

NUCLEIC ACID TARGETING ANGIOTENSINOGEN AND USE THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present disclosure claims priority to Chinese Patent Application No. 202310718430.6, filed on June 16, 2023, which is incorporated herein by reference in its entirety.

TECHNICAL FIELD

[0002] The present disclosure pertains to the field of biotechnologies, and specifically, relates to an angiotensinogen-targeting nucleic acid and use thereof.

BACKGROUND

[0003] The renin-angiotensin-aldosterone system (RAAS) is a complex network regulatory system formed by various proteases and short peptides, and serves as a crucial regulatory factor for cardiovascular and renal functions. Excessive activation of the system constitutes a central link in many common pathological conditions, including hypertension, heart failure, and kidney diseases (Lu H et al., Hypertension Res. 39:492-500, 2016). The RAAS pathway initiates with renin cleaving its substrate angiotensinogen to generate the inactive peptide angiotensin I (Ang I), then angiotensin-converting enzyme (ACE) in endothelial cells converts Ang I into angiotensin II (Ang II), with ACE-mediated activation of Ang II being most prevalent in the lungs. Ang II induces vascular contraction and stimulates the adrenal glands to release aldosterone, thereby causing sodium retention and an increase in blood pressure.

[0004] RAAS inhibitors include ACE inhibitors, angiotensin II receptor blockers (ARBs), aldosterone antagonists, direct renin inhibitors, and the like. The RAAS inhibitors are existing important medications for treating hypertension and preventing and treating heart failure, and have wide clinical application (Schmieder RE et al., Lancet. 369 (9568):1208-1219; Antonaccio MJ., J Pharmacol., 14:29-45, 1983; Ruiz-Ortega M et al., Trends Cardiovasc Med. 17 (1): 19-25, 2007; Matsubara H., Cric Res. 83 (12):1182-1191, 1998). The ACE inhibitors and angiotensin II receptor blockers can activate compensatory pathways, which further cause reactivation of angiotensin and aldosterone escape. As a result, concentrations of Ang II and aldosterone in the blood return to pre-treatment levels or become even higher (Nobakht N et al., Nat Rev Nephrol. 7:356-359, 2011; Bomback AS and Klemmer PJ., Nat Clin Pract Nephrol. 3:486-492,2007). This may be a key factor causing poor response of patients with refractory hypertension and heart failure to RAAS-inhibiting drugs (Narayan H and Webb DJ., Curr Hypertens Rep. 18:34, 2016, Roig E et al., Eur Heart J. 21: 53-57, 2000). Therefore, a

more effective therapeutic strategy should be to target the upstream of RAAS enzymes and receptors to avoid compensatory mechanisms and intracrine pathways that limit therapeutic effects (Mullick AE et al., Hypertension. 70(3): 566-576, 2017).

[0005] Angiotensinogen (AGT; also known as SERPINA8, ANHU, and hFLT1) is the common precursor of all angiotensins (Wu C et al., Am J Med Sci. 4: 183-190, 2011; Lu H et al., Hypertens Res. 39: 492-500, 2016). The liver is the main source of AGT in the blood (Yiannilouris G et al., Hypertension. 66: 836-842, 2015; Matsusaka T et al., J Am Soc Nephrol. 23: 1181-1189, 2012). Multiple studies have confirmed that an increase in blood AGT concentration is significantly positively correlated with hypertension (Fasola AF et al., J Appl Physiol. 21:1709-1712, 1966). Reducing the blood AGT concentration can inhibit activity of the RAAS pathway and cause a decrease in blood pressure (Olearczyk J et al., Hypertension Res. 37:405-412, 2014). Intravenous infusion of AGT into rats can increase blood pressure, and the increase in blood pressure can be reversed by treatment with anti-AGT antibodies (Menard J et al., Hypertension. 18:705-707, 1991). AGT-knockout mice have reduced blood pressure, while overexpression of AGT causes an increase in the blood pressure (Kim HS et al., Proc Natl Acad Sci USA92:2735-2739, 1995; Kimura S et al., Embo J. 11:821-827, 1982). Regulating AGT levels to treat hypertension is a promising research and development direction. However, numerous difficulties have been encountered in targeting AGT in conventional methods (Morgan L et al., Int J Biochem Cell Biol.28:1211-1222, 1996).

[0006] RNA interference (RNAi) refers to a highly conserved phenomenon of efficient and specific degradation of homologous mRNA induced by double-stranded small interfering RNA (siRNA) in an evolutionary process. Therefore, developing AGT-targeting siRNA is of great significance.

SUMMARY OF THE INVENTION

[0007] An objective of the present disclosure is to overcome the problems existing in the prior art and provide a new AGT-targeting nucleic acid and use thereof.

[0008] A first aspect of the present disclosure provides a nucleic acid including a sense strand and an antisense strand, where the sense strand includes a sequence having at least 80% sequence identity with a sequence as set forth in any one of SEQ ID NOs: 1 to 26, and the antisense strand includes a sequence having at least 80% sequence identity with a sequence as set forth in any one of SEQ ID NOs: 27 to 52.

[0009] A second aspect of the present disclosure provides a targeted drug delivery system including a targeting moiety, a linking moiety, and the foregoing nucleic acid linked to the targeting moiety through the linking moiety.

[0010] A third aspect of the present disclosure provides a pharmaceutical composition including the foregoing nucleic acid or targeted drug delivery system, and a pharmaceutically acceptable carrier.

[0011] A fourth aspect of the present disclosure provides a method of inhibiting expression of angiotensin gene in a cell, including: contacting the cell with the foregoing nucleic acid, targeted drug delivery system, or pharmaceutical composition to inhibit the expression of the angiotensin gene in the cell.

[0012] A fifth aspect of the present disclosure provides use of the foregoing nucleic acid, targeted drug delivery system, or pharmaceutical composition in any one of the following aspects: (1) treatment and/or prevention of a disease associated with angiotensin; and (2) preparation of a medicament for treating and/or preventing a disease associated with angiotensin.

[0013] The inventors of the present disclosure have found that in the renin-angiotensin-aldosterone system, AGT is the most suitable target for RNAi. Therefore, the present disclosure provides a new AGT-targeting nucleic acid and use thereof. The nucleic acid in the present disclosure can effectively inhibit the expression of AGT and reduce the activation level of RAAS, and therefore, can be used to treat and/or prevent hypertension and other AGT-related diseases.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] To describe the technical solutions in the specific embodiments of the present disclosure or in the prior art more clearly, the following briefly describes the accompanying drawings required for describing the specific embodiments or the prior art. Apparently, the accompanying drawings in the following description show merely some embodiments of the present disclosure, and a person of ordinary skills in the art may still derive other drawings from these accompanying drawings without creative efforts.

