peptide-based cleavage group is generally limited to the peptide bond (i.e., the amide bond) formed between amino acids yielding peptides and proteins and does not include the entire amide functional group. Peptide-based cleavable linking groups have the general formula -NHCHRAC(O)NHCHRBC(O) -, where RA and RB

are the R groups of the two adjacent amino acids. These candidates can be evaluated using methods analogous to those described above.

[0087] Other types of linkers suitable for attaching ligands to the sense or antisense strands in the RNAi constructs of the invention are known in the art and can include the linkers described in U.S. Patent Nos. 7,723,509; 8,017,762; 8,828,956; 8,877,917; and 9,181,551, all of which are hereby incorporated by reference in their entireties.

[0088] In certain embodiments, the ligand covalently attached to the sense or antisense strand of the RNAi constructs of the invention comprises a GalNAc moiety, e.g, a multivalent GalNAc moiety. In some embodiments, the multivalent GalNAc moiety is a trivalent GalNAc moiety and is attached to the 3' end of the sense strand. In other embodiments, the multivalent GalNAc moiety is a trivalent GalNAc moiety and is attached to the 5' end of the sense strand. In yet other embodiments, the multivalent GalNAc moiety is a tetravalent GalNAc moiety and is attached to the 3' end of the sense strand. In still other embodiments, the multivalent GalNAc moiety is a tetravalent GalNAc moiety and is attached to the 5' end of the sense strand. In some embodiments, a GalNAc moiety is attached to the 5’ end of the sense strand of the odd numbered sequences of SEQ ID NOs: 1-159, 161-319, 321-461, or 463-603.

[0089] In some embodiments, the RNAi constructs of the invention may be delivered to a cell or tissue of interest by administering a vector that encodes and controls the intracellular expression of the RNAi construct. A "vector" (also referred to herein as an "expression vector”) is a composition of matter which can be used to deliver a nucleic acid of interest to the interior of a cell. Numerous vectors are known in the art including, but not limited to, linear polynucleotides, polynucleotides associated with ionic or amphiphilic compounds, plasmids, and viruses. Thus, the term "vector" includes an autonomously replicating plasmid or a virus. Examples of viral vectors include, but are not limited to, adenoviral vectors, adeno-associated viral vectors, retroviral vectors, and the like. A vector can be replicated in a living cell, or it can be made synthetically.

[0090] Generally, a vector for expressing an RNAi construct of the invention will comprise one or more promoters operably linked to sequences encoding the RNAi construct. The phrase" operably linked" or "under transcriptional control" as used herein means that the promoter is in the correct location and orientation in relation to a polynucleotide sequence to control the initiation of transcription by RNA polymerase and expression of the polynucleotide

sequence. A "promoter" refers to a sequence recognized by the synthetic machinery of the cell, or introduced synthetic machinery, required to initiate the specific transcription of a gene sequence. Suitable promoters include, but are not limited to, RNA pol I, pol II, HI or U6 RNA pol III, and viral promoters (e.g. human cytomegalovirus (CMV) immediate early gene promoter, the SV40 early promoter, and the Rous sarcoma virus long terminal repeat). In some embodiments, a HI or U6RNA pol III promoter is preferred. The promoter can be a tissue-specific or inducible promoter. Of particular interest are liver-specific promoters, such as promoter sequences from human alpha-1 antitrypsin gene, albumin gene, hemopexin gene, and hepatic lipase gene. Inducible promoters include promoters regulated by ecdysone, estrogen, progesterone, tetracycline, and isopropyl-PD1-thiogalactopyranoside (IPTG).

[0091] In some embodiments in which the RNAi construct comprises a siRNA, the two separate strands (sense and antisense strand) can be expressed from a single vector or two separate vectors. For example, in one embodiment, the sequence encoding the sense strand is operably linked to a promoter on a first vector and the sequence encoding the antisense strand is operably linked to a promoter on a second vector. In such an embodiment, the first and second vectors are co-introduced, e.g., by infection or transfection, into a target cell, such that the sense and antisense strands, once transcribed, will hybridize intracellularly to form the siRNA molecule. In another embodiment, the sense and antisense strands are transcribed from two separate promoters located in a single vector. In some such embodiments, the sequence encoding the sense strand is operably linked to a first promoter and the sequence encoding the antisense strand is operably linked to a second promoter, wherein the first and second promoters are located in a single vector. In one embodiment, the vector comprises a first promoter operably linked to a sequence encoding the siRNA molecule, and a second promoter operably linked to the same sequence in the opposite direction, such that transcription of the sequence from the first promoter results in the synthesis of the sense strand of the siRNA molecule and transcription of the sequence from the second promoter results in synthesis of the antisense strand of the siRNA molecule.

[0092] In other embodiments in which the RNAi construct comprises a shRNA, a sequence encoding the single, at least partially self-complementary RNA molecule is operably linked to a promoter to produce a single transcript. In some embodiments, the sequence encoding the shRNA comprises an inverted repeat joined by a linker polynucleotide sequence to produce the stem and loop structure of the shRNA following transcription.

[0093] In some embodiments, the vector encoding an RNAi construct of the invention is a viral vector. Various viral vector systems that are suitable to express the RNAi constructs described herein include, but are not limited to, adenoviral vectors, retroviral vectors (e.g., lentiviral vectors, maloney murine leukemia virus), adeno- associated viral vectors; herpes simplex viral vectors; SV 40 vectors; polyoma viral vectors; papilloma viral vectors; picornaviral vectors; and pox viral vectors (e.g. vaccinia virus). In certain embodiments, the viral vector is a retroviral vector (e.g. lentiviral vector).

[0094] Various vectors suitable for use in the invention, methods for inserting nucleic acid sequences encoding siRNA or shRNA molecules into vectors, and methods of delivering the vectors to the cells of interest are within the skill of those in the art. See, e.g., Dornburg , Gene Therap., Vol. 2: 301-310, 1995; Eglitis, Biotechniques, Vol. 6: 608-614, 1988; Miller, HumGene Therap., Vol. 1: 5-14, 1990; Anderson, Nature, Vol. 392: 25-30, 1998; Rubinson D A et al., Nat. Genet., Vol. 33: 401-406, 2003; Brummelkamp et al., Science, Vol. 296: 550-553, 2002;Brummelkamp et al., Cancer Cell, Vol. 2: 243-247, 2002; Lee et al., Nat Biotechnol, Vol. 20:500-505, 2002; Miyagishi et al., Nat Biotechnol, Vol. 20: 497-500, 2002; Paddison et al., GenesDev, Vol. 16: 948-958, 2002; Paul et al., Nat Biotechnol, Vol. 20: 505-508, 2002; Sui et al., ProcNatl Acad Sci USA, Vol. 99: 5515-5520, 2002; and Yu et al., Proc Natl Acad Sci USA, Vol. 99:6047-6052, 2002, all of which are hereby incorporated by reference in their entireties.

[0095] The present invention also includes pharmaceutical compositions and formulations comprising the RNAi constructs described herein and pharmaceutically acceptable carriers, excipients, or diluents. Such compositions and formulations are useful for reducing expression of SCAP in a subject in need thereof. Where clinical applications are contemplated, pharmaceutical compositions and formulations will be prepared in a form appropriate for the intended application. Generally, this will entail preparing compositions that are essentially free of pyrogens, as well as other impurities that could be harmful to humans or animals.

[0096] The phrases "pharmaceutically acceptable" or "pharmacologically acceptable" refer to molecular entities and compositions that do not produce adverse, allergic, or other untoward reactions when administered to an animal or a human. As used herein, "pharmaceutically acceptable carrier, excipient, or diluent" includes solvents, buffers, solutions, dispersion media, coatings, antibacterial and antifungal agents, isotonic and

absorption delaying agents and the like acceptable for use in formulating pharmaceuticals, such as pharmaceuticals suitable for administration to humans. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the RNAi constructs of the present invention, its use in therapeutic compositions is contemplated. Supplementary active ingredients also can be incorporated into the compositions, provided they do not inactivate the vectors or RNAi constructs of the compositions.

[0097] Compositions and methods for the formulation of pharmaceutical compositions depend on a number of criteria, including, but not limited to, route of administration, type and extent of disease or disorder to be treated, or dose to be administered. In some embodiments, the pharmaceutical compositions are formulated based on the intended route of delivery. For instance, in certain embodiments, the pharmaceutical compositions are formulated for parenteral delivery. Parenteral forms of delivery include intravenous, intraarterial, subcutaneous, intrathecal, intraperitoneal or intramuscular injection or infusion. In one embodiment, the pharmaceutical composition is formulated for intravenous delivery. In such an embodiment, the pharmaceutical composition may include a lipid-based delivery vehicle. In another embodiment, the pharmaceutical composition is formulated for subcutaneous delivery. In such an embodiment, the pharmaceutical composition may include a targeting ligand (e.g. GalNAc containing ligands described herein).

[0098] In some embodiments, the pharmaceutical compositions comprise an effective amount of an RNAi construct described herein. An "effective amount" is an amount sufficient to produce a beneficial or desired clinical result. In some embodiments, an effective amount is an amount sufficient to reduce SCAP expression in hepatocytes of a subject. In some embodiments, an effective amount may be an amount sufficient to only partially reduce SCAP expression, for example, to a level comparable to expression of the wild-type SCAP allele in human heterozygotes.

[0099] An effective amount of an RNAi construct of the invention may be from about 0.01mg/kg body weight to about 100 mg/kg body weight, about 0.05 mg/kg body weight to about 75mg/kg body weight, about 0.1 mg/kg body weight to about 50 mg/kg body weight, about 1mg/kg to about 30 mg/kg body weight, about 2.5 mg/kg of body weight to about 20 mg/kg bodyweight, or about 5 mg/kg body weight to about 15 mg/kg body weight. In certain embodiments, a single effective dose of an RNAi construct of the invention may be about 0.1

mg/kg, about 0.5mg/kg, about 1 mg/kg, about 2 mg/kg, about 3 mg/kg, about 4 mg/kg, about 5 mg/kg, about 6mg/kg, about 7 mg/kg, about 8 mg/kg, about 9 mg/kg, or about 10 mg/kg. The pharmaceutical composition comprising an effective amount of RNAi construct can be administered weekly, biweekly, monthly, quarterly, or biannually. The precise determination of what would be considered an effective amount and frequency of administration may be based on several factors, including a patient's size, age, and general condition, type of disorder to be treated (e.g. myocardial infarction, heart failure, coronary artery disease, hypercholesterolemia), particular RNAi construct employed, and route of administration. Estimates of effective dosages and in vivo half-lives for any particular RNAi construct of the invention can be ascertained using conventional methods and/or testing in appropriate animal models.

[0100] Administration of the pharmaceutical compositions of the present invention may be via any common route so long as the target tissue is available via that route. Such routes include, but are not limited to, parenteral (e.g., subcutaneous, intramuscular, intraperitoneal or intravenous), oral, nasal, buccal, intradermal, transdermal, and sublingual routes, or by direct injection into liver tissue or delivery through the hepatic portal vein. In some embodiments, the pharmaceutical composition is administered parenterally. For instance, in certain embodiments, the pharmaceutical composition is administered intravenously. In other embodiments, the pharmaceutical composition is administered subcutaneously.

[0101] Colloidal dispersion systems, such as macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems, including oil-in-water emulsions, micelles, mixed micelles, and liposomes, may be used as delivery vehicles for the RNAi constructs of the invention or vectors encoding such constructs. Commercially available fat emulsions that are suitable for delivering the nucleic acids of the invention include Intralipid®, Liposyn®, Liposyn®II, Liposyn®III, Nutrilipid, and other similar lipid emulsions. A preferred colloidal system for use as a delivery vehicle in vivo is a liposome (i.e., an artificial membrane vesicle). The RNAi constructs of the invention may be encapsulated within liposomes or may form complexes thereto, in particular to cationic liposomes. Alternatively, RNAi constructs of the invention may be complexed to lipids, in particular to cationic lipids. Suitable lipids and liposomes include neutral (e.g., dioleoylphosphatidyl ethanolamine (DOPE), dimyristoylphosphatidyl choline (DMPC), and dipalmitoyl phosphatidylcholine (DPPC)), distearolyphosphatidyl choline), negative (e.g., dimyristoylphosphatidyl glycerol (DMPG)),

andcationic (e.g., dioleoyltetramethylaminopropyl (DOTAP) and dioleoylphosphatidyl ethanolamine (DOTMA)). The preparation and use of such colloidal dispersion systems is well known in the art. Exemplary formulations are also disclosed in U.S. Pat. No. 5,981,505; U.S. Pat. No.6,217,900; U.S. Pat. No. 6,383,512; U.S. Pat. No. 5,783,565; U.S. Pat. No. 7,202,227; U.S. Pat. No. 6,379,965; U.S. Pat. No. 6,127,170; U.S. Pat. No. 5,837,533; U.S. Pat. No. 6,747,014; andW003/093449.

[0102] In some embodiments, the RNAi constructs of the invention are fully encapsulated in a lipid formulation, e.g., to form a SPLP, pSPLP, SNALP, or other nucleic acid-lipid particle. As used herein, the term "SNALP" refers to a stable nucleic acid-lipid particle, including SPLP. As used herein, the term "SPLP" refers to a nucleic acid-lipid particle comprising plasmid DNA encapsulated within a lipid vesicle. SNALPs and SPLPs typically contain a cationic lipid, a noncationic lipid, and a lipid that prevents aggregation of the particle (e.g., a PEG-lipid conjugate). SNALPs and SPLPs are exceptionally useful for systemic applications, as they exhibit extended circulation lifetimes following intravenous injection and accumulate at distal sites (e.g., sites physically separated from the administration site). SPLPs include "pSPLP," which include an encapsulated condensing agent-nucleic acid complex as set forth in PCT Publication No. WO00/03683. The nucleic acid-lipid particles typically have a mean diameter of about 50 nm to about 150 nm, about 60 nm to about 130 nm, about 70 nm to about 110 nm, or about 70 nm to about 90 nm, and are substantially nontoxic. In addition, the nucleic acids when present in the nucleic acid-lipid particles are resistant in aqueous solution to degradation with a nuclease. Nucleic acid-lipid particles and their method of preparation are disclosed in, e.g., U.S. Patent Nos. 5,976,567; 5,981,501; 6,534,484; 6,586,410; 6,815,432; and PCT Publication No. WO96/40964.

[0103] The pharmaceutical compositions suitable for injectable use include, for example, sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. Generally, these preparations are sterile and fluid to the extent that easy injectability exists. Preparations should be stable under the conditions of manufacture and storage and should be preserved against the contaminating action of microorganisms, such as bacteria and fungi. Appropriate solvents or dispersion media may contain, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the

maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial an antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

[0104] Sterile injectable solutions may be prepared by incorporating the active compounds in an appropriate amount into a solvent along with any other ingredients (for example as enumerated above) as desired, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the desired other ingredients, e.g., as enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation include vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient(s) plus any additional desired ingredient from a previously sterile-filtered solution thereof

[0105] The compositions of the present invention generally may be formulated in a neutral or salt form. Pharmaceutically-acceptable salts include, for example, acid addition salts (formed with free amino groups) derived from inorganic acids (e.g., hydrochloric or phosphoric acids), or from organic acids (e.g., acetic, oxalic, tartaric, mandelic, and the like). Salts formed with the free carboxyl groups can also be derived from inorganic bases (e.g., sodium, potassium, ammonium, calcium, or ferric hydroxides) or from organic bases (e.g., isopropylamine, trimethylamine, histidine, procaine and the like).