[0015] FIG. 1 shows the efficacy of SN-682160, SN-682172, SN-682173, SN-682175, and SN-682176 in human AGT transgenic mice in an example of the present disclosure.

[0016] FIG. 2 shows the efficacy of SN-682726 and SN-682728 in human AGT transgenic mice in an example of the present disclosure.

[0017] FIG. 3 shows the efficacy of SN-682726 and SN-2073 in human AGT transgenic mice in an example of the present disclosure.

DETAILED DESCRIPTION

[0018] The specific embodiments of the present disclosure are described in detail hereinafter. It should be understood that the specific embodiments described herein are only intended to illustrate and explain the present disclosure, rather than limiting the present disclosure. A person skilled in the art can make various modifications and changes to the present disclosure without departing from the scope or spirit of the present disclosure. For example, features illustrated or described as a part of an embodiment can be used in another embodiment to create still another embodiment.

[0019] Term Interpretation

[0020] Unless otherwise specified, all terms (including technical and scientific terms) used to disclose the present disclosure have the same meanings as commonly understood by a person of ordinary skill in the art to which the present disclosure belongs. With reference to further guidance, the following definitions are used to better understand teachings in the present disclosure. The terms used herein in the description of the present disclosure are only for the purpose of describing specific examples, and are not intended to limit the present disclosure.

[0021] The term "and/or" as used herein refers to a selection range that includes any one of the listed items among two or more related items, as well as any and all combinations of those listed items. These combinations may include any two of the related items, any greater number of the related items, or all of the related items. In the present disclosure, it should be understood that when at least two items are connected by "and/or", the technical solutions undoubtedly include technical solutions connected by logical "AND", and also undoubtedly include technical solutions connected by logical "OR". For example, "A and/or B" includes three parallel solutions: A, B, and both A and B. For another example, technical solutions of "A, B, C and/or D" include any one of A, B, C and D (namely, a technical solution connected by logical "OR"), and also include a combination of any one and all of A, B, C and D, that is, a combination of any two or three of A, B, C and D and a combination of four of A, B, C and D (namely, technical solutions connected by logical "AND").

[0022] The terms "comprise", "include", and "contain", as well as their variations, as used herein are synonymous, and are inclusive or open-ended, and do not exclude additional uncited members, elements, or method steps.

[0023] In the present disclosure, a numerical range defined by endpoints includes all values and fractions within the range, as well as the cited endpoints.

[0024] In the present disclosure, concentration values refer to values that may fluctuate within a certain range. For example, fluctuations may occur within the corresponding precision range. For instance, a value of 2% may allow fluctuations within $\pm 0.1\%$. For larger values or values that do not require precise control, the meaning may include greater fluctuations. For example, a value of 100 mM may allow fluctuations within $\pm 1\%$, $\pm 2\%$, $\pm 5\%$, etc. For molecular weight, the meaning may include fluctuations within $\pm 10\%$.

[0025] In the present disclosure, descriptions such as "multiple" and "various" refer to two or more than two, unless otherwise specified.

[0026] In the present disclosure, technical features described in an open-ended manner encompass both closed technical solutions consisting of the listed features and open technical solutions that include the listed features.

[0027] In the present disclosure, expressions such as "preferably", "more preferably", "most preferably", or "desirably" are used solely to describe embodiments or examples with better effects, and shall not be construed as limiting the scope of protection of the invention.

[0028] In the present disclosure, terms such as "optionally", "optional", or equivalents thereof indicate that the feature may or may not be present, i.e., selected from two parallel options: presence or absence. Where multiple instances of "optional" features appear in a technical solution, unless otherwise specified and provided there is no contradiction or mutual exclusivity, each "optional" feature shall be considered independently.

[0029] In the present disclosure, the term "nucleic acid" refers to a composition containing RNA or RNA-like (for example, chemically modified RNA) oligonucleotide molecules, and the oligonucleotide molecules are capable of degrading or inhibiting (for example, degrading or inhibiting under proper conditions) translation of messenger RNA (mRNA) transcripts of the target mRNA in a sequence-specific manner. The nucleic acid can exert an effect through the RNA interference mechanism (that is, RNA interference induced by interaction via an RNA interference pathway mechanism (RNA-induced silencing complex, RISC) of mammalian cells) or any alternative mechanism or pathway. The scope of lower limitations for the nucleic acids that include a sense strand and an antisense strand and that are disclosed herein includes, but is not limited to, short (or small) interfering RNA (siRNA), double-stranded RNA (dsRNA), microRNA (miRNA), short hairpin RNA (shRNA), and dicer substrates.

[0030] In the present disclosure, when referring to the expression of a given gene, the term "silencing", "reduction", "inhibition", "downregulation" or "knockdown" refers to a decrease

in gene expression, as measured by the level of RNA transcribed from the gene or the level of polypeptide, protein, or protein subunit translated from the mRNA, in cells, cell populations, tissues, organs, or subjects in which the gene is transcribed, when treated with the nucleic acid described herein, as compared to untreated cells, cell populations, tissues, organs, or subjects.

5 **[0031]** In the present disclosure, the term "fully complementary" means that, in a hybridized pair of nucleobases or nucleotide sequence molecules, all (100%) bases in a contiguous sequence of the first oligonucleotide hybridize with the same number of bases in a contiguous sequence of the second oligonucleotide. The contiguous sequence may include all or part of the first nucleotide sequence or the second nucleotide sequence.

10 **[0032]** In the present disclosure, the term "partially complementary" means that, in a hybridized pair of nucleobases or nucleotide sequence molecules, at least 70%, but not all, of the bases in a contiguous sequence of the first oligonucleotide hybridize with the same number of bases in a contiguous sequence of the second oligonucleotide. The contiguous sequence may include all or part of the first nucleotide sequence or the second nucleotide sequence.

15 **[0033]** In the present disclosure, the term "substantially complementary" means that, in a hybridized pair of nucleobases or nucleotide sequence molecules, at least 85%, but not all, of the bases in a contiguous sequence of the first oligonucleotide hybridize with the same number of bases in a contiguous sequence of the second oligonucleotide. The contiguous sequence may include all or part of the first nucleotide sequence or the second nucleotide sequence.

20 **[0034]** In the present disclosure, the term "at least partially complementary" means that, in a hybridized pair of nucleobases or nucleotide sequence molecules, the first oligonucleotide and the second oligonucleotide are partially, substantially, or fully complementary.

25 **[0035]** In the present disclosure, the term "treatment" refers to a method or step taken to relieve or alleviate the number, severity, and/or frequency of one or more disease symptoms in a subject. The treatment may include the prevention, management, prophylactic treatment, and/or inhibition or reduction of the number, severity, and/or frequency of one or more disease symptoms in a subject.

30 **[0036]** In the present disclosure, the term "link" means that two compounds or molecules are joined through a covalent bond. Unless otherwise specified, as used herein, the term "link" may refer to a connection between a first compound and a second compound with or without any intermediate atom or group of atoms.

[0037] Nucleic Acid

[0038] The present disclosure provides a nucleic acid (modified or unmodified) including a sense strand and an antisense strand, the sense strand includes a sequence having at least 80%

(for example, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) sequence identity with the sequence as set forth in any one of SEQ ID NOs: 1 to 26, and the antisense strand includes a sequence having at least 80% (for example, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) sequence identity with the sequence as set forth in any one of SEQ ID NOs: 27 to 52.

[0039] In some embodiments, the antisense strand has 15 to 30 (for example, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides (bases).

[0040] In some embodiments, the sense strand has 15 to 30 (for example, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides (bases).

[0041] During specific implementation, a person skilled in the art can combine the sequences provided in the present disclosure in consideration of the complementarity of the sense strand and the antisense strand, thereby obtaining the combined nucleic acid (siRNA).

[0042] In a preferred embodiment of the present disclosure, as shown in Table 1, the nucleic acid is selected from at least one of: siRNA-1 with a sense strand sequence of SEQ ID NO: 1 and an antisense strand sequence of SEQ ID NO: 27, siRNA-2 with a sense strand sequence of SEQ ID NO: 2 and an antisense strand sequence of SEQ ID NO: 28, siRNA-3 with a sense strand sequence of SEQ ID NO: 3 and an antisense strand sequence of SEQ ID NO: 29, siRNA-4 with a sense strand sequence of SEQ ID NO: 4 and an antisense strand sequence of SEQ ID NO: 30, siRNA-5 with a sense strand sequence of SEQ ID NO: 5 and an antisense strand sequence of SEQ ID NO: 31, ..., siRNA-24, siRNA-25, and siRNA-26.

[0043] In some preferred embodiments, the antisense strand includes at least 15, 16, 17, 18, 19, 20, 21, 22, or 23 consecutive nucleotides that differ from the sequence as set forth in any one of SEQ ID NOs: 27 to 52 by no more than 0, 1, 2, or 3 nucleotides, and the sense strand includes a nucleotide sequence that is at least partially complementary (for example, partially, substantially, or fully complementary) to the antisense strand.

[0044] In some preferred embodiments, the sense strand includes at least 15, 16, 17, 18, 19, 20, or 21 consecutive nucleotides that differ from the sequence as set forth in any one of SEQ ID NOs: 1 to 26 by no more than 0, 1, 2, or 3 nucleotides.

[0045] In the present disclosure, the sense strand and the antisense strand may have the same length or different lengths.

[0046] All nucleotide groups in the foregoing nucleic acid may be unchemically modified, or may include at least one modified nucleotide group, and the modification may be present in a nucleotide at any position.

[0047] In some embodiments, the sense strand and the antisense strand may be partially, substantially, or fully complementary.

[0048] In some preferred embodiments, the antisense strand includes a nucleotide sequence that differs from the sequence as set forth in any one of SEQ ID NO: 29, SEQ ID NO: 32, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, and SEQ ID NO: 44 by 0, 1, or 2 nucleotides, and the sense strand includes a nucleotide sequence that is at least partially complementary (for example, partially, substantially, or fully complementary) to the antisense strand. When the antisense strand has the foregoing sequence, the double-stranded RNA exhibits a significantly better inhibitory effect on AGT.

[0049] In some preferred embodiments, the sense strand includes a nucleotide sequence that differs from the sequence as set forth in any one of SEQ ID NO: 3, SEQ ID NO: 6, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, and SEQ ID NO: 18 by 0, 1, or 2 nucleotides.

[0050] In some more preferred embodiments, the antisense strand includes a nucleotide sequence that differs from the sequence as set forth in any one of SEQ ID NO: 29, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 43, and SEQ ID NO: 44 by 0, 1, or 2 nucleotides, and the sense strand includes a nucleotide sequence that is at least partially complementary to the antisense strand.

[0051] In some preferred embodiments, the sense strand includes a nucleotide sequence that differs from the sequence as set forth in any one of SEQ ID NO: 3, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 17, and SEQ ID NO: 18 by 0, 1, or 2 nucleotides.

[0052] In some further preferred embodiments, the antisense strand includes a nucleotide sequence that differs from the sequence as set forth in SEQ ID NO: 40 by 0, 1, or 2 nucleotides, and the sense strand includes a nucleotide sequence that is at least partially complementary (for example, partially, substantially, or fully complementary) to the antisense strand. When the antisense strand has this sequence, the double-stranded RNAs with different modifications all exhibit a better inhibitory effect on AGT.

[0053] In some preferred embodiments, the sense strand includes a nucleotide sequence that differs from the sequence as set forth in SEQ ID NO: 14 by 0, 1, or 2 nucleotides.

[0054] In some preferred embodiments, a content of GC in the nucleic acid is 20%-45%, more preferably 20%-35%.

[0055] In some embodiments, when the sense strand or antisense strand of the nucleic acid has a sequence identity of less than 100% with the corresponding sequence according to the present disclosure or differs by one or more nucleotides, the sense strand or antisense strand of

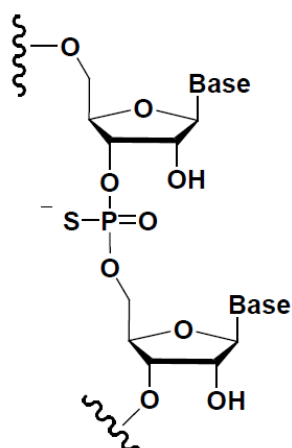
the nucleic acid still exhibits an inhibitory effect on AGT that is similar or equivalent to that of the corresponding sequence. For example, the UU linked to the 3' end of the antisense strand of the nucleic acid is replaced with AA, CC, GG, or UG, or a combination of any two nucleic acids. Such nucleic acid sequences also fall within the scope of protection of the present disclosure.

[0056] The effect advantages of the technical solutions related to naked sequences (that is, unmodified sequences) according to the present disclosure do not depend on the selection of the modification method or the targeting carrier. The applicable modification solutions and further preferred modification solutions are further introduced in detail as follows:

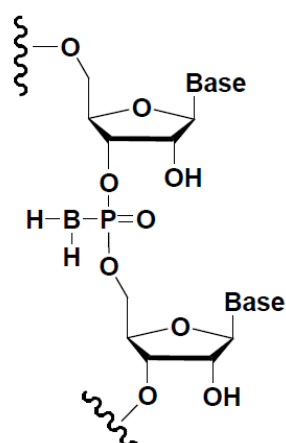
[0057] According to the nucleic acid of the present disclosure, the nucleic acid contains nucleotide groups as basic structural units, the nucleotide group includes a phosphate group, a ribose group, and a base, and preferably, the nucleic acid contains at least one modified nucleotide group. The inhibitory efficiency of the modified nucleic acid on AGT is not less than 50% (for example, 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 99%).