[0106] For parenteral administration in an aqueous solution, for example, the solution generally is suitably buffered and the liquid diluent first rendered isotonic for example with sufficient saline or glucose. Such aqueous solutions may be used, for example, for intravenous, intramuscular, subcutaneous and intraperitoneal administration. Preferably, sterile aqueous media are employed as is known to those of skill in the art, particularly in light of the present disclosure. By way of illustration, a single dose may be dissolved in 1 ml of isotonic NaCl solution and either added to 1000 ml of hypodermoclysis fluid or injected at the proposed site of infusion, (see for example, "Remington's Pharmaceutical Sciences" 15th Edition, pages 1035-1038 and 1570-1580). For human administration, preparations should meet sterility,

pyrogenicity, general safety and purity standards as required by FDA standards. In certain embodiments, a pharmaceutical composition of the invention comprises or consists of a sterile saline solution and an RNAi construct described herein. In other embodiments, a pharmaceutical composition of the invention comprises or consists of an RNAi construct described herein and sterile water (e.g. water for injection, WFI). In still other embodiments, a pharmaceutical composition of the invention comprises or consists of an RNAi construct described herein and phosphate-buffered saline (PBS).

[0107] In some embodiments, the pharmaceutical compositions of the invention are packaged with or stored within a device for administration. Devices for injectable formulations include, but are not limited to, injection ports, pre-filled syringes, auto injectors, injection pumps, on-body injectors, and injection pens. Devices for aerosolized or powder formulations include, but are not limited to, inhalers, insufflators, aspirators, and the like. Thus, the present invention includes administration devices comprising a pharmaceutical composition of the invention for treating or preventing one or more of the disorders described herein.

Methods for inhibiting SCAP expression

[0108] The present invention also provides methods of inhibiting expression of a SCAP gene in a cell. The methods include contacting a cell with an RNAi construct, e.g., double stranded RNAi construct, in an amount effective to inhibit expression of SCAP in the cell, thereby inhibiting expression of SCAP in the cell. Contacting of a cell with an RNAi construct, e.g., a double stranded RNAi construct, may be done in vitro or in vivo. Contacting a cell in vivo with the RNAi construct includes contacting a cell or group of cells within a subject, e.g., a human subject, with the RNAi construct. Combinations of in vitro and in vivo methods of contacting a cell are also possible.

[0109] The present invention provides methods for reducing or inhibiting expression of SCAP in a subject in need thereof as well as methods of treating or preventing conditions, diseases, or disorders associated with SCAP expression or activity. A "condition, disease, or disorder associated with SCAP expression" refers to conditions, diseases, or disorders in which SCAP expression levels are altered or where elevated expression levels of SCAP are associated with an increased risk of developing the condition, disease or disorder.

[0110] Contacting a cell may be direct or indirect, as discussed above. Furthermore, contacting a cell may be accomplished via a targeting ligand, including any ligand described

herein or known in the art. In preferred embodiments, the targeting ligand is a carbohydrate moiety, e.g., a GalNAc ligand, or a trivalent GalNAc moiety, or any other ligand that directs the RNAi construct to a site of interest.

[0111] In one embodiment, contacting a cell with an RNAi construct includes "introducing" or "delivering the RNAi construct into the cell" by facilitating or effecting uptake or absorption into the cell. Absorption or uptake of an RNAi construct can occur through unaided diffusive or active cellular processes, or by auxiliary agents or devices. Introducing an RNAi construct into a cell may be in vitro and/or in vivo. For example, for in vivo introduction, RNAi constructs can be injected into a tissue site or administered systemically. In vitro introduction into a cell includes methods known in the art such as electroporation and lipofection. Further approaches are described herein below and/or are known in the art.

[0112] The term "inhibiting," as used herein, is used interchangeably with "reducing," "silencing," "downregulating", "suppressing", and other similar terms, and includes any level of inhibition.

[0113] The phrase "inhibiting expression of a SCAP" is intended to refer to inhibition of expression of any SCAP gene (such as, e.g., a mouse SCAP gene, a rat SCAP gene, a monkey SCAP gene, or a human SCAP gene) as well as variants or mutants of a SCAP gene. Thus, the SCAP gene may be a wild-type SCAP gene, a mutant SCAP gene (such as a mutant SCAP gene giving rise to triglyceride deposition), or a transgenic SCAP gene in the context of a genetically manipulated cell, group of cells, or organism.

[0114] "Inhibiting expression of a SCAP gene" includes any level of inhibition of a SCAP gene, e.g., at least partial suppression of the expression of a SCAP gene. The expression of the SCAP gene may be assessed based on the level, or the change in the level, of any variable associated with SCAP gene expression, e.g., SCAP mRNA level, SCAP protein level, or the number or extent of triglyceride deposits. This level may be assessed in an individual cell or in a group of cells, including, for example, a sample derived from a subject.

[0115] Inhibition may be assessed by a decrease in an absolute or relative level of one or more variables that are associated with SCAP expression compared with a control level. The control level may be any type of control level that is utilized in the art, e.g., a pre-dose baseline level, or a level determined from a similar subject, cell, or sample that is untreated or treated with a control (such as, e.g., buffer only control or inactive agent control). In some

embodiments of the methods of the invention, expression of a SCAP gene is inhibited by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%,at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%. at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%.

[0116] Inhibition of the expression of a SCAP gene may be manifested by a reduction of the amount of mRNA expressed by a first cell or group of cells (such cells may be present, for example, in a sample derived from a subject) in which a SCAP gene is transcribed and which has or have been treated (e.g., by contacting the cell or cells with an RNAi construct of the invention, or by administering an RNAi construct of the invention to a subject in which the cells are or were present) such that the expression of a SCAP gene is inhibited, as compared to a second cell or group of cells substantially identical to the first cell or group of cells but which has not or have not been so treated (control cell(s)). In preferred embodiments, the inhibition is assessed by expressing the level of mRNA in treated cells as a percentage of the level of mRNA in control cells, using the following formula:

$$\frac{(\text{mRNA in control cells}) - (\text{mRNA in treated cells})}{(\text{mRNA in control cells})} \cdot 100\%$$

[0117] Alternatively, inhibition of the expression of a SCAP gene may be assessed in terms of a reduction of a parameter that is functionally linked to SCAP gene expression, e.g. , SCAP protein expression or SREBP pathway protein activities. SCAP gene silencing may be determined in any cell expressing SCAP, either constitutively or by genomic engineering, and by any assay known in the art.

[0118] Inhibition of the expression of a SCAP protein may be manifested by a reduction in the level of the SCAP protein that is expressed by a cell or group of cells (e.g., the level of protein expressed in a sample derived from a subject). As explained above, for the assessment of mRNA suppression, the inhibiton of protein expression levels in a treated cell or group of

cells may similarly be expressed as a percentage of the level of protein in a control cell or group of cells.

[0119] A control cell or group of cells that may be used to assess the inhibition of the expression of a SCAP gene includes a cell or group of cells that has not yet been contacted with an RNAi construct of the invention. For example, the control cell or group of cells may be derived from an individual subject (e.g., a human or animal subject) prior to treatment of the subject with an RNAi construct.

[0120] The level of SCAP mRNA that is expressed by a cell or group of cells, or the level of circulating SCAP mRNA, may be determined using any method known in the art for assessing mRNA expression. In one embodiment, the level of expression of SCAP in a sample is determined by detecting a transcribed polynucleotide, or portion thereof, e.g., mRNA of the SCAP gene. RNA may be extracted from cells using RNA extraction techniques including, for example, using acid phenol/guanidine isothiocyanate extraction (RNAzol B; Biogenesis), RNeasy RNA preparation kits (Qiagen) or PAXgene (PreAnalytix, Switzerland). Typical assay formats utilizing ribonucleic acid hybridization include nuclear run-on assays, RT-PCR, RNase protection assays (Melton et al., Nuc. Acids Res. 12:7035), Northern blotting, in situ hybridization, and microarray analysis. Circulating SCAP mRNA may be detected using methods the described in PCT/US2012/043584, the entire contents of which are hereby incorporated herein by reference.

[0121] In one embodiment, the level of expression of SCAP is determined using a nucleic acid probe. The term "probe", as used herein, refers to any molecule that is capable of selectively binding to a specific SCAP. Probes can be synthesized by one of skill in the art, or derived from appropriate biological preparations. Probes may be specifically designed to be labeled. Examples of molecules that can be utilized as probes include, but are not limited to, RNA, DNA, proteins, antibodies, and organic molecules.

[0122] Isolated mRNA can be used in hybridization or amplification assays that include, but are not limited to, Northern analyses, polymerase chain reaction (PCR) analyses and probe arrays. One method for the determination of mRNA levels involves contacting the isolated mRNA with a nucleic acid molecule (probe) that can hybridize to SCAP mRNA. In one embodiment, the mRNA is immobilized on a solid surface and contacted with a probe, for example by running the isolated mRNA on an agarose gel and transferring the mRNA from the

gel to a membrane, such as nitrocellulose. In an alternative embodiment, the probe(s) are immobilized on a solid surface and the mRNA is contacted with the probe(s), for example, in an Affymetrix gene chip array. A skilled artisan can readily adapt known mRNA detection methods for use in determining the level of SCAP mRNA.

[0123] An alternative method for determining the level of expression of SCAP in a sample involves the process of nucleic acid amplification and/or reverse transcriptase (to prepare cDNA) of for example mRNA in the sample, e.g., by RT-PCR (the experimental embodiment set forth in Mullis, 1987, U.S. Pat. No. 4,683,202), ligase chain reaction (Barany (1991) Proc. Natl. Acad. Sci. USA 88: 189-193), self sustained sequence replication (Guatelli et al. (1990) Proc. Natl. Acad. Sci. USA 87: 1874-1878), transcriptional amplification system (Kwoh et al. (1989) Proc. Natl. Acad. Sci. USA 86: 1173-1177), Q-Beta Replicase (Lizardi et al. (1988) Bio/Technology 6: 1197), rolling circle replication (Lizardi et al., U.S. Pat. No. 5,854,033) or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers. In particular aspects of the invention, the level of expression of SCAP is determined by quantitative fluorogenic RT-PCR (i.e., the TaqMan™ System). The expression levels of SCAP mRNA may be monitored using a membrane blot (such as used in hybridization analysis such as Northern, , dot, and the like), or microwells, sample tubes, gels, beads or fibers (or any solid support comprising bound nucleic acids). See U.S. Pat. Nos. 5,770,722, 5,874,219, 5,744,305, 5,677,195 and 5,445,934, which are incorporated herein by reference. The determination of SCAP expression level may also comprise using nucleic acid probes in solution.

[0124] In preferred embodiments, the level of mRNA expression is assessed using branched DNA (bDNA) assays or real time PCR (qPCR). The use of these methods is described and exemplified in the Examples presented herein.

[0125] The level of SCAP protein expression may be determined using any method known in the art for the measurement of protein levels. Such methods include, for example, electrophoresis, capillary electrophoresis, high performance liquid chromatography (HPLC), thin layer chromatography (TLC), hyperdiffusion chromatography, fluid or gel precipitin reactions, absorption spectroscopy, a colorimetric assays, spectrophotometric assays, flow cytometry, immunodiffusion (single or double), Immunoelectrophoresis, Western blotting,

radioimmunoassay (RIA), enzyme-linked immunosorbent assays (ELISAs), immunofluorescent assays, electrochemiluminescence assays, and the like.

[0126] In some embodiments, the efficacy of the methods of the invention can be monitored by detecting or monitoring a reduction in a symptom of a SCAP disease, such as reduction in edema swelling of the extremities, face, larynx, upper respiratory tract, abdomen, trunk, and genitals, prodrome; laryngeal swelling; nonpruritic rash; nausea; vomiting; or abdominal pain. These symptoms may be assessed in vitro or in vivo using any method known in the art.

[0127] In some embodiments of the methods of the invention, the RNAi construct is administered to a subject such that the RNAi construct is delivered to a specific site within the subject. The inhibition of expression of SCAP may be assessed using measurements of the level or change in the level of SCAP mRNA or SCAP protein in a sample derived from fluid or tissue from the specific site within the subject. In preferred embodiments, the site is selected from the group consisting of liver, choroid plexus, retina, and pancreas. The site may also be a subsection or subgroup of cells from any one of the aforementioned sites. The site may also include cells that express a particular type of receptor.

Methods of treating or preventing SCAP-Associated Diseases

[0128] The present invention provides therapeutic and prophylactic methods which include administering to a subject with a SCAP -associated disease, disorder, and/or condition, or prone to developing, a SCAP- associated disease, disorder, and/or condition, compositions comprising an RNAi construct, or pharmaceutical compositions comprising an RNAi construct, or vectors comprising an RNAi construct of the invention. Non-limiting examples of SCAP- associated diseases include, for example, 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). In one embodiment, the SCAP-associated disease is NAFLD. In another embodiment, the SCAP -associated disease is NASH. In another embodiment, the SCAP -associated disease is fatty liver (steatosis). In another embodiment, the SCAP-associated disease is insulin resistance. In another embodiment, the SCAP-associated disease is not insulin resistance. In some embodiments, SCAP RNAi can be used to treat hepatocellular carcinoma. Increase in SREBP activity has been documented in human HCC samples and

evidence points to a causal role in HCC growth. SCAP RNAi (eg. siRNA) treatment in rodent models of HCC (eg. xenograft implantation of HCC cells or hepatic expression of oncogenes) can lead to a reduction in hepatic tumor burden (ie tumor volume).

[0129] In certain embodiments, the present invention provides a method for reducing the expression of SCAP in a patient in need thereof comprising administering to the patient any of the RNAi constructs described herein. The term "patient," as used herein, refers to a mammal, including humans, and can be used interchangeably with the term "subject." Preferably, the expression level of SCAP in hepatocytes in the patient is reduced following administration of the RNAi construct as compared to the SCAP expression level in a patient not receiving the RNAi construct.

[0130] The methods of the invention are useful for treating a subject having a SCAP-associated disease, e.g., a subject that would benefit from reduction in SCAP gene expression and/or SCAP protein production. In one aspect, the present invention provides methods of reducing the level of SREBP Cleavage Activating Protein (SCAP) gene expression in a subject having nonalcoholic fatty liver disease (NAFLD). In another aspect, the present invention provides methods of reducing the level of SCAP protein in a subject with NAFLD. The present invention also provides methods of reducing the level of activity of the hedgehog pathway in a subject with NAFLD.

[0131] In another aspect, the present invention provides methods of treating a subject having an NAFLD. In one aspect, the present invention provides methods of treating a subject having an SCAP-associated disease, e.g., fatty liver (steatosis), nonalcoholic steatohepatitis (NASH), cirrhosis of the liver, accumulation of fat in the liver, inflammation of the liver, hepatocellular necrosis, liver fibrosis, obesity, hepatocellular carcinoma, myocardial infarction, heart failure, coronary artery disease, hypercholesterolemia, or nonalcoholic fatty liver disease (NAFLD). The treatment methods (and uses) of the invention include administering to the subject, e.g., a human, a therapeutically effective amount of an RNAi construct of the invention targeting a SCAP gene or a pharmaceutical composition comprising an RNAi construct of the invention targeting a SCAP gene or a vector of the invention comprising an RNAi construct targeting an SCAP gene.

[0132] In one aspect, the invention provides methods of preventing at least one symptom in a subject having NAFLD, e.g., the presence of elevated signaling pathways,

fatigue, weakness, weight loss, loss of apetite, nausea, abdominal pain, spider-like blood vessels, yellowing of the skin and eyes (jaundice), itching, fluid build up and swelling of the legs (edema), abdomen swelling (ascites), and mental confusion. The methods include administering to the subject a therapeutically effective amount of the RNAi construct, e.g. dsRNA, pharmaceutical compositions, or vectors of the invention, thereby preventing at least one symptom in the subject having a disorder that would benefit from reduction in SCAP gene expression.