[0058] According to the nucleic acid of the present disclosure, the modified nucleotide group is a nucleotide group with its phosphate group and/or ribose group modified. The modified site(s) may be present at at least 1, 2, 3, 4, 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, or 30 of positions 1, 2, 3, 4, 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, or 30 of the sense strand and/or the antisense strand.

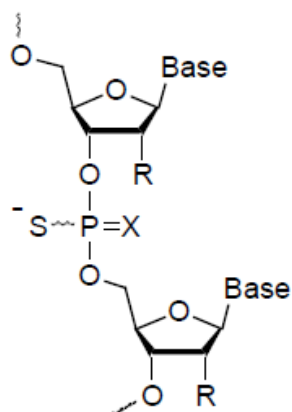
[0059] For example, modification of the phosphate group refers to the modification of oxygen in the phosphate group, including phosphorothioate modification, boranophosphate modification, and the like. As shown in the following formula, oxygen in the phosphate group is replaced with sulfur, borane, amino group, alkyl group, or alkoxy group, respectively. All these modifications can stabilize the structure of the nucleic acid and maintain high specificity and high affinity of base pairing.



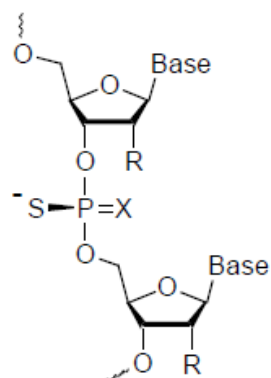
Phosphorothioate modification



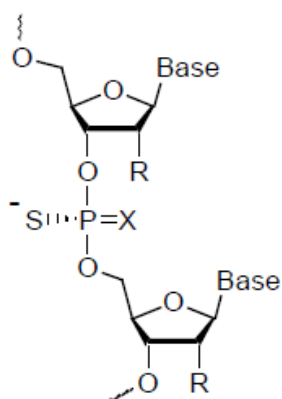
Boranophosphate modification



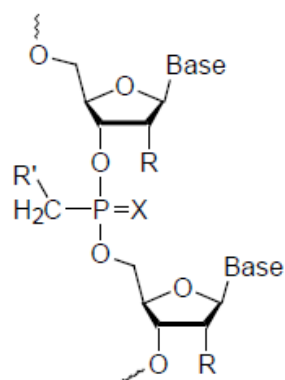
Non-stereospecific phosphorothioate modification



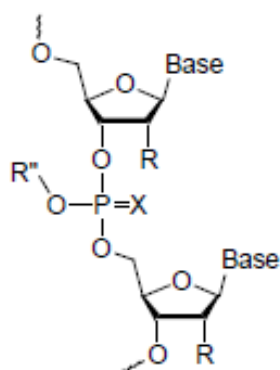
Stereospecific (Sp) phosphorothioate modification



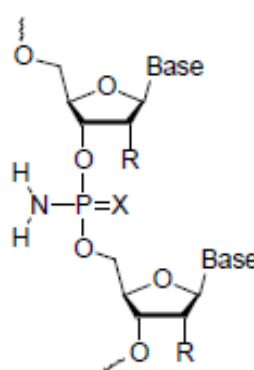
Stereospecific (Rp) phosphorothioate modification



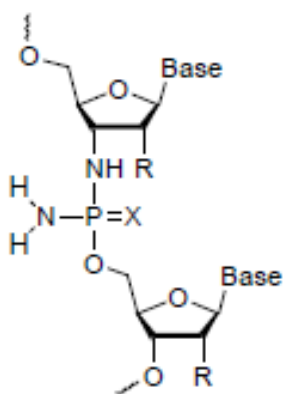
Alkyl phosphate modification



Alkoxy phosphate modification



Phosphoramidate modification

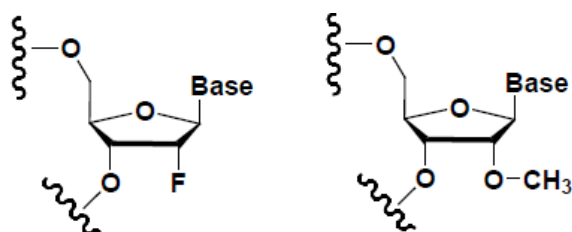


Phosphorodiamidate modification

[0060] In the above structural formula, Base represents the base A, U, C, G, or T. X may be oxygen (O) or sulfur (S). R may be the same or different in the above structure, such as hydrogen (H), fluorine (F), methoxy (OME) or methoxyethyl (MOE), hydroxyl, allyl, ethylamino, propargyl, amino, cyanoethyl, or acetyl.; R' and R'' may each independently be

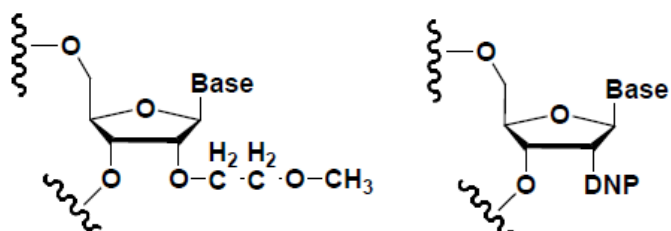
[0061] Modification of the ribose group refers to modification of the 2'-hydroxyl group (2'-OH) in the ribose group. After introducing certain substituents (e.g., a methoxy group or a fluoro group) into the 2'-hydroxyl of the ribose group, the nucleic acid is not readily cleaved by ribonuclease, thereby enhancing stability of the nucleic acid and allowing the nucleic acid to be more resistant to hydrolysis by the nuclease. Modifications of the 2'-hydroxyl group in the nucleotide pentose include 2'-fluoro modification (e.g., 2'-arabino-fluoro modification), 2'-methoxy modification (2'-OME), 2'-methoxyethyl modification (2'-MOE), 2'-2,4-dinitrophenol modification (2'-DNP modification), 2',4'-constrained ethyl modification, 2'-Amino modification, 2'-Deoxy modification, BNA, acyclic nucleic acid modification, mal-positioned nucleic acid modification, L-type nucleic acid modification and the like. BNA

(internal ring bridged nucleotide) refers to a constrained or inaccessible nucleotide. BNA may contain a bridging structure of a five-, six-, or seven-membered ring, with a "locked" C3'-endo sugar pucker. The bridge is typically incorporated into the 2', 4'-position of the ribose ring to provide the 2',4'-BNA nucleotides such as locked ethyl modification (LNA), constrained ethyl modification (ENA), and constrained ethyl bicyclic nucleic acid modification (cET BNA). Acyclic nucleic acid is a nucleotide formed after the ribose ring of the nucleotide is opened, such as unlocked nucleic acid (UNA) nucleotides and glycerol nucleic acid (GNA) nucleotides. Mal-positioned nucleic acid modification refers to a 3',5'-phosphate bond linkage substituted by the 2',5'-phosphate bond linkage. L-type nucleic acid modification refers to a naturally occurring D-type nucleic acid being substituted by its mirror-stereoscopic counterpart L-type nucleic acid.



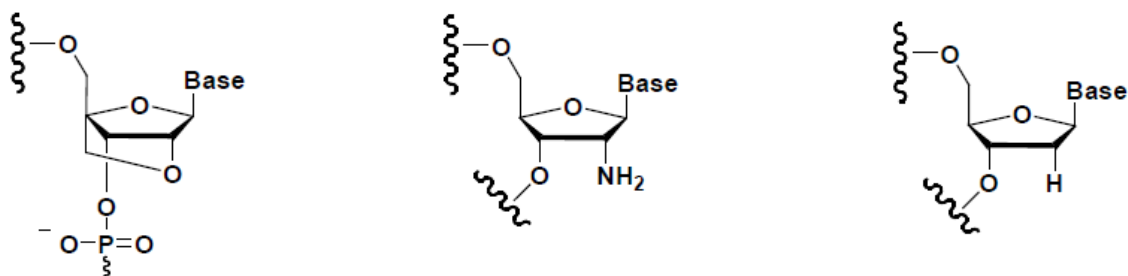
2'-fluoro modification

2'-OMe modification



2'-MOE modification

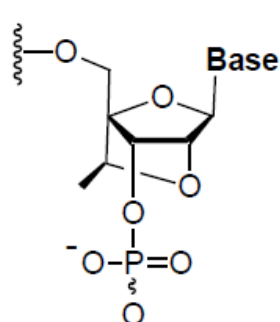
2'-DNP modification



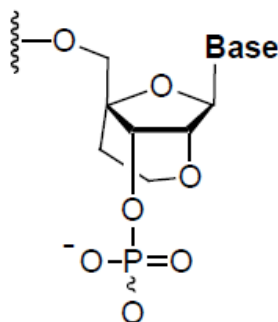
Locked nucleic acid modification

2'-amino modification

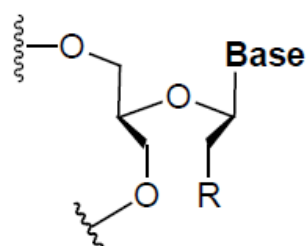
2'-Deoxy modification



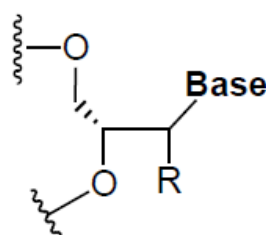
ENA modification



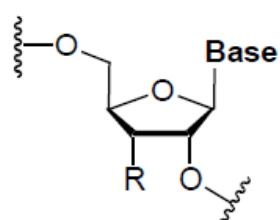
cET BNA modification



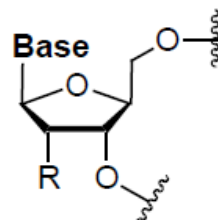
Unlocked nucleic acid modification



Glycerol nucleic acid modification



Mal-positioned nucleic acid modification



L-type nucleic acid modification

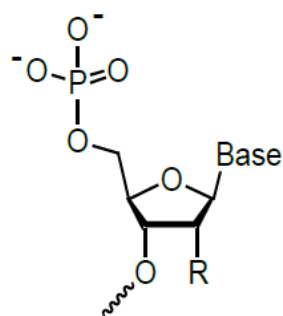
[0062] In the above structural formula, Base represents the base A, U, C, G, or T. R may be the same or different in the above structure, such as hydrogen (H), fluorine (F), methoxy (OME) or methoxyethyl (MOE), hydroxyl, allyl, ethylamino, propargyl, cyanoethyl, or acetyl.

5 **[0063]** According to the nucleic acid of the present disclosure, preferably, the nucleotide group with a modified ribose group is a nucleotide group in which the 2'-OH of the ribose group is replaced with a methoxy or fluoro group.

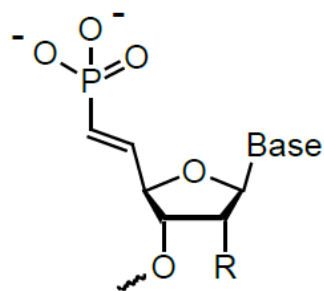
10 **[0064]** According to a particularly preferred embodiment of the present disclosure, the nucleotide groups containing uracil bases or cytosine bases in the sense strand of the nucleic acid are the nucleotide groups with their ribose groups modified. That is, 2'-OH of the ribose groups in the nucleotide groups containing uracil bases or cytosine bases in the sense strand of the nucleic acid is replaced with a methoxy or fluoro group. More preferably, the 3' ends of both the sense strand and the antisense strand of the nucleic acid may be linked to dTdT;

alternatively, the 3' end of the antisense strand of the nucleic acid may be linked to AA, UU, or a combination of any two nucleotides (which may be, but not limited to, CC, GG, or UG), so that the sequence has specificity as a trigger for mRNA degradation. Nucleic acids with the foregoing modifications exhibit better *in vivo* inhibitory effects, and the foregoing modifications can further reduce the immunogenicity of the nucleic acids in the present disclosure *in vivo*.

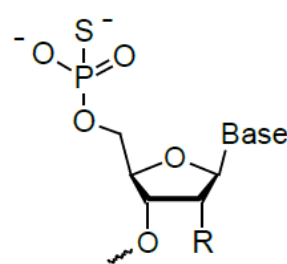
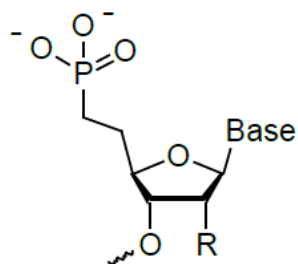
[0065] The nucleic acid in the present disclosure may further include the modification in which a monophosphate nucleoside is linked to the 5'-end of the antisense strand. The 5'-monophosphate at the end of the guide strand of the siRNA is important for RISC recognition. Phosphorylation of the 5'-hydroxyl group plays a certain role in whether the siRNA can be effectively loaded on the intracellular Ago2. The monophosphate at the 5'-end of the guide strand in the siRNA interacts with Argonaute-2(Ago2) through a hydrogen bond, in order to ensure accurate targeting and precise cleavage of the mRNA target. Several derivatives of the 5'-monophosphate nucleosides are commonly used, this type of derivative of the phosphate nucleoside has been proven to exhibit certain stability in the biological metabolism medium and to play a certain role in facilitating the loading of siRNA guide strand on the intracellular Ago2 (*Nucleic Acids Research*, 2015, 43, 2993-3011). According to the nucleic acid of the present disclosure, preferably, trans-vinyl phosphate (VP) is used as the first choice, and derivatives of nucleoside monophosphate other than those mentioned above may also be included.