[0133] In another aspect, the present invention provides uses of a therapeutically effective amount of an RNAi construct of the invention for treating a subject, e.g., a subject that would benefit from a reduction and/or inhibition of SCAP gene expression. In a further aspect, the present invention provides uses of an RNAi construct, e.g., a dsRNA, of the invention targeting an SCAP gene or pharmaceutical composition comprising an RNAi construct targeting an SCAP gene in the manufacture of a medicament for treating a subject, e.g., a subject that would benefit from a reduction and/or inhibition of SCAP gene expression and/or SCAP protein production, such as a subject having a disorder that would benefit from reduction in SCAP gene expression, e.g., a SCAP- associated disease.

[0134] In another aspect, the invention provides uses of an RNAi, e.g., a dsRNA, of the invention for preventing at least one symptom in a subject suffering from a disorder that would benefit from a reduction and/or inhibition of SCAP gene expression and/or SCAP protein production.

[0135] In a further aspect, the present invention provides uses of an RNAi construct of the invention in the manufacture of a medicament for preventing at least one symptom in a subject suffering from a disorder that would benefit from a reduction and/or inhibition of SCAP gene expression and/or SCAP protein production, such as a SCAP-associated disease.

[0136] In one embodiment, an RNAi construct targeting SCAP is administered to a subject having a SCAP-associated disease, e.g., nonalcoholic fatty liver disease (NAFLD), such that the expression of a SCAP gene, e.g. , in a cell, tissue, blood or other tissue or fluid of the subject are reduced by at least about 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 62%, 64%, 65%, 66%,

67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91 %, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least about 99% or more when the dsRNA agent is administered to the subject.

[0137] The methods and uses of the invention include administering a composition described herein such that expression of the target SCAP gene is decreased, such as for about 1, 2, 3, 4 5, 6, 7, 8, 12, 16, 18, 24, 28, 32, 36, 40, 44, 48, 52, 56, 60, 64, 68, 72, 76, or about 80 hours. In one embodiment, expression of the target SCAP gene is decreased for an extended duration, e.g., at least about two, three, four, five, six, seven days or more, e.g., about one week, two weeks, three weeks, or about four weeks or longer.

[0138] Administration of the RNAi construct according to the methods and uses of the invention may result in a reduction of the severity, signs, symptoms, and/or markers of such diseases or disorders in a patient with a SCAP- associated disease, e.g., nonalcoholic fatty liver disease (NAFLD). By "reduction" in this context is meant a statistically significant decrease in such level. The reduction can be, for example, at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or about 100%. Efficacy of treatment or prevention of disease can be assessed, for example by measuring disease progression, disease remission, symptom severity, reduction in pain, quality of life, dose of a medication required to sustain a treatment effect, level of a disease marker or any other measurable parameter appropriate for a given disease being treated or targeted for prevention. It is well within the ability of one skilled in the art to monitor efficacy of treatment or prevention by measuring any one of such parameters, or any combination of parameters. For example, efficacy of treatment of NAFLD may be assessed, for example, by periodic monitoring of NAFLD symptoms, liver fat levels, or expression of downstream genes. Comparison of the later readings with the initial readings provide a physician an indication of whether the treatment is effective. It is well within the ability of one skilled in the art to monitor efficacy of treatment or prevention by measuring any one of such parameters, or any combination of parameters. In connection with the administration of an RNAi construct targeting SCAP or pharmaceutical composition thereof, "effective against" an SCAP - associated disease indicates that administration in a clinically appropriate manner results in a beneficial effect for at least a statistically significant fraction of patients, such as improvement of symptoms, a cure, a reduction in disease, extension of life, improvement in quality of life,

or other effect generally recognized as positive by medical doctors familiar with treating NAFLD and/or an SCAP -associated disease and the related causes.

[0139] A treatment or preventive effect is evident when there is a statistically significant improvement in one or more parameters of disease status, or by a failure to worsen or to develop symptoms where they would otherwise be anticipated. As an example, a favorable change of at least 10% in a measurable parameter of disease, and preferably at least 20%, 30%, 40%, 50% or more can be indicative of effective treatment. Efficacy for a given RNAi drug or formulation of that drug can also be judged using an experimental animal model for the given disease as known in the art. When using an experimental animal model, efficacy of treatment is evidenced when a statistically significant reduction in a marker or symptom is observed.

[0140] Administration of the RNAi construct can reduce the presence of SCAP protein levels, e.g. , in a cell, tissue, blood, urine or other compartment of the patient by at least about 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31 %, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least about 99% or more.

[0141] Before administration of a full dose of the RNAi construct, patients can be administered a smaller dose, such as a 5% infusion, and monitored for adverse effects, such as an allergic reaction. In another example, the patient can be monitored for unwanted immunostimulatory effects, such as increased cytokine (e.g. , TNF-alpha or IFN-alpha) levels.

[0142] Owing to the inhibitory effects on SCAP expression, a composition according to the invention or a pharmaceutical composition prepared therefrom can enhance the quality of life.

[0143] An RNAi construct of the invention may be administered in "naked" form, where the modified or unmodified RNAi construct is directly suspended in aqueous or suitable buffer solvent, as a "free RNAi." A free RNAi is administered in the absence of a pharmaceutical composition.

[0144] An RNAi may be in a pharmaceutical composition with a suitable buffer solution. The buffer solution may comprise acetate, citrate, prolamine, carbonate, or phosphate, or any combination thereof. In one embodiment, the buffer solution is phosphate buffered saline (PBS). The pH and osmolality of the buffer solution containing the RNAi construct can be adjusted such that it is suitable for administering to a subject.

[0145] Alternatively, an RNAi construct of the invention may be administered as a pharmaceutical composition, such as a RNAi construct liposomal formulation.

[0146] Subjects that would benefit from a reduction and/or inhibition of SCAP gene expression are those having nonalcoholic fatty liver disease (NAFLD) and/or an SCAP-associated disease or disorder as described herein.

[0147] Treatment of a subject that would benefit from a reduction and/or inhibition of SCAP gene expression includes therapeutic and prophylactic treatment.

[0148] The invention further provides methods and uses of an RNAi construct or a pharmaceutical composition thereof for treating a subject that would benefit from reduction and/or inhibition of SCAP gene expression, e.g., a subject having a SCAP-associated disease, in combination with other pharmaceuticals and/or other therapeutic methods, e.g. , with known pharmaceuticals and/or known therapeutic methods, such as, for example, those which are currently employed for treating these disorders.

[0149] For example, in certain embodiments, an RNAi construct targeting a SCAP gene is administered in combination with, e.g., an agent useful in treating an SCAP-associated disease as described elsewhere herein. For example, additional therapeutics and therapeutic methods suitable for treating a subject that would benefit from reduction in SCAP expression, e.g., a subject having a SCAP-associated disease, include an RNAi construct targeting a different portion of the SCAP gene, a therapeutic agent, and/or procedures for treating a SCAP -associated disease or a combination of any of the foregoing.

[0150] In certain embodiments, a first RNAi construct targeting a SCAP gene is administered in combination with a second RNAi construct targeting a different portion of the SCAP gene. For example, the first RNAi construct comprises a first sense strand and a first antisense strand forming a double stranded region, wherein substantially all of the nucleotides of said first sense strand and substantially all of the nucleotides of the first antisense strand are modified nucleotides, wherein said first sense strand is conjugated to a ligand attached at the

3'- terminus, and wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker; and the second RNAi construct comprises a second sense strand and a second antisense strand forming a double stranded region, wherein substantially all of the nucleotides of the second sense strand and substantially all of the nucleotides of the second antisense strand are modified nucleotides, wherein the second sense strand is conjugated to a ligand attached at the 3 '-terminus, and wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker.

[0151] In one embodiment, all of the nucleotides of the first and second sense strand and/or all of the nucleotides of the first and second antisense strand comprise a modification.

[0152] In one embodiment, the at least one of the modified nucleotides is selected from the group consisting of a 3 '-terminal deoxy-thymine (dT) nucleotide, a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a locked nucleotide, an unlocked nucleotide, a conformationally restricted nucleotide, a constrained ethyl nucleotide, an abasic nucleotide, a 2' -amino-modified nucleotide, a 2' -O-allyl-modified nucleotide, 2'-C-alkyl-modified nucleotide, 2' -hydroxly- modified nucleotide, a 2'- methoxyethyl modified nucleotide, a 2'-0-alkyl-modified nucleotide, a morpholino nucleotide, a phosphoramidate, a non-natural base comprising nucleotide, a tetrahydropyran modified nucleotide, a 1,5-anhydrohexitol modified nucleotide, a cyclohexenyl modified nucleotide, a nucleotide comprising a phosphorothioate group, a nucleotide comprising a methylphosphonate group, a nucleotide comprising a 5 '-phosphate, and a nucleotide comprising a 5'-phosphate mimic.

[0153] In certain embodiments, a first RNAi construct targeting a SCAP gene is administered in combination with a second RNAi construct targeting a gene that is different from the SCAP gene. For example, the RNAi construct targeting the SCAP gene may be administered in combination with an RNAi construct targeting the SCAP gene. The first RNAi construct targeting a SCAP gene and the second RNAi construct targeting a gene different from the SCAP gene, e.g. , the SCAP gene, may be administered as parts of the same pharmaceutical composition. Alternatively, the first RNAi construct targeting a SCAP gene and the second RNAi construct targeting a gene different from the SCAP gene, e.g. , the SCAP gene, may be administered as parts of different pharmaceutical compositions.

[0154] The RNAi construct and an additional therapeutic agent and/or treatment may be administered at the same time and/or in the same combination, e.g. , parenterally, or the

additional therapeutic agent can be administered as part of a separate composition or at separate times and/or by another method known in the art or described herein.

[0155] The present invention also provides methods of using an RNAi construct of the invention and/or a composition containing an RNAi construct of the invention to reduce and/or inhibit SCAP expression in a cell. In other aspects, the present invention provides an RNAi construct of the invention and/or a composition comprising an RNAi construct of the invention for use in reducing and/or inhibiting SCAP gene expression in a cell. In yet other aspects, use of an RNAi construct of the invention and/or a composition comprising an RNAi construct of the invention for the manufacture of a medicament for reducing and/or inhibiting SCAP gene expression in a cell are provided. In still other aspects, the the present invention provides an RNAi construct of the invention and/or a composition comprising an RNAi construct of the invention for use in reducing and/or inhibiting SCAP protein production in a cell. In yet other aspects, use of an RNAi construct of the invention and/or a composition comprising an RNAi construct of the invention for the manufacture of a medicament for reducing and/or inhibiting SCAP protein production in a cell are provided. The methods and uses include contacting the cell with an RNAi construct of the invention and maintaining the cell for a time sufficient to obtain degradation of the mRNA transcript of an SCAP gene, thereby inhibiting expression of the SCAP gene or inhibiting SCAP protein production in the cell.

[0156] Reduction in gene expression can be assessed by any methods known in the art. For example, a reduction in the expression of SCAP may be determined by determining the mRNA expression level of SCAP using methods routine to one of ordinary skill in the art, e.g., Northern blotting, qRT-PCR, by determining the protein level of SCAP using methods routine to one of ordinary skill in the art, such as Western blotting, immunological techniques, flow cytometry methods, ELISA, and/or by determining a biological activity of SCAP.

[0157] In the methods and uses of the invention the cell may be contacted in vitro or in vivo, i.e., the cell may be within a subject.

[0158] A cell suitable for treatment using the methods of the invention may be any cell that expresses an SCAP gene, e.g., a cell from a subject having NAFLD or a cell comprising an expression vector comprising a SCAP gene or portion of a SCAP gene. A cell suitable for use in the methods and uses of the invention may be a mammalian cell, e.g., a primate cell (such as a human cell or a non-human primate cell, e.g., a monkey cell or a chimpanzee cell),

a non-primate cell (such as a cow cell, a pig cell, a camel cell, a llama cell, a horse cell, a goat cell, a rabbit cell, a sheep cell, a hamster, a guinea pig cell, a cat cell, a dog cell, a rat cell, a mouse cell, a lion cell, a tiger cell, a bear cell, or a buffalo cell), a bird cell (e.g., a duck cell or a goose cell), or a whale cell. In one embodiment, the cell is a human cell.

[0159] SCAP gene expression may be inhibited in the cell by at least about 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or about 100%.

[0160] SCAP protein production may be inhibited in the cell by at least about 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or about 100%.

[0161] The in vivo methods and uses of the invention may include administering to a subject a composition containing an RNAi construct, where the RNAi construct includes a nucleotide sequence that is complementary to at least a part of an RNA transcript of the SCAP gene of the mammal to be treated. When the organism to be treated is a human, the composition can be administered by any means known in the art including, but not limited to subcutaneous, intravenous, oral, intraperitoneal, or parenteral routes, including intracranial (e.g., intraventricular, intraparenchymal and intrathecal), intramuscular, transdermal, airway (aerosol), nasal, rectal, and topical (including buccal and sublingual) administration. In certain embodiments, the compositions are administered by subcutaneous or intravenous infusion or injection. In one embodiment, the compositions are administered by subcutaneous injection.

[0162] In some embodiments, the administration is via a depot injection. A depot injection may release the RNAi in a consistent way over a prolonged time period. Thus, a depot injection may reduce the frequency of dosing needed to obtain a desired effect, e.g., a desired

inhibition of SCAP, or a therapeutic or prophylactic effect. A depot injection may also provide more consistent serum concentrations. Depot injections may include subcutaneous injections or intramuscular injections. In preferred embodiments, the depot injection is a subcutaneous injection.

[0163] In some embodiments, the administration is via a pump. The pump may be an external pump or a surgically implanted pump. In certain embodiments, the pump is a subcutaneously implanted osmotic pump. In other embodiments, the pump is an infusion pump. An infusion pump may be used for intravenous, subcutaneous, arterial, or epidural infusions. In preferred embodiments, the infusion pump is a subcutaneous infusion pump. In other embodiments, the pump is a surgically implanted pump that delivers the RNAi construct to the subject.

[0164] The mode of administration may be chosen based upon whether local or systemic treatment is desired and based upon the area to be treated. The route and site of administration may be chosen to enhance targeting.

[0165] In one aspect, the present invention also provides methods for inhibiting the expression of an SCAP gene in a mammal, e.g., a human. The present invention also provides a composition comprising an RNAi construct that targets an SCAP gene in a cell of a mammal for use in inhibiting expression of the SCAP gene in the mammal. In another aspect, the present invention provides use of an RNAi construct that targets an SCAP gene in a cell of a mammal in the manufacture of a medicament for inhibiting expression of the SCAP gene in the mammal.

[0166] The methods and uses include administering to the mammal, e.g., a human, a composition comprising an RNAi construct that targets an SCAP gene in a cell of the mammal and maintaining the mammal for a time sufficient to obtain degradation of the mRNA transcript of the SCAP gene, thereby inhibiting expression of the SCAP gene in the mammal.

[0167] Reduction in gene expression can be assessed in peripheral blood sample of the RNAi-administered subject by any methods known it the art, e.g. qRT-PCR, described herein. Reduction in protein production can be assessed by any methods known it the art and by methods, e.g., ELISA or Western blotting, described herein. In one embodiment, a tissue sample serves as the tissue material for monitoring the reduction in SCAP gene and/or protein expression. In another embodiment, a blood sample serves as the tissue material for monitoring the reduction in SCAP gene and/or protein expression.

[0168] In one embodiment, verification of RISC medicated cleavage of target in vivo following administration of RNAi construct is done by performing 5 '-RACE or modifications of the protocol as known in the art (Lasham A et al., (2010) Nucleic Acid Res., 38 (3) p-el9) (Zimmermann et al. (2006) Nature 441: 111-4).

[0169] It is understood that all ribonucleic acid sequences disclosed herein can be converted to deoxyribonucleic acid sequences by substituting a thymine base for a uracil base in the sequence. Likewise, all deoxyribonucleic acid sequences disclosed herein can be converted to ribonucleic acid sequences by substituting a uracil base for a thymine base in the sequence. Deoxyribonucleic acid sequences, ribonucleic acid sequences, and sequences containing mixtures of deoxyribonucleotides and ribonucleotides of all sequences disclosed herein are included in the invention.