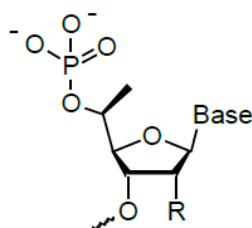
5'-monophosphate modification



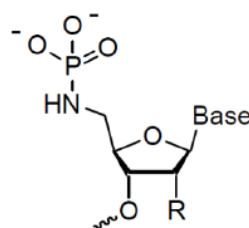
5'-trans vinyl phosphate modification



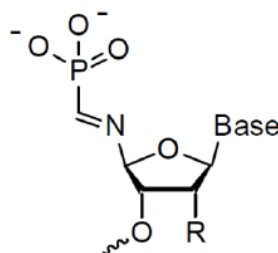
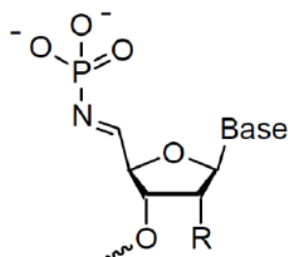
5'-ethyl phosphate modification



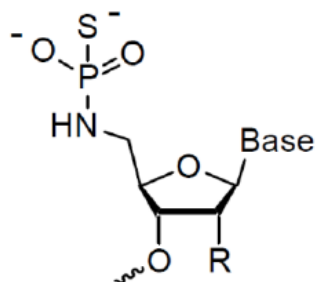
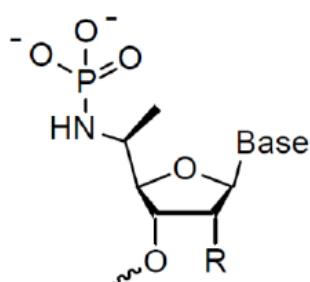
5'-phosphorothioate modification



5'-methoxymethylphosphonic acid modification 5'-phosphoramidate modification



5'-trans-phosphorimidate modification 5'-trans-formyl phosphate imidate modification



5'-methylphosphoramidate modification 5'-thiophosphoramidate modification

[0066] In the above structural formula, Base represents the base A, U, C, G or T. R may be the same or different in the above structure, such as hydrogen (H), fluorine (F), methoxy (OME) or methoxyethyl (MOE), hydroxyl, allyl, ethylamino, propargyl, cyanoethyl, amino, or acetyl.

[0067] In some embodiments, at least one nucleotide in the nucleic acid is a modified nucleotide or includes a modified internucleotide linkage.

[0068] In some preferred embodiments, the modified nucleotide is one or more selected from 2'-O-methyl nucleotide, 2'-fluoro nucleotide, 2'-deoxy nucleotide, 2',3'-seco nucleotide mimetic, locked nucleotide, 2'-F-arabinonucleotide, 2'-methoxyethyl nucleotide, abasic nucleotide, ribitol, inverted nucleotide, inverted 2'-O-methyl nucleotide, inverted 2'-deoxy nucleotide, 2'-amino modified nucleotide, 2'-alkyl modified nucleotide, morpholino

nucleotide, nucleotide containing vinyl phosphonate, nucleotide containing cyclopropyl phosphonate, and 3'-O-methyl nucleotide; and the modified nucleotide is further preferably one or more selected from 2'-O-methyl nucleotide and 2'-fluoro nucleotide.

[0069] In some preferred embodiments, the modified internucleotide linkage is preferably one or more selected from phosphorothioate internucleotide linkage and methylphosphonate internucleotide linkage; and the modified internucleotide linkage is further preferably one or more selected from phosphorothioate monoester internucleotide linkage and phosphorothioate diester internucleotide linkage.

[0070] In some embodiments, the nucleotides at positions 5 and 6 (counted from the 5' end) of the antisense strand are 2'-O-methyl nucleotides, and the nucleotide at position 7 is 2'-fluoro nucleotide. Based on any sequence according to the present disclosure, the foregoing modifications to the antisense strand can further significantly improve the inhibitory effect of siRNA on AGT.

[0071] In some preferred embodiments, the 5' end and 3' end of the antisense strand each contain 2 phosphorothioate internucleotide linkages, nucleotides at positions 1, 2, 7, 9, 14, and 16 (counted from the 5' end) are 2'-fluoro nucleotides, and all of the remaining nucleotides are 2'-O-methyl nucleotides.

[0072] In some embodiments, the nucleotide at position 2 (counted from the 5' end) of the sense strand is a 2'-O-methyl nucleotide or a 2'-fluoro nucleotide, preferably a 2'-O-methyl nucleotide. The above modifications to the sense strand can further improve the inhibitory effect of siRNA on AGT.

[0073] In some preferred embodiments, the 5' end of the sense strand contains 2 phosphorothioate internucleotide linkages, and the nucleotides at positions 4, 7, 9, 10, and 11 (counted from the 5' end) are 2'-fluoro nucleotides, and all of the remaining nucleotides are 2'-O-methyl nucleotide.

[0074] In some embodiments, the antisense strand has at least 15, 16, 17, 18, 19, 20, 21, 22, or 23 consecutive nucleotides that differ from the antisense strand sequence as set forth in any one in Table 3 or Table 8 by no more than 0, 1, 2, or 3 nucleotides; and the sense strand includes at least 15, 16, 17, 18, 19, 20, or 21 consecutive nucleotides that differ from the sense strand sequence as set forth in any one in Table 3 or Table 8 by no more than 0, 1, 2, or 3 nucleotides.

[0075] In some embodiments, the sense strand and the antisense strand form an siRNA as set forth in any one in Table 4 or 8.

[0076] In some embodiments, the antisense strand includes, from the 5' end to the 3' end, a nucleotide sequence that differs from the following nucleotide sequence by 0, 1, or 2 nucleotides:

[0077] AfsCfsacuuUfuUfuguuUfcAfcaaaacsasa;

5 **[0078]** the sense strand includes a nucleotide sequence that is at least partially complementary to the antisense strand.

[0079] In some embodiments, the sense strand includes, from the 5' end to the 3' end, a nucleotide sequence that differs from any one of the following nucleotide sequences by 0, 1, or 2 nucleotides:

10 **[0080]** gsusuUfguGfaAfAfCfaaaaaagugu; or

[0081] gsUfsuUfguGfaAfAfCfaaaaaagugu.

[0082] In each sequence of the present disclosure, the nucleotide represented by a lowercase letter is a 2'-O-methyl nucleotide; "f" indicates that the adjacent nucleotide on the left side is a 2'-fluoro nucleotide; and "s" indicates that the two adjacent nucleotides on the left and right
15 sides are linked by a phosphorothioate diester bond.