[0170] Additionally, any nucleic acid sequences disclosed herein may be modified with any combination of chemical modifications. One of skill in the art will readily appreciate that such designation as "RNA" or "DNA" to describe modified polynucleotides is, in certain instances, arbitrary. For example, a polynucleotide comprising a nucleotide having a 2'-OH substituent on the ribose sugar and a thymine base could be described as a DNA molecule having a modified sugar (2'-OH for the natural 2'-H of DNA) or as an RNA molecule having a modified base(thymine (methylated uracil) for natural uracil of RNA).

[0171] Accordingly, nucleic acid sequences provided herein, including, but not limited to those in the sequence listing, are intended to encompass nucleic acids containing any combination of natural or modified RNA and/or DNA, including, but not limited to such nucleic acids having modified nucleobases. By way of a further example and without limitation, a polynucleotide having the sequence "ATCGATCG" encompasses any polynucleotides having such a sequence, whether modified or unmodified, including, but not limited to, such compounds comprising RNA bases, such as those having sequence "AUCGAUCG" and those having some DNA bases and some RNA bases such as "AUCGATCG" and polynucleotides having other modified bases, such as "ATmeCGAUCG," wherein meC indicates a cytosine base comprising a methyl group at the 5-position.

[0172] The following examples, including the experiments conducted and the results achieved, are provided for illustrative purposes only and are not to be construed as limiting the scope of the appended claims.

INCORPORATION BY REFERENCE

[0173] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference. However, the citation of a reference herein should not be construed as an acknowledgement that such reference is prior art to the present invention. To the extent that any of the definitions or terms provided in the references incorporated by reference differ from the terms and discussion provided herein, the present terms and definitions control.

EQUIVALENTS

[0174] The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. The foregoing description and examples detail certain preferred embodiments of the invention and describe the best mode contemplated by the inventors. It will be appreciated, however, that no matter how detailed the foregoing may appear in text, the invention may be practiced in many ways and the invention should be construed in accordance with the appended claims and any equivalents thereof.

[0175] The following examples, including the experiments conducted and results achieved, are provided for illustrative purposes only and are not to be construed as limiting the present invention.

EXAMPLE 1: Selection, Design and Synthesis of Modified SCAP siRNA molecules

[0176] The identification and selection of optimal sequences for therapeutic siRNA molecules targeting sterol regulatory element binding protein (SREBP) cleavage-activating protein (SCAP) were identified using bioinformatics analysis of a human SCAP transcript (NM_012235). Table 1 shows sequences identified as having therapeutic properties. Throughout the various sequences, "invAb" is an inverted abasic nucleotide.

Table 1. siRNA sequences directed to SCAP

Duplex No.	Sense sequence (5'-3')	SEQ ID NO: (sense)	Antisense sequence (5'-3')	SEQ ID NO: (antisense)
D-1000	UGGAUUGGCAUCCUGGUAUUU	1	AUACCAGGAUGCCAAUCCAUU	2
D-1001	GGCUGUGUCUCCUUUUGGUUU	3	ACCAAAAGGAGACACAGCCUU	4
D-1002	GCCUACAUCUACUUCUCCAUU	5	UGGAGAAGUAGAUGUAGGCUU	6
D-1003	UUGGCAUCCUGGUAUACAUUU	7	AUGUAUACCAGGAUGCCAAUU	8
D-1004	GUGCAAGCUUGGGUGUCAUUU	9	AUGACACCCAAGCUUGCACUU	10
D-1005	CCUACAUCUACUUCUCCAUUU	11	AUGGAGAAGUAGAUGUAGGUU	12
D-1006	UUCCUUCCGAAACCUGCGUUU	13	ACGCAGGUUUCGGAAGGAAUU	14
D-1007	CUUCCUUCCGAAACCUGCUUU	15	AGCAGGUUUCGGAAGGAAGUU	16
D-1008	GGACCUGUUACAGACAGUUUU	17	AACUGUCUGUAACAGGUCCUU	18
D-1009	GACCUGUUACAGACAGUCUUU	19	AGACUGUCUGUAACAGGUCUU	20
D-1010	GGGACCUGUUACAGACAGUUU	21	ACUGUCUGUAACAGGUCCCUU	22
D-1011	CCAUCUUCCCACCUGAUGUUU	23	ACAUCAGGUGGGAAGAUGGUU	24
D-1012	GUGGUGCAAGCUUGGGUGUUU	25	ACACCCAAGCUUGCACCACUU	26
D-1013	ACCGCAGCACAGGCAUCAAUU	27	UUGAUGCCUGUGCUGCGGUUU	28
D-1014	GGGGACCUGUUACAGACAUUU	29	AUGUCUGUAACAGGUCCCCUU	30
D-1015	AUUGUCUGCAACUUUGGCAUU	31	UGCCAAAGUUGCAGACAAUUU	32
D-1016	CCAUGGUCACUUUCCGGGAUU	33	UCCCGGAAAGUGACCAUGGUU	34
D-1017	UCUACUUCCUGGCCCGCAUUU	35	AUGCGGGCCAGGAAGUAGAUU	36
D-1018	UGACCCUGACUGAAAGGCUUU	37	AGCCUUUCAGUCAGGGUCAUU	38
D-1019	UGGCCAGUGGAGGACAAGAUU	39	UCUUGUCCUCCACUGGCCAUU	40
D-1020	GCUGGUCCAUCAUGAAGAAUU	41	UUCUUCAUGAUGGACCAGCUU	42
D-1021	AGGAAAUUGUCCUUCCGCUUU	43	AGCGGAAGGACAAUUUCCUUU	44
D-1022	UCCAUCUUCCCACCUGAUUUU	45	AAUCAGGUGGGAAGAUGGAUU	46
D-1023	GCCAGUGGAGGACAAGAUUUU	47	AAUCUUGUCCUCCACUGGCUU	48
D-1024	AGCUGGUCCAUCAUGAAGAUU	49	UCUUCAUGAUGGACCAGCUUU	50
D-1025	GCGGCCGGCUGGAGGUGUUUU	51	AACACCUCCAGCCGGCCGCUU	52
D-1026	UGGAGGAAAUUGUCCUUCUUU	53	AGAAGGACAAUUUCCUCCAUU	54
D-1027	AGAGCUGGUCCAUCAUGAAUU	55	UUCAUGAUGGACCAGCUCUUU	56
D-1028	CUGUGGUGCAAGCUUGGGUUU	57	ACCCAAGCUUGCACCACAGUU	58

D-1029	UGUGGUGCAAGCUUGGGUUUU	59	AACCCAAGCUUGCACCACAUU	60
D-1030	GGCCAGUGGAGGACAAGAUUU	61	AUCUUGUCCUCCACUGGCCUU	62
D-1031	UCUUCCUUCCGAAACCUGUUU	63	ACAGGUUUCGGAAGGAAGAUU	64
D-1032	GCUGUGGUGCAAGCUUGGUUU	65	ACCAAGCUUGCACCACAGCUU	66
D-1033	CAUGGUCACUUUCCGGGAUUU	67	AUCCCGGAAAGUGACCAUGUU	68
D-1034	GAGAGCUGGUCCAUCAUGAUU	69	UCAUGAUGGACCAGCUCUCUU	70
D-1035	GGCUGUGGUGCAAGCUUGUUU	71	ACAAGCUUGCACCACAGCCUU	72
D-1036	GAGCUGGGCAUCAUCCUCAUU	73	UGAGGAUGAUGCCCAGCUCUU	74
D-1037	GUCUCCUACACCAUCACCUUU	75	AGGUGAUGGUGUAGGAGACUU	76
D-1038	CCAGUGGAGGACAAGAUGUUU	77	ACAUCUUGUCCUCCACUGGUU	78
D-1039	GUCACUUUCCGGGAUGGCAUU	79	UGCCAUCCCGGAAAGUGACUU	80
D-1040	UCUGGAUUGGCAUCCUGGUAinvAb	81	AUACCAGGAUGCCAAUCCAGAUU	82
D-1041	AGGGCUGUGUCUCCUUUUGGinvAb	83	ACCAAAAGGAGACACAGCCCUUU	84
D-1042	UUGCCUACAUCUACUUCUCCinvAb	85	UGGAGAAGUAGAUGUAGGCAAUU	86
D-1043	GAUUGGCAUCCUGGUAUACAinvAb	87	AUGUAUACCAGGAUGCCAAUCUU	88
D-1044	UGGUGCAAGCUUGGGUGUCAinvAb	89	AUGACACCCAAGCUUGCACCAUU	90
D-1045	UGCCUACAUCUACUUCUCCAinvAb	91	AUGGAGAAGUAGAUGUAGGCAUU	92
D-1046	UCUUCCUUCCGAAACCUGCGinvAb	93	ACGCAGGUUUCGGAAGGAAGAUU	94
D-1047	GUCUUCCUUCCGAAACCUGCinvAb	95	AGCAGGUUUCGGAAGGAAGACUU	96
D-1048	GGGGACCUGUUACAGACAGUinvAb	97	AACUGUCUGUAACAGGUCCCCUU	98
D-1049	GGGACCUGUUACAGACAGUCinvAb	99	AGACUGUCUGUAACAGGUCCCUU	100
D-1050	CGGGGACCUGUUACAGACAGinvAb	101	ACUGUCUGUAACAGGUCCCCGUU	102
D-1051	CUCCAUCUUCCCACCUGAUGinvAb	103	ACAUCAGGUGGGAAGAUGGAGUU	104
D-1052	CUGUGGUGCAAGCUUGGGUGinvAb	105	ACACCCAAGCUUGCACCACAGUU	106
D-1053	GGACCGCAGCACAGGCAUCAinvAb	107	UUGAUGCCUGUGCUGCGGUCCUU	108
D-1054	ACGGGGACCUGUUACAGACAinvAb	109	AUGUCUGUAACAGGUCCCCGUUU	110
D-1055	CCAUUGUCUGCAACUUUGGCinvAb	111	UGCCAAAGUUGCAGACAAUGGUU	112
D-1056	AUCCAUGGUCACUUUCCGGGinvAb	113	UCCCGGAAAGUGACCAUGGAUUU	114
D-1057	UGUCUACUUCCUGGCCCGCAinvAb	115	AUGCGGGCCAGGAAGUAGACAUU	116
D-1058	GAUGACCCUGACUGAAAGGCinvAb	117	AGCCUUUCAGUCAGGGUCAUCUU	118
D-1059	GCUGGCCAGUGGAGGACAAGinvAb	119	UCUUGUCCUCCACUGGCCAGCUU	120
D-1060	GAGCUGGUCCAUCAUGAAGAinvAb	121	UUCUUCAUGAUGGACCAGCUCUU	122

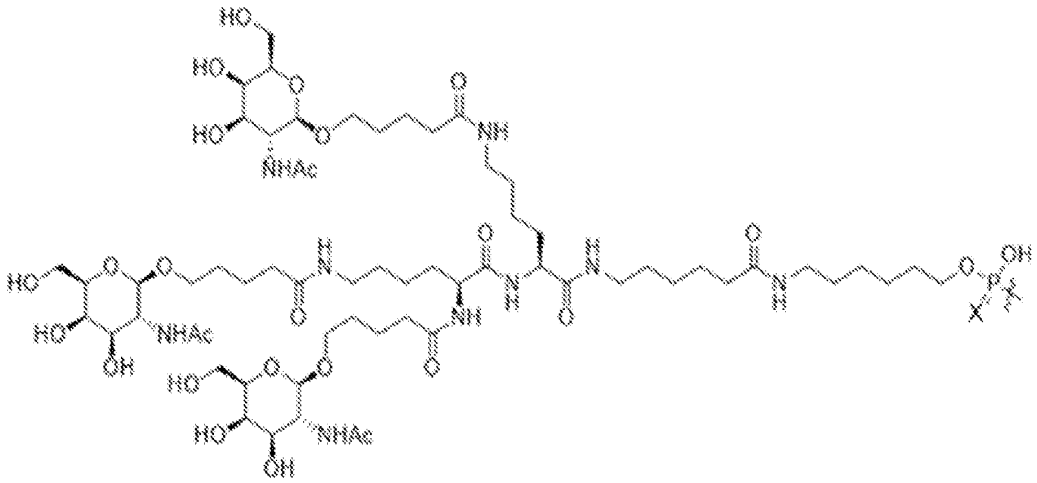
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D-1062	UCUCCAUCUUCCCACCUGAUinvAb	125	AAUCAGGUGGGAAGAUGGAGAUU	126
D-1063	UGGCCAGUGGAGGACAAGAUinvAb	127	AAUCUUGUCCUCCACUGGCCAUU	128
D-1064	AGAGCUGGUCCAUCAUGAAGinvAb	129	UCUUCAUGAUGGACCAGCUCUUU	130
D-1065	CAGCGGCCGGCUGGAGGUGUinvAb	131	AACACCUCCAGCCGGCCGCUGUU	132
D-1066	UUUGGAGGAAAUUGUCCUUCinvAb	133	AGAAGGACAAUUUCCUCCAAAUU	134
D-1067	CGAGAGCUGGUCCAUCAUGAinvAb	135	UUCAUGAUGGACCAGCUCUCGUU	136
D-1068	GGCUGUGGUGCAAGCUUGGGinvAb	137	ACCCAAGCUUGCACCACAGCCUU	138
D-1069	GCUGUGGUGCAAGCUUGGGUinvAb	139	AACCCAAGCUUGCACCACAGCUU	140
D-1070	CUGGCCAGUGGAGGACAAGAinvAb	141	AUCUUGUCCUCCACUGGCCAGUU	142
D-1071	CGUCUUCCUUCCGAAACCUGinvAb	143	ACAGGUUUCGGAAGGAAGACGUU	144
D-1072	GGGCUGUGGUGCAAGCUUGGinvAb	145	ACCAAGCUUGCACCACAGCCCUU	146
D-1073	UCCAUGGUCACUUUCCGGGAinvAb	147	AUCCCGGAAAGUGACCAUGGAUU	148
D-1074	GCGAGAGCUGGUCCAUCAUGinvAb	149	UCAUGAUGGACCAGCUCUCGCUU	150
D-1075	UGGGCUGUGGUGCAAGCUUGinvAb	151	ACAAGCUUGCACCACAGCCCAUU	152
D-1076	CGGAGCUGGGCAUCAUCCUCinvAb	153	UGAGGAUGAUGCCCAGCUCCGUU	154
D-1077	UGGUCUCCUACACCAUCACCinvAb	155	AGGUGAUGGUGUAGGAGACCAUU	156
D-1078	GGCCAGUGGAGGACAAGAUGinvAb	157	ACAUCUUGUCCUCCACUGGCCUU	158
D-1079	UGGUCACUUUCCGGGAUGGCinvAb	159	UGCCAUCCCGGAAAGUGACCAUU	160
D-1080	UGUGUGCCAGGGUGAUCCinvAb	321	UGGAUCACCCUGGCACACAUU	322
D-1081	AUAUCUCGGGCCUUCUACinvAb	323	UGUAGAAGGCCCGAGAUAUUU	324
D-1082	GGACCUGUGGAAUUCACCinvAb	325	UGGUGAAUUCCACAGGUCCUU	326
D-1083	UCUACUUCUCCACGCGGAinvAb	327	UUCCGCGUGGAGAAGUAGAUU	328
D-1084	GCGAGAUUUUCCCCUACCinvAb	329	AGGUAGGGGAAAAUCUCGCUU	330
D-1085	CCUGUCCAUUGACAUUCGinvAb	331	ACGAAUGUCAAUGGACAGGUU	332
D-1086	CUGUCCAUUGACAUUCGCinvAb	333	AGCGAAUGUCAAUGGACAGUU	334
D-1087	GUCCAUUGACAUUCGCCGinvAb	335	ACGGCGAAUGUCAAUGGACUU	336
D-1088	UCCAUUGACAUUCGCCGGinvAb	337	UCCGGCGAAUGUCAAUGGAUU	338
D-1089	CCAUUGACAUUCGCCGGAinvAb	339	AUCCGGCGAAUGUCAAUGGUU	340
D-1090	CAUUGACAUUCGCCGGAUinvAb	341	AAUCCGGCGAAUGUCAAUGUU	342
D-1091	CCGUCUUCCUUCCGAAACinvAb	343	AGUUUCGGAAGGAAGACGGUU	344
D-1092	UGGCUGGCACCGUUGUCUinvAb	345	AAGACAACGGUGCCAGCCAUU	346

D-1093	CCCAUGCCCGUGCCUAGUinvAb	347	AACUAGGCACGGGCAUGGGUU	348
D-1094	CACUGGCCGACGCUCUUCinvAb	349	UGAAGAGCGUCGGCCAGUGUU	350
D-1095	GCGACGACUACGGCUAUGinvAb	351	ACAUAGCCGUAGUCGUCGCUU	352
D-1096	CUCAACGGUUCCCUUGAUinvAb	353	AAUCAAGGGAACCGUUGAGUU	354
D-1097	UCAACGGUUCCCUUGAUUinvAb	355	AAAUCAAGGGAACCGUUGAUU	356
D-1098	AACGGUUCCCUUGAUUUCinvAb	357	AGAAAUCAAGGGAACCGUUUU	358
D-1099	UGCAGUUUAGAGGGACCCinvAb	359	AGGGUCCCUCUAAACUGCAUU	360
D-1100	UGCACACCAAAAACCCAUinvAb	361	AAUGGGUUUUUGGUGUGCAUU	362
D-1101	UGGGAUGUACUGACUGGCinvAb	363	UGCCAGUCAGUACAUCCCAUU	364
D-1102	GGAUGUACUGACUGGCAGinvAb	365	ACUGCCAGUCAGUACAUCCUU	366
D-1103	GGACCUAAACUACGGGGAinvAb	367	AUCCCCGUAGUUUAGGUCCUU	368
D-1104	GACCUAAACUACGGGGACinvAb	369	AGUCCCCGUAGUUUAGGUCUU	370
D-1105	ACCUAAACUACGGGGACCinvAb	371	AGGUCCCCGUAGUUUAGGUUU	372
D-1106	UAAACUACGGGGACCUGUinvAb	373	AACAGGUCCCCGUAGUUUAUU	374
D-1107	GGAAAGAGCCGAGUAUCUinvAb	375	AAGAUACUCGGCUCUUUCCUU	376
D-1108	AAAGAGCCGAGUAUCUUCinvAb	377	AGAAGAUACUCGGCUCUUUUU	378
D-1109	AGCCGAGUAUCUUCCAGCinvAb	379	AGCUGGAAGAUACUCGGCUUU	380
D-1110	UCUGGAUUGGCAUCCUGGUAinvAb	381	AUACCAGGAUGCCAAUCCAGAUU	382
D-1111	UCUGGAUUGGCAUCCUGGUAinvAb	383	AUACCAGGAUGCCAAUCCAGAUU	384
D-1112	UCUGGAUUGGCAUCCUGGUAinvAb	385	AUACCAbGGAUGCCAAUCCAGAUU	386
D-1113	UCUGGAUUGGCAUCCUGGUAinvAb	387	AUACCAGGAUGCCAAUCCAGAUU	388
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D-1115	UGCCUACAUCUACUUCUCCAinvAb	391	AUGGAGAAGUAGAUGUAGGCAUU	392
D-1116	UGCCUACAUCUACUUCUCCAinvAb	393	AUGGAbGAAGUAGAUGUAGGCAU U	394
D-1117	UUGCCUACAUCUACUUCUCCinvAb	395	UGGAGAAGUAGAUGUAGGCAAUU	396
D-1118	UUGCCUACAUCUACUUCUCCinvAb	397	UGGAAbAAGUAGAUGUAGGCAAU U	398
D-1119	UUGCCUACAUCUACUUCUCCinvAb	399	UGGAGAAGUAGAUGUAGGCAAUU	400
D-1120	AGGGCUGUGUCUCCUUUUGGinvAb	401	ACCAAAAGGAGACACAGCCCUUU	402
D-1121	AGGGCUGUGUCUCCUUUUGGinvAb	403	ACCAAAAGGAGACACAGCCCUUU	404
D-1122	UGGUGCAAGCUUGGGUGUCAinvAb	405	AUGACACCCAAGCUUGCACCAUU	406

D-1123	UGGUGCAAGCUUGGGUGUCAinvAb	407	AUGACAAbCCAAGCUUGCACCAUU	408
D-1124	UGGUGCAAGCUUGGGUGUCAinvAb	409	AUGACAbCCCAAGCUUGCACCAUU	410
D-1125	GCCUACAUCUACUUCUCCAUU	411	UGGAGAAGUAGAUGUAGGCUU	412
D-1126	GCCUACAUCUACUUCUCCA	413	UGGAGAAGUAGAUGUAGGCUU	414
D-1127	GCCUACAUCUACUUCUCCinvAb	415	UGGAGAAGUAGAUGUAGGCUU	416
D-1128	GCCUACAUCUACUUCUCCinvAb	417	UGGAGAAGUAGAUGUAGGCUU	418
D-1129	CCUACAUCUACUUCUCCAUUU	419	AUGGAGAAGUAGAUGUAGGUU	420
D-1130	UGCCUACAUCUACUUCUCCAinvAb	421	AUGGAGAAGUAGAUGUAGGCAUU	422
D-1131	UGCCUACAUCUACUUCUCCAinvAb	423	AUGGAGAAGUAGAUGUAGGCAUU	424
D-1132	UGCCUACAUCUACUUCUCCAinvAb	425	AUGGAGAAGUAGAUGUAGGCAUU	426
D-1133	UGGAUUGGCAUCCUGGUAinvAb	427	AUACCAGGAUGCCAAUCCAUU	428
D-1134	UCUGGAUUGGCAUCCUGGUAinvAb	429	AUACCAGGAUGCCAAUCCAGAUU	430
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D-1137	GGCUGUGUCUCCUUUUGGUUU	435	ACCAAAAGGAGACACAGCCUU	436
D-1138	GGCUGUGUCUCCUUUUGGUUU	437	ACCAAAAGGAGACACAGCCUU	438
D-1139	GGCUGUGUCUCCUUUUGGinvAb	439	ACCAAAAGGAGACACAGCCUU	440
D-1140	GGCUGUGUCUCCUUUUGGinvAb	441	ACCAAAAGGAGACACAGCCUU	442
D-1141	GGCUGUGUCUCCUUUUGGinvAb	443	ACCAAAAGGAGACACAGCCUU	444
D-1142	GUGCAAGCUUGGGUGUCAUUU	445	AUGACACCCAAGCUUGCACUU	446
D-1143	GUGCAAGCUUGGGUGUCAUUU	447	AUGACACCCAAGCUUGCACUU	448
D-1144	GUGCAAGCUUGGGUGUCAinvAb	449	AUGACACCCAAGCUUGCACUU	450
D-1145	GUGCAAGCUUGGGUGUCAinvAb	451	AUGACACCCAAGCUUGCACUU	452
D-1146	GUGCAAGCUUGGGUGUCAinvAb	453	AUGACACCCAAGCUUGCACUU	454
D-1147	UCUGGAUUGGGUACCUGGUAinvAb	455	AUACCAGGUACCCAAUCCAGAUU	456
D-1148	GCCUACAUGAUCUUCUCCA	457	UGGAGAAGAUCAUGUAGGCUU	458
D-1149	GGCUGUGUGAGCUUUUGGinvAb	459	ACCAAAAGCUCACACAGCCUU	460
D-1150	GUGCAAGCAACGGUGUCAinvAb	461	AUGACACCGUUGCUUGCACUU	462

[0177] To improve the potency and in vivo stability of SCAP siRNA sequences, chemical modifications were incorporated into SCAP siRNA molecules. Specifically, 2'-O-methyl and 2'-fluoro modifications of the ribose sugar were incorporated at specific positions within the SCAP siRNAs. Phosphorothioate internucleotide linkages were also incorporated at

the terminal ends of the antisense and/ or sense sequences. Table 2 below depicts the modifications in the sense and antisense sequences for each of the modified SCAP siRNAs. The nucleotide sequences in Table 2 are listed according to the following notations: A, U, G, and C =corresponding ribonucleotide; dC and dG= corresponding deoxyribonucleotide; dT = deoxythymidine; a, u, g, and c = corresponding 2'-O-methyl ribonucleotide; Af, Uf, Gf, and Cf = corresponding 2'-deoxy-2'-fluoro ("2'-fluoro") ribonucleotide; [InvAb] is an inverted abasic residue; [Ab] is an abasic residue; GNA is a glycol nucleic acid and bases with the GNA backbone are shown as AgN, UgN, CgN, and GgN. Insertion of an "s" in the sequence indicates that the two adjacent nucleotides are connected by a phosphorothiodiester group (e.g. a phosphorothioate internucleotide linkage). Unless indicated otherwise, all other nucleotides are connected by 3'-5' phosphodiester groups. Each of the siRNA compounds in Table 2 comprises a 19-21 base pair duplex region with either a 2 nucleotide overhang at the 3' end of both strands or bluntmer at one or both ends. Each [Phosphate] has been linked to the GalNAc structure below:



, wherein X = O or S.

Table 2. siRNA sequences directed to SCAP with modifications

Duplex No.	Sense sequence (5'-3')	SEQ ID NO: (sense)	Antisense sequence (5'-3')	SEQ ID NO: (antisense)
D-2000	[Phosphate] usgsgauuGfgCfAfUfCfcugguaususu	161	[Phosphate] asUfsaCfcAfGfgaugCfcAfauccasusu	162

D-2001	[Phosphate] gsgscuguGfuCfUfCfCfuuuuggususu	163	[Phosphate] asCfscAfaAfAfggagAfcAfcagccsusu	164
D-2002	[Phosphate] gscscuacAfuCfUfAfCfuucuccasusu	165	[Phosphate] usGfsgAfgAfAfguagAfuGfuaggcsusu	166
D-2003	[Phosphate] ususggcaUfcCfUfGfGfuauacaususu	167	[Phosphate] asUfsgUfaUfAfccagGfaUfgccaasusu	168
D-2004	[Phosphate] gsusgcaaGfcUfUfGfGfgugucaususu	169	[Phosphate] asUfsgAfcAfCfccaaGfcUfugcacsusu	170
D-2005	[Phosphate] cscsuacaUfcUfAfCfUfucuccaususu	171	[Phosphate] asUfsgGfaGfAfaguaGfaUfguaggsusu	172
D-2006	[Phosphate] ususccuuCfcGfAfAfAfccugcgususu	173	[Phosphate] asCfsgCfaGfGfuuucGfgAfaggaasusu	174
D-2007	[Phosphate] csusuccuUfcCfGfAfAfaccugcususu	175	[Phosphate] asGfscAfgGfUfuucgGfaAfggaagsusu	176
D-2008	[Phosphate] gsgsaccuGfuUfAfCfAfgacaguususu	177	[Phosphate] asAfscUfgUfCfuguaAfcAfgguccsusu	178
D-2009	[Phosphate] gsasccugUfuAfCfAfGfacagucususu	179	[Phosphate] asGfsaCfuGfUfcuguAfaCfaggucsusu	180
D-2010	[Phosphate] gsgsgaccUfgUfUfAfCfagacagususu	181	[Phosphate] asCfsuGfuCfUfguaaCfaGfgucccsusu	182
D-2011	[Phosphate] cscsaucuUfcCfCfAfCfcugaugususu	183	[Phosphate] asCfsaUfcAfGfguggGfaAfgauggsusu	184
D-2012	[Phosphate] gsusggugCfaAfGfCfUfugggugususu	185	[Phosphate] asCfsaCfcCfAfagcuUfgCfaccacsusu	186
D-2013	[Phosphate] ascscgcaGfcAfCfAfGfgcaucaasusu	187	[Phosphate] usUfsgAfuGfCfcuguGfcUfgcggususu	188
D-2014	[Phosphate] gsgsggacCfuGfUfUfAfcagacaususu	189	[Phosphate] asUfsgUfcUfGfuaacAfgGfucccсsusu	190
D-2015	[Phosphate] asusugucUfgCfAfAfCfuuuggcasusu	191	[Phosphate] usGfscCfaAfAfguugCfaGfacaaususu	192
D-2016	[Phosphate] cscsauggUfcAfCfUfUfuccgggasusu	193	[Phosphate] usCfscCfgGfAfaaguGfaCfcauggsusu	194
D-2017	[Phosphate] uscsuacuUfcCfUfGfGfcccgcaususu	195	[Phosphate] asUfsgCfgGfGfccagGfaAfguagasusu	196
D-2018	[Phosphate] usgsacccUfgAfCfUfGfaaaggcususu	197	[Phosphate] asGfscCfuUfUfcaguCfaGfggucasusu	198
D-2019	[Phosphate] usgsgccaGfuGfGfAfGfgacaagasusu	199	[Phosphate] usCfsuUfgUfCfcuccAfcUfggccasusu	200
D-2020	[Phosphate] gscsugguCfcAfUfCfAfugaagaasusu	201	[Phosphate] usUfscUfuCfAfugauGfgAfccagcsusu	202
D-2021	[Phosphate] asgsgaaaUfuGfUfCfCfuuccgcususu	203	[Phosphate] asGfscGfgAfAfggacAfaUfuuccususu	204
D-2022	[Phosphate] uscscaucUfuCfCfCfAfccugauususu	205	[Phosphate] asAfsuCfaGfGfugggAfaGfauggasusu	206
D-2023	[Phosphate] gscscaguGfgAfGfGfGfAfcaagauususu	207	[Phosphate] asAfsuCfuUfGfuccuCfcAfcuggcsusu	208
D-2024	[Phosphate] asgscuggUfcCfAfUfCfaugaagasusu	209	[Phosphate] usCfsuUfcAfUfgaugGfaCfcagcususu	210