[0083] The nucleic acids according to the present disclosure can be obtained in conventional methods in the art, for example solid-phase synthesis and liquid-phase synthesis. Commercially customized services for the solid-phase synthesis are already available, and therefore, can be obtained through commercial purchase. The modified nucleotide groups can
20 be introduced via nucleotide monomers with corresponding modifications.

[0084] Based on the foregoing synthesized nucleic acids (siRNA), in the present disclosure, expression plasmids of shRNA that have the same or similar functions as the foregoing siRNA can be further constructed. The methods for constructing such expression plasmids are well-known to a person skilled in the art and are not described in detail herein again.

25 **[0085]** The present disclosure also provides target gene loci of the foregoing nucleic acids. In some embodiments, the target gene loci are as indicated in any one of the items in Column 1 of Table 1.

Table 1

Target gene loci	SEQ ID NO.	Sense strand (5'-3')	SEQ ID NO.	Antisense strand (5'-3')
778	1	CCACGCUCUCUGGACUUCACA	27	UGUGAAGUCCAGAGAGCGUGGGA
977	2	UGGACAACAGCACCUCAGUGU	28	ACACUGAGGUGCUGUUGUCCAUU
1145	3	GUCUCACUUUCCAGCAAAACU	29	AGUUUUGCUGGAAAGUGAGACUU
1167	4	CCUCAACUGGAUGAAGAAACU	30	AGUUUCUUCAUCCAGUUGAGGGA
1450	5	UUCCUGUUUGCUGUGUAUGAU	31	AUCAUACACAGCAAACAGGAAUG

1763	6	GCUGGGUUUAUUUUAGAGAAU	32	AUUCUCUAAAAUAAACCCAGCUU
1764	7	CUGGGUUUAUUUUAGAGAAUG	33	CAUUCUCUAAAAUAAACCCAGUU
1843	8	CCAACCGACCAGCUUGUUUGU	34	ACAAACAAGCUGGUCGGUUGGAA
1847	9	CCGACCAGCUUGUUUGUGAAA	35	UUUCACAAACAAGCUGGUCGGUU
1849	10	GACCAGCUUGUUUGUGAAACA	36	UGUUUCACAAACAAGCUGGUCGG
1849	11	GACCAGCUUGUUUGUGAAACA	37	UGUUUCACAAACAAGCUGGUCUU
1851	12	CCAGCUUGUUUGUGAAACAAA	38	UUUGUUUCACAAACAAGCUGGUC
1854	13	GCUUGUUUGUGAAACAAAAA	39	UUUUUUGUUUCACAAACAAGCUG
1858	14	GUUUGUGAAACAAAAAAGUGU	40	ACACUUUUUUGUUUCACAAACAA
1868	15	CAAAAAAGUGUCCCUUUUCA	41	UGAAAAGGGAACACUUUUUUGUU
1880	16	CCCUUUUCAAGUUGAGAACAA	42	UUGUUCUCAACUUGAAAAGGGUU
1893	17	GAGAACAAAAAUUGGGUUUUA	43	UAAAACCCAAUUUUUGUUCUCAA
1895	18	GAACAAAAAUUGGGUUUUAAA	44	UUUAAAACCCAAUUUUUGUUCUC
1934	19	GCAUUGCCUUCGGUUUGUAUU	45	AAUACAAACCGAAGGCAAUGCAA
1935	20	CAUUGCCUUCGGUUUGUAUUU	46	AAAUACAAACCGAAGGCAAUGCA
1940	21	CCUUCGGUUUGUAUUUAGUGU	47	ACACUAAAUACAAACCGAAGGCA
1945	22	GGUUUGUAUUUAGUGUCUUGA	48	UCAAGACACUAAAUACAAACCGA
1950	23	GUAUUUAGUGUCUUGAAUGUA	49	UACAUUCAAGACACUAAAUACUU
1959	24	GUGUCUUGAAUGUAAGAACAU	50	AUGUUCUUAACAUUCAAGACACU
1961	25	GUCUUGAAUGUAAGAACAUGA	51	UCAUGUUCUUAACAUUCAAGACAC
2085	26	CCAUAGCUGGUUAUUUCUCCU	52	AGGAGAAAUAACCAGCUAUGGUU

Note: Column 1 refers to positions of the first bases of the target genes in the AGT gene sequence, and so on; and the numbers in Columns 2 and 4 represent sequence numbers. For example, "1" represents SEQ ID NO: 1.

Coding sequence of human AGT (NM_001382817.3, SEQ ID NO: 53):

```

5  1 agaaagtaga cctcaccag gcatggaatc tgccagtgcc ttgatcttgg acttcagct
61 tccagaactg gtatgcggaa gcgagcaccc cagtctgaga tggctcctgc cgggtgtgagc
121 ctgagggcca ccatcctctg cctcctggcc tgggctggcc tggctgcagg tgaccgggtg
181 tacatacacc ccttcacct cgtcatccac aatgagagta cctgtgagca gctggcaaa
241 gccatgccc ggaagccaa agacccacc ttcatactg ctcaattca ggccaagaca
10 301 tccctgtgg atgaaaaggc cctacaggac cagtgtgtgc tagtctgtgc aaaacttgac
361 accgaagaca agttgagggc cgcaatggc gggatgctgg ccaactctt gggttccgt
421 atatattgca tgcacagtga gctatggggc gtgttccatg ggccaccgt cctctccca
481 acggtgtct tggcacctt ggctctctc tatctgggag ccttgacca cacagtgcac
541 aggtacagg caatcctggg tttccttgg aaggacaaga actgcacctc cggctggat
15 601 gcgcacaagg tctgtctgc cctgcaggct gtacagggcc tctagtggc ccagggcagg
661 gctgatagcc aggccagct gctgtgtcc acggtggtgg gcgtgtcac agccccaggc
721 ctgcacctga agcagccgtt tgtgcagggc ctggctctct ataccctgt ggtcctcca
781 cgctctctgg acttcacaga actggatgtt gctgctgaga agattgacag gttcatgcag
841 gctgtgacag gatggaagac tggctgtcc ctgatgggag ccagtgtgga cagcacctg
20 901 gctttcaaca cctactgcca ctccaaggg aagatgaagg gcttctcct gctggccgag
961 cccaggagt tctgggtgga caacagcacc tcagtgtctg tcccatgct ccttgcatg
1021 ggcaccttc agcactggag tgacatccag gacaactct cggtgactca agtgccctc
1081 actgagagcg cctgctgtg gctgatccag cctcatatg cctctgacct ggacaagggtg
1141 gaggtgtcct cttccagca aaactccctc aactggatga agaaactatc tccccggacc
25 1201 atccacctga ccatgcccc actggtgtctg caaggatctt atgacctgca ggacctgtc
1261 gccaggctg agctggccgc cattctgcac accgagctga acctgcaaaa attgagcaat
1321 gaccgcatca ggggtgggga ggtgctgaac agcattttt ttgagcttga agcggatgag
1381 agagagccca cagagtctac ccaacagctt aacaagcctg aggtcttga ggtgacctg