D-2025	[Phosphate] gscsggccGfgCfUfGfGfagguguususu	211	[Phosphate] asAfscAfcCfUfccagCfcGfgccgcsusu	212
D-2026	[Phosphate] usgsgaggAfaAfUfUfGfuccuucususu	213	[Phosphate] asGfsaAfgGfAfcaauUfuCfcuccasusu	214
D-2027	[Phosphate] asgsagcuGfgUfCfCfAfucaugaasusu	215	[Phosphate] usUfscAfuGfAfuggaCfcAfgcucususu	216
D-2028	[Phosphate] csusguggUfgCfAfAfGfcuugggususu	217	[Phosphate] asCfscCfaAfGfcuugCfaCfcacagsusu	218
D-2029	[Phosphate] usgsugguGfcAfAfGfCfuuggguususu	219	[Phosphate] asAfscCfcAfAfgcuuGfcAfccacasusu	220
D-2030	[Phosphate] gsgsccagUfgGfAfGfGfacaagaususu	221	[Phosphate] asUfscUfuGfUfccucCfaCfuggccsusu	222
D-2031	[Phosphate] uscsuuccUfuCfCfGfAfaaccugususu	223	[Phosphate] asCfsaGfgUfUfucggAfaGfgaagasusu	224
D-2032	[Phosphate] gscsugugGfuGfCfAfAfgcuuggususu	225	[Phosphate] asCfscAfaGfCfuugcAfcCfacagcsusu	226
D-2033	[Phosphate] csasugguCfaCfUfUfUfccgggaususu	227	[Phosphate] asUfscCfcGfGfaaagUfgAfccaugsusu	228
D-2034	[Phosphate] gsasgagcUfgGfUfCfCfaucaugasusu	229	[Phosphate] usCfsaUfgAfUfggacCfaGfcucucsusu	230
D-2035	[Phosphate] gsgscuguGfgUfGfCfAfagcuugususu	231	[Phosphate] asCfsaAfgCfUfugcaCfcAfcagccsusu	232
D-2036	[Phosphate] gsasgcugGfgCfAfUfCfauccucasusu	233	[Phosphate] usGfsaGfgAfUfgaugCfcCfagcucsusu	234
D-2037	[Phosphate] gsuscuccUfaCfAfCfCfaucaccususu	235	[Phosphate] asGfsgUfgAfUfggugUfaGfgagacsusu	236
D-2038	[Phosphate] cscsagugGfaGfGfAfCfaagaugususu	237	[Phosphate] asCfsaUfcUfUfguccUfcCfacuggsusu	238
D-2039	[Phosphate] gsuscacuUfuCfCfGfGfgauggcasusu	239	[Phosphate] usGfscCfaUfCfccggAfaAfgugacsusu	240
D-2040	[Phosphate]ucuggauuGfgCfAfUfCfcug guas[invAb]	241	asUfsaccaGfgaugCfcAfauccagasusu	242
D-2041	[Phosphate]agggcuguGfuCfUfCfCfuuu uggs[invAb]	243	asCfscaaaAfggagAfcAfcagcccususu	244
D-2042	[Phosphate]uugccuacAfuCfUfAfCfuuc uccs[invAb]	245	usGfsgagaAfguagAfuGfuaggcaasusu	246
D-2043	[Phosphate]gauuggcaUfcCfUfGfGfuau acas[invAb]	247	asUfsguauAfccagGfaUfgccaaucsusu	248
D-2044	[Phosphate]uggugcaaGfcUfUfGfGfgug ucas[invAb]	249	asUfsgacaCfccaaGfcUfugcaccasusu	250
D-2045	[Phosphate]ugccuacaUfcUfAfCfUfucu ccas[invAb]	251	asUfsggagAfaguaGfaUfguaggcasusu	252
D-2046	[Phosphate]ucuuccuuCfcGfAfAfAfccu gcgs[invAb]	253	asCfsgcagGfuuucGfgAfaggaagasusu	254
D-2047	[Phosphate]gucuuccuUfcCfGfAfAfacc ugcs[invAb]	255	asGfscaggUfuucgGfaAfggaagacsusu	256
D-2048	[Phosphate]ggggaccuGfuUfAfCfAfgac agus[invAb]	257	asAfscuguCfuguaAfcAfgguccccsusu	258

D-2049	[Phosphate]gggaccugUfuAfCfAfGfacagucs[invAb]	259	asGfsacugUfcuguAfaCfaggucccsusu	260
D-2050	[Phosphate]cggggaccUfgUfUfAfCfagacags[invAb]	261	asCfsugucUfguaaCfaGfgucccgsusu	262
D-2051	[Phosphate]cuccaucuUfcCfCfAfCfcugaugs[invAb]	263	asCfsaucaGfguggGfaAfgauggagsusu	264
D-2052	[Phosphate]cuguggugCfaAfGfCfUfugggugs[invAb]	265	asCfsacccAfagcuUfgCfaccacagsusu	266
D-2053	[Phosphate]ggaccgcaGfcAfCfAfGfgcaucas[invAb]	267	usUfsgaugCfcuguGfcUfgcgguccsusu	268
D-2054	[Phosphate]acggggacCfuGfUfUfAfcagacas[invAb]	269	asUfsgucuGfuaacAfgGfucccgususu	270
D-2055	[Phosphate]ccauugucUfgCfAfAfCfuuuggcs[invAb]	271	usGfsccaaAfguugCfaGfacaauggsusu	272
D-2056	[Phosphate]auccauggUfcAfCfUfUfuccgggs[invAb]	273	usCfsccggAfaaguGfaCfcauggaususu	274
D-2057	[Phosphate]ugucuacuUfcCfUfGfGfcccgcas[invAb]	275	asUfsgcggGfccagGfaAfguagacasusu	276
D-2058	[Phosphate]gaugacccUfgAfCfUfGfaaaggcs[invAb]	277	asGfsccuuUfcaguCfaGfggucaucsusu	278
D-2059	[Phosphate]gcuggccaGfuGfGfAfGfgacaags[invAb]	279	usCfsuuguCfcuccAfcUfggccagcsusu	280
D-2060	[Phosphate]gagcuggcCfcAfUfCfAfugaagas[invAb]	281	usUfscuucAfugauGfgAfccagcucsusu	282
D-2061	[Phosphate]ggaggaaaUfuGfUfCfCfuuccgcs[invAb]	283	asGfscggaAfggacAfaUfuuccuccsusu	284
D-2062	[Phosphate]ucuccaucUfuCfCfCfAfccugaus[invAb]	285	asAfsucagGfugggAfaGfauggagasusu	286
D-2063	[Phosphate]uggccaguGfgAfGfGfAfcaagaus[invAb]	287	asAfsucuuGfuccuCfcAfcuggccasusu	288
D-2064	[Phosphate]agagcuggUfcCfAfUfCfaugaags[invAb]	289	usCfsuucaUfgaugGfaCfcagcucususu	290
D-2065	[Phosphate]cagcggccGfgCfUfGfGfaggugus[invAb]	291	asAfscaccUfccagCfcGfgccgcugsusu	292
D-2066	[Phosphate]uuuggaggAfaAfUfUfGfuccuucs[invAb]	293	asGfsaaggAfcaauUfuCfcuccaaasusu	294
D-2067	[Phosphate]cgagagcuGfgUfCfCfAfucaugas[invAb]	295	usUfscaugAfuggaCfcAfgcucucgsusu	296
D-2068	[Phosphate]ggcuguggUfgCfAfAfGfcuugggs[invAb]	297	asCfsccaaGfcuugCfaCfcacagccsusu	298
D-2069	[Phosphate]gcugugguGfcAfAfGfCfuugggus[invAb]	299	asAfscccaAfgcuuGfcAfccacagcsusu	300
D-2070	[Phosphate]cuggccagUfgGfAfGfGfacaagas[invAb]	301	asUfscuugUfccucCfaCfuggccagsusu	302
D-2071	[Phosphate]cgucuuccUfuCfCfGfAfaaccugs[invAb]	303	asCfsagguUfucggAfaGfgaagacgsusu	304
D-2072	[Phosphate]gggcugugGfuGfCfAfAfgcuuggs[invAb]	305	asCfscaagCfuugcAfcCfacagcccsusu	306

D-2073	[Phosphate]uccaugguCfaCfUfUfUfccggas[invAb]	307	asUfscccgGfaaagUfgAfccauggasusu	308
D-2074	[Phosphate]gcgagagcUfgGfUfCfCfaucaugs[invAb]	309	usCfsaugaUfggacCfaGfcucucgcsusu	310
D-2075	[Phosphate]ugggcuguGfgUfGfCfAfagcuugs[invAb]	311	asCfsaagcUfugcaCfcAfcagcccasusu	312
D-2076	[Phosphate]cggagcugGfgCfAfUfCfauccucs[invAb]	313	usGfsaggaUfgaugCfcCfagcuccgsusu	314
D-2077	[Phosphate]uggucuccUfaCfAfCfCfauccaccs[invAb]	315	asGfsgugaUfggugUfaGfgagaccasusu	316
D-2078	[Phosphate]ggccagugGfaGfGfAfCfaagaugs[invAb]	317	asCfsaucuUfguccUfcCfacuggccsusu	318
D-2079	[Phosphate]uggucacuUfuCfCfGfGfgauggcs[invAb]	319	usGfsccauCfccggAfaAfgugaccasusu	320
D-2080	[Phosphate] usgsugugCfcAfGfGfGfugaucscs[invAb]	463	usGfsgaucAfcccuGfgCfacacasusu	464
D-2081	[Phosphate] asusaucuCfgGfGfCfCfuucuascs[invAb]	465	usGfsuagaAfggccCfgAfgauaususu	466
D-2082	[Phosphate] gsgsaccuGfuGfGfAfAfuucacscs[invAb]	467	usGfsgugaAfuuccAfcAfgguccsusu	468
D-2083	[Phosphate] uscsuacuUfcUfCfCfAfcgcggsas[invAb]	469	usUfsccgcGfuggaGfaAfguagasusu	470
D-2084	[Phosphate] gscsgagaUfuUfUfCfCfccuacscs[invAb]	471	asGfsguagGfggaaAfaUfcucgcsusu	472
D-2085	[Phosphate] cscsugucCfaUfUfGfAfcauucsgs[invAb]	473	asCfsgaauGfucaaUfgGfacaggsusu	474
D-2086	[Phosphate] csusguccAfuUfGfAfCfauucgscs[invAb]	475	asGfscgaaUfgucaAfuGfgacagsusu	476
D-2087	[Phosphate] gsusccauUfgAfCfAfUfucgccsgs[invAb]	477	asCfsggcgAfauguCfaAfuggacsusu	478
D-2088	[Phosphate] uscscauuGfaCfAfUfUfcgccgsgs[invAb]	479	usCfscggcGfaaugUfcAfauggasusu	480
D-2089	[Phosphate] cscsauugAfcAfUfUfCfgccggsas[invAb]	481	asUfsccggCfgaauGfuCfaauggsusu	482
D-2090	[Phosphate] csasuugaCfaUfUfCfGfccggasus[invAb]	483	asAfsuccgGfcgaaUfgUfcaaugsusu	484
D-2091	[Phosphate] cscsgucuUfcCfUfUfCfcgaaascs[invAb]	485	asGfsuuucGfgaagGfaAfgacggsusu	486
D-2092	[Phosphate] usgsgcugGfcAfCfCfGfuugucsus[invAb]	487	asAfsgacaAfcgguGfcCfagccasusu	488
D-2093	[Phosphate] cscscaugCfcCfGfUfGfccuagsus[invAb]	489	asAfscuagGfcacgGfgCfaugggsusu	490
D-2094	[Phosphate] csascuggCfcGfAfCfGfcucuuscs[invAb]	491	usGfsaagaGfcgucGfgCfcagugsusu	492
D-2095	[Phosphate] gscsgacgAfcUfAfCfGfgcuausgs[invAb]	493	asCfsauagCfcguaGfuCfgucgcsusu	494
D-2096	[Phosphate] csuscaacGfgUfUfCfCfcuugasus[invAb]	495	asAfsucaaGfggaaCfcGfuugagsusu	496

D-2097	[Phosphate] uscsaacgGfuUfCfCfCfuugausus[invAb]	497	asAfsaucaAfgggaAfcCfguugasusu	498
D-2098	[Phosphate] asascgguUfcCfCfUfUfgauuuscs[invAb]	499	asGfsaaauCfaaggGfaAfccguususu	500
D-2099	[Phosphate] usgscaguUfuAfGfAfGfggaccscs[invAb]	501	asGfsggucCfcucuAfaAfcugcasusu	502
D-2100	[Phosphate] usgscacaCfcAfAfAfAfacccasus[invAb]	503	asAfsugggUfuuuuGfgUfgugcasusu	504
D-2101	[Phosphate] usgsggauGfuAfCfUfGfacuggscs[invAb]	505	usGfsccagUfcaguAfcAfucccasusu	506
D-2102	[Phosphate] gsgsauguAfcUfGfAfCfuggcasgs[invAb]	507	asCfsugccAfgucaGfuAfcauccsusu	508
D-2103	[Phosphate] gsgsaccuAfaAfCfUfAfcggggsas[invAb]	509	asUfsccccGfuaguUfuAfgguccsusu	510
D-2104	[Phosphate] gsasccuaAfaCfUfAfCfggggascs[invAb]	511	asGfsucccCfguagUfuUfaggucsusu	512
D-2105	[Phosphate] ascscuaaAfcUfAfCfGfgggacscs[invAb]	513	asGfsguccCfcguaGfuUfuaggususu	514
D-2106	[Phosphate] usasaacuAfcGfGfGfGfaccugsus[invAb]	515	asAfscaggUfccccGfuAfguuuasusu	516
D-2107	[Phosphate] gsgsaaagAfgCfCfGfAfguaucsus[invAb]	517	asAfsgauaCfucggCfuCfuuuccsusu	518
D-2108	[Phosphate] asasagagCfcGfAfGfUfaucuuscs[invAb]	519	asGfsaagaUfacucGfgCfucuuususu	520
D-2109	[Phosphate] asgsccgaGfuAfUfCfUfuccagscs[invAb]	521	asGfscuggAfagauAfcUfcggcususu	522
D-2110	[Phosphate] ucuggauuGfgCfAfUfCfcugguas[invAb]	523	asUfsac[CgN]aGfgaugCfcAfauccagasusu	524
D-2111	[Phosphate] ucuggauuGfgCfAfUfCfcugguas[invAb]	525	asUfsaccaGf[GgN]augCfcAfauccagasusu	526
D-2112	[Phosphate] ucuggauuGfgCfAfUfCfcugguas[invAb]	527	asUfsacc[Ab]GfgaugCfcAfauccagasusu	528
D-2113	[Phosphate] ucuggauuGfgCfAfUfCfcugguas[invAb]	529	asUfsacca[GgN]gaugCfcAfauccagasusu	530
D-2114	[Phosphate] ugccuacaUfcUfAfCfUfucuccas[invAb]	531	asUfs[GgN]gagAfaguaGfaUfguaggcasusu	532
D-2115	[Phosphate] ugccuacaUfcUfAfCfUfucuccas[invAb]	533	asUfsgg[AgN]gAfaguaGfaUfguaggcasusu	534
D-2116	[Phosphate] ugccuacaUfcUfAfCfUfucuccas[invAb]	535	asUfsgg[Ab]gAfaguaGfaUfguaggcasusu	536
D-2117	[Phosphate] uugccuacAfuCfUfAfCfuucuccs[invAb]	537	usGfsga[GgN]aAfguagAfuGfuaggcaasusu	538
D-2118	[Phosphate] uugccuacAfuCfUfAfCfuucuccs[invAb]	539	usGfsga[Ab]aAfguagAfuGfuaggcaasusu	540
D-2119	[Phosphate] uugccuacAfuCfUfAfCfuucuccs[invAb]	541	usGfs[GgN]agaAfguagAfuGfuaggcaasusu	542
D-2120	[Phosphate] agggcuguGfuCfUfCfCfuuuuggs[invAb]	543	asCfscaa[AgN]AfggagAfcAfcagcccususu	544

D-2121	[Phosphate] agggcuguGfuCfUfCfCfuuuuggs[invAb]	545	asCfsca[AgN]aAfggagAfcAfcagcccususu	546
D-2122	[Phosphate] uggugcaaGfcUfUfGfGfgugucas[invAb]	547	asUfs[GgN]acaCfccaaGfcUfugcaccasus u	548
D-2123	[Phosphate] uggugcaaGfcUfUfGfGfgugucas[invAb]	549	asUfsgaca[Ab]ccaaGfcUfugcaccasusu	550
D-2124	[Phosphate] uggugcaaGfcUfUfGfGfgugucas[invAb]	551	asUfsgac[Ab]CfccaaGfcUfugcaccasusu	552
D-2125	[Phosphate] gccuacAfuCfUfAfCfuucuccasusu	553	usGfsgAfgAfAfguagAfuGfuaggcsusu	554
D-2126	[Phosphate] gccuacAfuCfUfAfCfuucucscsa	555	usGfsgAfgAfAfguagAfuGfuaggcsusu	556
D-2127	[Phosphate] gccuacAfuCfUfAfCfuucucscs[invAb]	557	usGfsgagaAfguagAfuGfuaggcsusu	558
D-2128	[Phosphate] gccuacAfuCfUfAfCfuucucscs[invAb]	559	usGfsgAfgAfAfguagAfuGfuaggcsusu	560
D-2129	[Phosphate] ccuacaUfcUfaCfUfucuccaususu	561	asUfsgGfaGfAfaguaGfaUfguaggsusu	562
D-2130	[Phosphate] ugccuacaUfcUfaCfUfucuccas[invAb]	563	asUfsgGfaGfAfaguaGfaUfguaggcasusu	564
D-2131	[Phosphate] ugccuacaUfcUfaCfUfucuccas[invAb]	565	asUfsggagAfagUfaGfaUfguaggcasusu	566
D-2132	[Phosphate] ugccuacaUfcUfaCfUfucuccas[invAb]	567	asUfsggagAfaguaGfaUfguaggcasusu	568
D-2133	[Phosphate] uggauuGfgCfAfUfCfcuggusas[invAb]	569	asUfsaccaGfgaugCfcAfauccasusu	570
D-2134	[Phosphate] ucuggauuGfgCfaUfCfcugguas[invAb]	571	asUfsaCfcAfGfgaugCfcAfauccagasusu	572
D-2135	[Phosphate] ucuggauuGfgCfaUfCfcugguas[invAb]	573	asUfsaccaGfgaugCfcAfauccagasusu	574
D-2136	[Phosphate] ucuggauuGfgCfaUfCfcugguas[invAb]	575	asUfsaccaGfgaUfgCfcAfauccagasusu	576
D-2137	[Phosphate] ggcuguGfuCfUfCfCfuuuuggususu	577	asCfscAfaAfAfggagAfcAfcagccsusu	578
D-2138	[Phosphate] ggcuguGfuCfuCfCfuuuuggususu	579	asCfscAfaAfAfggagAfcAfcagccsusu	580
D-2139	[Phosphate] ggcuguGfuCfUfCfCfuuuugsgs[invAb]	581	asCfscAfaAfAfggagAfcAfcagccsusu	582
D-2140	[Phosphate] ggcuguGfuCfUfCfCfuuuugsgs[invAb]	583	asCfscaaaAfggagAfcAfcagccsusu	584
D-2141	[Phosphate] ggcuguGfuCfUfdCCfuuuuggs[invAb]	585	asCfscaaaAfggagAfcAfcagccsusu	586
D-2142	[Phosphate] gugcaaGfcUfUfGfGfgugucaususu	587	asUfsgAfcAfCfccaaGfcUfugcacsusu	588
D-2143	[Phosphate] gugcaaGfcUfuGfGfgugucaususu	589	asUfsgAfcAfCfccaaGfcUfugcacsusu	590
D-2144	[Phosphate] gugcaaGfcUfUfGfGfgugucsas[invAb]	591	asUfsgAfcAfCfccaaGfcUfugcacsusu	592

D-2145	[Phosphate] gugcaaGfcUfUfGfGfgugucsas[invAb]	593	asUfsgacaCfccaaGfcUfugcacsusu	594
D-2146	[Phosphate] gugcaaGfcUfUfdGGfgugucas[invAb]	595	asUfsgacaCfccaaGfcUfugcacsusu	596
D-2147	[Phosphate] ucuggauuGfgGfUfAfCfcugguas[invAb]	597	asUfsaccaGfguacCfcAfauccagasusu	598
D-2148	[Phosphate] gccuacAfuGfAfUfCfuucucscsa	599	usGfsgAfgAfAfgaucAfuGfuaggcsusu	600
D-2149	[Phosphate] ggcuguGfuGfAfGfCfuuuugsgs[invAb]	601	asCfscaaaAfgcucAfcAfcagccsusu	602
D-2150	[Phosphate] gugcaaGfcAfAfCfGfgugucsas[invAb]	603	asUfsgacaCfcguuGfcUfugcacsusu	604

EXAMPLE 2: Efficacy of select SCAP siRNA molecules in RNA FISH assay

[0178] A panel of fully chemically modified siRNA were prepared and tested for potency and selectivity of mRNA knockdown in vitro. Each siRNA duplex consisted of two strands, the sense or 'passenger' strand and the antisense or 'guide' strand, and are described in Example 1 with substitution of the natural 2'-OH in the ribose of certain nucleotides. Optionally, phosphodiester internucleotide linkages at one or both strands were replaced with phosphorothioates to reduce degradation by exonucleases.

[0179] RNA FISH (fluorescence in situ hybridization) Assay was carried out to measure SCAP mRNA knockdown by test siRNAs. Hep3B cells (purchased from ATCC) were cultured in minimal essential medium (MEM, Corning) supplemented with 10% Fetal Bovine Serum (FBS, Sigma) and 1% penicillin-streptomycin (P-S, Corning). The siRNA transfection was performed as follows: 1 μL of test siRNAs and 4 μL of plain MEM were added to PDL-coated CellCarrier-384 Ultra assay plates (PerkinElmer) by BioMek FX (Beckman Coulter). 5 μL of Lipofectamine RNAiMAX (Thermo Fisher Scientific), pre-diluted in plain MEM (0.035 μL of RNAiMAX in 5 μL MEM), was then dispensed into the assay plates by Multidrop Combi Reagent Dispenser (Thermo Fisher Scientific). After 20 mins incubation of the siRNA/RNAiMAX mixture at room temperature (RT), 30 μL of Hep3B cells (2000 cells per well) in MEM supplemented with 10% FBS and 1% P-S were added to the transfection complex using Multidrop Combi Reagent Dispenser and the assay plates were sit at RT for 20 mins prior to moving them to an incubator. Cells were incubated for 72 hrs at 37 °C and 5% CO2. ViewRNA ISH Cell Assay was performed following manufacture's protocol (Thermo Fisher Scientific) using an in-house assembled automated FISH assay platform for liquid

handling. In brief, cells were fixed in 4% formaldehyde (Thermo Fisher Scientific) for 15 mins at RT, permeabilized with detergent for 3 mins at RT and then treated with protease solution for 10 mins at RT. Incubation of target-specific probe pairs (Thermo Fisher Scientific) was done for 3 hrs, while for Preamplifiers, Amplifiers and Label Probes (Thermo Fisher Scientific) were for 1 hr each. All hybridization steps were carried out at 40 °C in Cytomat 2 C-LIN automated incubator (Thermo Fisher Scientific). After hybridization reactions, cells were stained for 30 mins with Hoechst and CellMask Blue (Thermo Fisher Scientific) and then imaged on Opera Phenix (PerkinElmer). The images were analyzed using Columbus Image Data Storage and Analysis System (PerkinElmer) to obtain mean spot counts per cell. The spot counts were normalized using the high (containing phosphate-buffered saline, Corning) and low (without target probe pairs) control wells. The normalized values against the total siRNA concentrations were plotted and the data were fit to a four-parameter sigmoidal model in Genedata Screener (Genedata) to obtain IC50 and maximum activity.

[0180] The results of the RNA FISH assay for Hep3B cells are shown in Table 3. The values represent knockdown of SCAP mRNA.

Table 3. RNA FISH assay on Hep3B cells

Duplex No.	IC50 (νM)	Max activity
D-2000	6.05	83
D-2001	0.26	72.8
D-2002	4.4	69.6
D-2003	2.86	81.4
D-2004	4.08	83
D-2005	1.62	84.1
D-2006	7.73	86.2
D-2007	0.91	83.8
D-2008	118	70
D-2009	3.03	86.7
D-2010	12.1	86.2
D-2011	6.71	75.5
D-2012	90.6	80.8
D-2013	1.24	75.1
D-2014	29.8	80.5
D-2015	0.58	72.3
D-2016	2.5	83.2
D-2017	2.32	78
D-2018	14.9	66.8

D-2019	90.9	69.4
D-2020	1.84	77.6
D-2021	20.9	78.4
D-2022	11.8	66
D-2023	79.8	87.8
D-2024	3.25	80.7
D-2025	93.6	79.5
D-2026	6.06	82.4
D-2027	3.36	84.3
D-2028	3.57	78.5
D-2029	3.26	69.7
D-2030	48.1	78.9
D-2031	8.12	85.1
D-2032	74.9	74.8
D-2033	9.28	64.3
D-2034	7.32	71.5
D-2035	4.85	72.9
D-2036	11.2	78.7
D-2037	67.3	80.4
D-2038	10.5	71.4
D-2039	16.3	77.3

Example 3: In Vivo silencing study to test silencing efficacy of SCAP siRNA sequences

[0181] C57Bl6 males of 9-10 weeks of age were procured from Charles River Laboratories and housed according to Amgen guidelines and Institutional Animal Care and Use Committees (IACUC) protocol. These animals were randomized according to their body weight and 6 were randomly assigned to each siRNA trigger sequence. On Day 0, the cohort was single dosed subcutaneously either with PBS or with specific siRNA compounds at 3mg/kg body weight. On day 29, mice were euthanized under CO2 and the left lobe of the liver was harvested from each animal. The tissue was cut into small pieces and immediately snap frozen in liquid nitrogen for further downstream assays.

[0182] RNA was isolated using the TRIzol reagent (Invitrogen) according to the manufacturer's guidelines. 2ug of RNA was treated with DNAse I (Promega) and subjected to quantitative PCR reaction using the Taqman RNA to CT, One Step Kit (Thermo Fisher Scientific). Gene specific Taqman Probes for mouse SCAP and GAPDH were used to quantitate the mRNA expression. GAPDH was used as the internal control. The qPCR

experiment was performed using the QuantStudio7 Flex Real Time PCR System from Invitrogen. Expression levels were calculated using the delta CT method.

[0183] In vivo screening with Ob/Ob mice (Ob/Ob on the Bl6 background) of 9-10 weeks of age from Jackson Laboratories was performed using the same method as described for C57Bl6 mice.

[0184] All animal experiments described herein were approved by the Institutional Animal Care and Use Committee (IACUC) of Amgen and cared for in accordance to the *Guide for the Care and Use of Laboratory Animals, 8th* Edition (National Research Council (U.S.). Committee for the Update of the Guide for the Care and Use of Laboratory Animals., Institute for Laboratory Animal Research (U.S.), and National Academies Press (U.S.) (2011) Guide for the care and use of laboratory animals. 8th Ed., National Academies Press, Washington, D.C. Mice were single-housed in an air-conditioned room at 22±2°C with a twelve-hour light; twelve-hour darkness cycle (0600-1800 hours). Animals had *ad libitum* access to a regular chow diet (Envigo, 2920X) and to water (reverse osmosis-purified) via automatic watering system, unless otherwise indicated. At termination, blood was collected by cardiac puncture under deep anesthesia, and then, following Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) guidelines, euthanized by a secondary physical method.

[0185] Data for relative knockdown is shown in Table 4, showing relative knockdown at day 25, at a dose of 3 mg/kg. SCAP knockdown is a percentage of decrease in SCAP mRNA levels.

Table 4. Day 25 SCAP knockdown assay

Duplex number	SCAP knockdown (%)
D-2040	74.5
D-2041	69.2
D-2042	66.5
D-2043	66
D-2044	64.8
D-2045	62.6
D-2046	55.9
D-2047	51.7
D-2048	50.2
D-2049	50.2

D-2050	49.3
D-2051	48.5
D-2052	47.4
D-2053	44.5
D-2054	42.4
D-2055	39.4
D-2056	37.1
D-2057	36.5
D-2058	33
D-2059	31.9
D-2060	25.4
D-2061	24.2
D-2062	21.4
D-2063	20.7
D-2064	18.8
D-2065	16.4
D-2066	16.4
D-2067	16.2
D-2068	16.2
D-2069	15.2
D-2070	14.1
D-2071	11.8
D-2072	4.9
D-2073	4.7
D-2074	-3.3
D-2075	-6.8
D-2076	-7.2
D-2077	-15.5
D-2078	-16.7
D-2079	-38.5

EXAMPLE 4: Efficacy of select SCAP siRNA molecules in RNA FISH assay

[0186] A panel of fully chemically modified siRNA were prepared and tested for potency and selectivity of mRNA knockdown in vitro. Each siRNA duplex consisted of two strands, the sense or 'passenger' strand and the antisense or 'guide' strand, and are described in Example 1 with substitution of the natural 2'-OH in the ribose of certain nucleotides. Optionally, phosphosdiester internucleotide linkages at one or both strands were replaced with phosphorothioates to reduce degradation by exonucleases.

[0187] RNA FISH was performed as described in Example 2. Cells were incubated for 72 hrs at 37 °C and 5% CO2. ViewRNA ISH Cell Assay was performed following manufacture's protocol (Thermo Fisher Scientific) using an in-house assembled automated FISH assay platform for liquid handling. In brief, cells were fixed in 4% formaldehyde (Thermo Fisher Scientific) for 15 mins at RT, permeabilized with detergent for 3 mins at RT and then treated with protease solution for 10 mins at RT. Incubation of target-specific probe pairs (Thermo Fisher Scientific) was done for 3 hrs, while for Preamplifiers, Amplifiers and Label Probes (Thermo Fisher Scientific) were for 1 hr each. All hybridization steps were carried out at 40 °C in Cytomat 2 C-LIN automated incubator (Thermo Fisher Scientific). After hybridization reactions, cells were stained for 30 mins with Hoechst and CellMask Blue (Thermo Fisher Scientific) and then imaged on Opera Phenix (PerkinElmer). The images were analyzed using Columbus Image Data Storage and Analysis System (PerkinElmer) to obtain mean spot counts per cell. The spot counts were normalized using the high (containing phosphate-buffered saline, Corning) and low (without target probe pairs) control wells. The normalized values against the total siRNA concentrations were plotted and the data were fit to a four-parameter sigmoidal model in Genedata Screener (Genedata) to obtain IC50 and maximum activity.

[0188] The results of the RNA FISH assay for Hep3B cells are shown in Table 5 for duplexes D-2080 to D-2109, Table 6 for Triggers D-2110 to D-2124, and Table 7 for Triggers D-2125 to D2146. Negative values for Max activity indicate knockdown of activity.

Table 5. RNA FISH assay on Hep3B cells

Duplex No.	IC50 (nM)	Max activity
D-2080	3.31	-71.4
D-2081	7.39	-81.2
D-2082	2.16	-82.4
D-2083	0.791	-87.4
D-2084	0.524	-90.4
D-2085	2.13	-88.4
D-2086	4.55	-72.6
D-2087	3.27	-90.1
D-2088	1.92	-82.8
D-2089	1.67	-76.8
D-2090	4.43	-77.8
D-2091	1.15	-93.1
D-2092	12.7	-71.8

D-2093	8.21	-83.8
D-2094	2.01	-79.9
D-2095	14.3	-84.6
D-2096	1.63	-84.3
D-2097	4.95	-89.5
D-2098	3.02	-90.8
D-2099	2.17	-79.7
D-2100	0.561	-92.9
D-2101	3.6	-89.0
D-2102	9.75	-77.9
D-2103	6.06	-81.2
D-2104	9.87	-77.4
D-2105	2.04	-83.2
D-2106	1.79	-85.0
D-2107	3.83	-82.8
D-2108	1.06	-89.8
D-2109	0.152	-85.6

Table 6. RNA FISH assay on Hep3B cells

Duplex No.	IC50 (nM)	Max activity
D-2110	13.4	-74.1
D-2111	20.8	-73.8
D-2112	9.2	-71.4
D-2113	16.8	-70.2
D-2114	7.81	-80.3
D-2115	11.9	-62
D-2116	31.6	-58.5
D-2117	1.75	-79.6
D-2118	13.4	-66
D-2119	10.7	-65.5
D-2120	2.8	-56.4
D-2121	3.9	-51.7
D-2122	14.2	-67.9
D-2123	4.3	-63.3
D-2124	8.7	-62.2

Table 7. RNA FISH assay on Hep3B cells

Duplex No.	IC50 (nM)	Max activity
D-2125	2.77	-63.4
D-2126	5.68	-74.4
D-2127	1.21	-81
D-2128	1.52	-69.1
D-2129	9.21	-90.2
D-2130	0.691	-84.6
D-2131	1.54	-85.4
D-2132	1.5	-83.4
D-2133	4.58	-89.3
D-2134	1.35	-70
D-2135	2.37	-75.4
D-2136	1.22	-71.5
D-2137	2.87	-67.4
D-2138	2.36	-62.4
D-2139	1.46	-68
D-2140	0.942	-71.7
D-2141	0.769	-79.7
D-2142	5.57	-60.9
D-2143	3.5	-64.5
D-2144	3.39	-64.1
D-2145	3.07	-75.3
D-2146	5.77	-72

Example 5: Screening of siRNA triggers modified with destabilizing bases

[0189] C57Bl6/J male mice between 9-10 wks of age were obtained from Charles River Laboratories and acclimatized in house. Mice were weighed and randomized into groups of 8 animals. These animals were subcutaneously dosed with SCAP siRNA triggers at 3 mg per kg body weight. Stock siRNA compounds were diluted in phosphate buffer solution without calcium and magnesium (Thermo Fischer Scientific, 14190-136) right before dosing. 30 days after siRNA treatment, animals were euthanized, and liver harvested. Freshly isolated left lobe of the liver was immediately snap frozen in liquid nitrogen. 30-50mg of liver tissue was used to isolate RNA using the QIAcube HT instrument RNeasy 96 QIAcube HT kits according to manufacturer's protocol. 2-4ug of RNA was treated with RQ1 RNase-Free DNase (Promega, M6101). 10ng of DNAse digested RNA was subjected to Real Time qPCR using the TaqMan RNA to CT 1 step kit (Applied Biosystems) run on the Quant Studio Real Time PCR machine.

TaqMan probes for mouse SCAP (Mm01250176_m1) and GAPDH (4352932E) were used to calculate the fold change of SCAP expression in the SCAP siRNA treated groups compared to the PBS (buffer control) group. Data is represented as percent knockdown in the siRNA treated group with respect to the PBS group. 5 trigger sequences, D-2040, D-2041, D-2042, D-2044, and D-2045 were tested, with various destabilizing modifications. The destabilizing base modification included both GNA and abasic modification patterns. The data are shown in Table 8. In each case, the modification patterns containing destabilizing bases resulted in lower SCAP mRNA expression compared to the parental trigger modification. Duplexes are shown with % SCAP knockdown.

Table 8. Silencing in siRNA with destabilizing base modifications.

Group	Duplex No.	% SCAP knockdown
1	D-2040	65.7
2	D-2110	25.6
3	D-2111	45.44
4	D-2112	16.51
5	D-2113	25
6	D-2045	65.83
7	D-2114	38.62
8	D-2115	44.6
9	D-2116	28.37
10	D-2042	64.16
11	D-2117	39.55
12	D-2118	19.95
13	D-2119	21.54
14	D-2041	50.6
15	D-2120	24.31
16	D-2121	31.93
17	D-2044	55.27
18	D-2122	23.31
19	D-2123	29.65
20	D-2124	17.29

Example 6: Screening of siRNA triggers in C57Bl6/J male mice

[0190] D-2040, D-2045, D-2042, D-2041 and D-2044 sequences were additionally modified with different chemical modification patterns. These modified triggers were compared to the original trigger modification pattern. Group 1 through 5 includes the trigger sequence D-2040 and variations of modification patterns. Group 6 through 10 includes the trigger sequence D-2045 and variations of modification patterns. Group 11 through 15 includes the trigger sequence D-2042 and variation of modification patterns. Group 16 through 21

includes the trigger sequence D-2041 and variation of modification patterns. Group 22 through 27 includes the trigger sequence D-2044 and variation of modification patterns. For the live phase of the in vivo study, C57Bl6/J male mice between 13-15 wks of age were obtained from Charles River Laboratories and acclimatized in house. Mice were weighed and randomized into groups each with 8 animals. These animals were subcutaneously dosed with SCAP siRNA triggers at 3 mg per kg body weight. Stock siRNA compounds were diluted in phosphate buffer solution without calcium and magnesium (Thermo Fischer Scientific, 14190-136) before dosing. 30 days after siRNA treatment, animals were euthanized, and liver harvested. Freshly isolated left lobe of the liver was immediately snap frozen in liquid nitrogen. 30-50mg of liver tissue was used to isolate RNA using the QIAcube HT instrument RNeasy 96 QIAcube HT kits according to manufacturer's protocol. 2-4ug of RNA was treated with RQ1 RNase-Free DNase (Promega, M6101). 10ng of DNAse digested RNA was subjected to Real Time qPCR using the TaqMan RNA to CT 1 step kit (Applied Biosystems) run on the Quant Studio Real Time PCR machine. TaqMan probes for mouse SCAP (Mm01250176_m1) and GAPDH (4352932E) were used to calculate the fold change of SCAP expression in the siRNA treated groups compared to the PBS (buffer control) group. Data is shown in Table 9 and represented as percent knockdown in the siRNA treated group with respect to the PBS group. As shown, different modification patterns produce different levels of silencing.

Table 9. Silencing in siRNA with different modification patterns.

Group	Duplex No.	% silencing
1	D-2042	77.42
2	D-2125	89.01
3	D-2126	86.53
4	D-2127	85.41
5	D-2128	75.89
6	D-2045	79.51
7	D-2129	78.08
8	D-2130	77.45
9	D-2132	79.94
10	D-2131	77.63
11	D-2040	70.64
12	D-2133	65.37
13	D-2134	59.97
14	D-2135	66.46
15	D-2136	62.19
16	D-2041	60.59
17	D-2137	49.87
18	D-2138	47.39

19	D-2139	68.76
20	D-2140	81.5
21	D-2141	71.83
22	D-2044	72.5
23	D-2142	67.22
24	D-2143	69.74
25	D-2144	61.39
26	D-2145	75.35
27	D-2146	60.32

Example 7: siRNA trigger silencing of SCAP in Ob/Ob animals

[0191] 10-12 weeks old male B6.V-Lep ob/J (632) mice, also known as Ob/Ob mice were obtained from Jackson Laboratories. Following acclimatization, these animals were randomized into groups of n=8. Mice were treated with SCAP siRNA triggers which were modified with chemical modifications as identified from the screening experiments in the above examples. Mice were subcutaneously dosed with 3 milligram siRNA per Kg body weight. 20 days and 30 days following siRNA dosing, animals were sacrificed. Following euthanasia, the left lobe of the liver was isolated and immediately snap frozen in liquid nitrogen. 30-50 mg of liver tissue was used to isolate RNA using the QIAcube HT instrument RNeasy 96 QIAcube HT kits according to manufacturer's protocol. 2-4ug of RNA was treated with RQ1 RNase-Free DNase (Promega, M6101). 10ng of DNAse digested RNA was subjected to Real Time qPCR using the TaqMan RNA to CT 1 step kit (Applied Biosystems) run on the Quant Studio Real Time PCR machine. TaqMan probes for mouse SCAP (Mm01250176_m1) and GAPDH (4352932E) were used to calculate the fold change of SCAP expression in the siRNA treated groups compared to the PBS (buffer control) group. Data is shown in Table 10 and represented as percent knockdown in the siRNA treated group with respect to the PBS group.

Table 10. siRNA knockdown of SCAP in ob/ob animals

Duplex No.	Day 20 harvest % silencing	Day 30 harvest % silencing
D-2040	80.6	71.0
D-2126	84.4	77.2
D-2140	74.0	60.4
D-2145	80.7	74.5
D-2147	16.4	-21.3

D-2148	8.3	-1.0
D-2149	-5.0	-14.9
D-2150	22.4	-14.6

Example 8: Efficacy studies with SCAP triggers

Prevention and rescue of NASH phenotype in efficacy models

1] Amylin (AMLN) AMLYN model

[0192] The Amylin Liver NASH model (AMLN) model was developed by feeding 5 week old obese male mice (Ob/Ob) obtained from Jackson Laboratories, Strain: B6.V-Lep ob/J (632) with a high fat, high cholesterol diet. The 45% fat, 36% carbohydrate and 2% cholesterol diet was obtained from Envigo, catalog number: TD170748. Regular water was replaced with a sugar solution containing 55% fructose and 46% glucose in water. Mice were randomized into groups of 8 and treated with Trigger D-2040 or with Trigger D-2147 or with PBS. D-2147 is a seed sequence matched control to Trigger D-2040 where the nucleotides 9 through 11 has been switched. Mice were subcutaneously dosed Q2D with 3 milligram per kg body weight dose on week 8, week-10 and week-12 of the AMLN diet. Stock siRNA compounds were diluted in phosphate buffer solution without calcium and magnesium (Thermo Fischer Scientific, 14190-136) right before dosing. Mice were harvested on week-14 of the diet, after 6 weeks of continued SCAP silencing. During harvest, following euthanasia under isoflurane, the medial lobe of the liver was fixed in 10% neutral buffered formalin. The formalin fixed medial lobe was further processed for Hematoxylin and Eosin (Dako, CS70030-2, CS70130-2), Trichrome staining and alpha smooth muscle actin (aSMA) expression by immunohistochemistry (IHC) according to the manufacturer's instruction. NASH readout was done by scoring for fibrosis and stellate cell activation and the reading was performed by a board certified pathologist.

[0193] The left lobe of the liver was snap frozen in liquid nitrogen. The snap frozen tissue was further processed for RNA extraction and evaluation of gene expression as detailed in Example 7. Further, hepatic triglyceride content was measured by homogenizing 50-100 mg of snap frozen liver tissue in isopropanol. Samples were homogenized and incubated in ice for 1 hour and then spun at 10000 rpm for 10 minutes. The supernatant was transferred to a clean deep well 96 well plate. Triglyceride content was determined by a colorimetric assay (Infinity Triglyceride Reagent, Thermo Fisher Scientific, TR22421) and using the standard (Pointe

Scientific T7531-STD) according to the manufacturer's instruction. The data is represented as milligrams of triglyceride per milligram of tissue.

[0194] Additional endpoints captured during the harvest include measuring the liver weight. The ratio of whole liver weight (in grams) and the terminal body weight (in grams) was analyzed to monitor alterations in liver mass. SCAP silencing inhibits PCSK9 expression. Serum PCSK9 levels were measured as a biomarker using the ELISA assay (R&D Systems, MPC900).

[0195] Figure 1A depicts the expression of SCAP mRNA represented as fold change over PBS control group. Trigger D-2040 treated group achieved ~85% SCAP silencing (85.3%), while there was no significant change in the D-2147 treated group. Figure 1B shows significant reduction in the terminal liver weight: body weight ratio in Trigger D-2040 treated mice. Figure 1C shows liver triglyceride lowering in the Trigger D-2040 treated mice, while it remained unchanged in the D-2147 treated group. In Figure 1D, serum PCSK9 levels were measured. Efficient SCAP silencing reduced the serum PCSK9 levels significantly, . In Figure 1E and 1F, the pathology readout of fibrosis (Trichrome stain) and stellate cell activation (aSMA immunohistochemistry) are shown. SCAP silencing with trigger D-2040 significantly reduced the fibrosis scores, suggesting improved NASH outcome. Statistical significance was measured by One-way ANOVA using Dunnett's multiple comparison test, with asterisks indicating adjusted p value(**** p value <0.0001. *** p value<0.001).

2] ALIOS model

[0196] The American Lifestyle Induced Obesity Syndrome mouse model (ALIOS) was also used to test the efficacy of SCAP triggers. C57Bl6 male mice obtained from Charles River Laboratories at 5 weeks of age were fed with the ALIOS diet, which is similar to the AMLN diet except for a reduced cholesterol content. The ALIOS diet contained 0.2% cholesterol and was obtained from Envigo (Catalog number TD130885). Regular water was replaced with a sugar solution containing 55% fructose and 46% glucose in water. 18 weeks after feeding animals on the ALIOS diet, mice were subcutaneously dosed bi-weekly with 3 milligram per kg body weight for 6 weeks. Mice were randomized into groups of n=4-5 and treated with Trigger D-2040, Trigger D-2042 or with PBS. Mice were harvested after 6 months of diet and 6 weeks of SCAP silencing. Similar to the previous efficacy study, end point analyses included SCAP message levels, terminal liver weight body weight ratio, hepatic triglyceride levels,

serum PCSK9 levels and pathological readout of fibrosis using Trichrome staining and stellate cell activation using alpha smooth muscle actin immunohistochemistry staining.

[0197] Figure 2A shows SCAP mRNA expression. Data is represented as a fold change over the PBS group. Both SCAP triggers D-2040 and D-2042 exhibited above 85% reduction in SCAP mRNA. In Figure 2B, significant reductions were observed in terminal liver weight/body weight (LW/BW) ratio in the groups treated with SCAP triggers D-2040 and D-2042 Figure 2C shows the hepatic triglyceride levels in the different groups. Administration of SCAP triggers D-2040 and D-2042 significantly reduced the hepatic triglyceride content when compared to the buffer control group. In Figure 2D, serum PCSK9 was measured using the ELISA kit described above. The levels of serum PCSK9 in SCAP siRNA treated groups were significantly lower compared to the PBS group. Figures 2E and 2F show pathology readout of fibrosis, measured by trichrome stains and immunostaining of alpha smooth muscle actin indicating stellate cell activation. Either readouts show reduction after SCAP silencing, suggesting rescue of NASH phenotype after SCAP silencing. Statistical significance was measured by One-way ANOVA using Dunnett's multiple comparison test, with asterisks indicating adjusted p value (****$p<0.0001$, ***$p<0.005$, **$p<0.01$ and *$p<0.05$)

Example 9: Utilizing the DIAMOND model to test SCAP siRNA efficacy to treat hepatocellular carcinoma

[0198] More than 50% of hepatocellular carcinoma patients have non-alcoholic fatty liver disease. In order to test the efficacy of SCAP siRNA in preventing further progression of HCC, a model where HCC is manifested with prolonged NASH diet, without using a chemical modifier, is implemented. Such models better represent the human pathophysiology. One such model is the Diet Induced Animal Model Of Non alcoholic fatty liver Disease (or DIAMOND) model. It is developed using a unique isogenic animal strain obtained from C57Bl/6J and 129ISvImJ backgrounds. Beginning at week 8 of age, male mice from this intergenic colony are fed with a high fat, high carbohydrate diet (42% kcal from fat) containing 0.1% cholesterol. In addition, the drinking water is also replaced with a high fructose-glucose solution. After 32 weeks of this diet, the model develops HCC. By week 51, the DIAMOND model liver tissues exhibit large areas of tumor and focus of alteration within the hepatocytes. The model is also highly penetrant. To test efficacy, SCAP siRNA and vehicle control are administered on week-40 of the diet. Mice are re-administered vehicle or SCAP siRNA at regular intervals for 6-10 weeks to ensure SCAP gene expression reduction. Endpoint analyses include pathological

examination of hepatocellular tumor burden metastatic tumor index, assessment of tumor proliferation using Ki67 expression and extent of tumor angiogenesis using CD31 expression. In addition, qPCR and protein analysis are evaluated to confirm efficient silencing of the target and the downstream pathway.

Example 10: Evaluation of SCAP siRNA in the Huh-7 Liver Xenograft model of HCC

[0199] SCAP siRNA are evaluated utilizing an orthotopic Huh-7 liver xenograft model. 6-week old BALB/c athymic nude mice are injected intrahepatically with 1 million Huh-7 cells suspended in cell culture media with 33% Matrigel. Subsequently, mice are divided into groups for treatment with either vehicle or SCAP siRNA. Vehicle or SCAP siRNA are re-administered at regular intervals (e.g., biweekly) to ensure sustained reduction of SCAP mRNA. At various timepoints (e.g., 4 weeks) following the initial vehicle or siRNA treatment, mice are euthanized, and the livers are harvested and fixed in 4% paraformaldehyde. Tumor burden is measured to understand the efficacy of SCAP siRNA treatment. In addition, qPCR and protein analysis are evaluated to confirm efficient silencing of the target.