```

1441 aaccgcccac tctgtttgc tgtgtatgat caaagcgcca ctgccctgca cttcctgggc
 1501 cgctgtggcca acccgctgag cacagcatga ggccagggcc ccagaacaca gtgcctggca
 1561 aggcctctgc ccttgccctt ttaggcaaag gccagcagca gataacaacc ccggacaaat
 1621 cagcgatgtg tcacccccag tctccacct tttctctaa tgagtcgact ttgagctgga
 5 1681 aagcagccgt ttctcctgg tctaagtgtg ctgcattggag tgagcagtag aagcctgcag
 1741 cggcacaat gcacctcca gtttctggg tttattttag agaattggggg tggggaggca
 1801 agaaccagtg tttagcgagg gactactgtt ccaaaaagaa ttccaaccga ccagctgtt
 1861 tgtgaaacaa aaaagtgttc cttttcaag ttgagaacaa aaattgggtt taaaattaa
 1921 agtatacatt ttgcattgc ctctggttg tatttagtgt ctggaatga agaactgac
 10 1981 ctccgtgtag tgtctgaat accttagttt ttccacaga tgcctgtgat tttgaacaa
 2041 tacgtgaag atgcaagcac ctgaatttct gttgaaatgc ggaaccatag ctggttattt
 2101 ctccctgtg ttagtaataa acgtcttgcc acaataagcc tccaaaaa

[0086] Targeted drug delivery system

[0087] The present disclosure further provides a targeted drug delivery system, where the
 15 targeted drug delivery system includes a targeting moiety, a linking moiety, and the foregoing
 nucleic acid linked to the targeting moiety through the linking moiety.

[0088] The targeting moiety can further enhance the targeting ability of oligonucleotides,
 and may be provided by monosaccharides (for example, glucose, mannose, allose, altrose,
 galactose, galactosamine, N-acetylgalactosamine, talose, fructose, and idose) and/or
 20 polypeptides (for example, proteins, monoclonal antibodies, and nanobodies). The linking
 moiety may be selected from -O-[CH₂CH₂O]_n-, -[CH₂]_m-CONH-[CH₂]_nO-, -O-[CH₂CH₂O]_m-
 CONH-[CH₂]_nO-, and -O-[CH₂]_m-CONH-[CH₂H₂O]_nO-. Herein, m and n may be
 independently an integer from 1 to 10.

[0089] When applied to different targeted drug delivery systems with reference to common
 25 knowledge in the art, the nucleic acids (siRNA) in the present disclosure all have better
 inhibitory effects. In other words, the effects of the naked sequences and modified sequences
 intended to be protected in the present disclosure do not depend on the selection of the targeting
 carriers. To further improve the bioavailability and therapeutic efficacy of siRNA, in the
 present disclosure, the targeted drug delivery system is also optimized to obtain the following
 30 technical solutions.

[0090] In some embodiments, the linking moiety is linked to the 3' end or 5' end of the sense
 strand or the antisense strand of the nucleic acid.

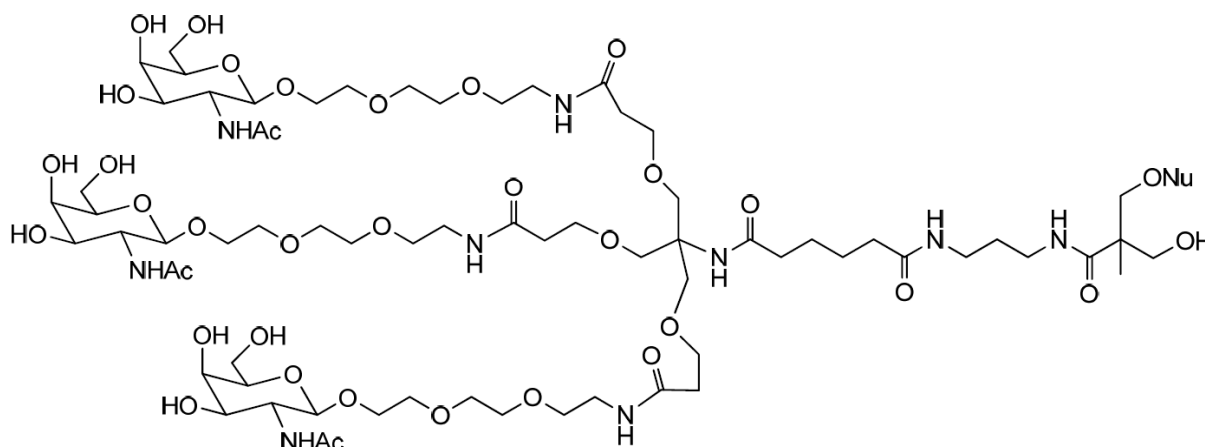
[0091] In some specific embodiments, the linking moiety is linked to the 3' end of the sense
 strand of the nucleic acid.

35 **[0092]** In some embodiments, the targeted drug delivery system includes a ligand and the
 nucleic acid linked to the ligand.

[0093] In some embodiments, the ligand is a GalNAc derivative.

[0094] In some embodiments, the ligand is one or more GalNAc derivatives connected via
 a single-stranded, double-stranded, or triple-stranded branched linker.

[0095] In some preferred embodiments, a structure of the targeted drug delivery system is represented by Formula I below:



Formula I

[0096] In Formula I, Nu represents the nucleic acid (siRNA). In some embodiments, the compound portion of the system can be linked to the 5' end or 3' end of the sense strand of the siRNA via a phosphodiester bond, or can be linked to the 5' end or 3' end of the antisense strand of the siRNA via a phosphodiester bond. This targeted drug delivery system, with its structural features on the left side, enhances the cellular penetration of nucleic acid drugs (Nu), improves their intracellular stability, and can be prepared through a simple process with strong practical applicability.

[0097] Pharmaceutical Composition

[0098] The present disclosure further provides a pharmaceutical composition including the nucleic acid or targeted drug delivery system as described above, and a pharmaceutically acceptable carrier.

[0099] The pharmaceutical composition can be prepared from said nucleic acid and the pharmaceutically acceptable carrier using conventional methods. For example, the pharmaceutical composition may be an injection solution. The injection solution can be used for subcutaneous, intramuscular, or intravenous injection.

[0100] For the pharmaceutical composition according to the present disclosure, there are no special requirements for the amounts of the nucleic acid, the targeted drug delivery system, or the pharmaceutically acceptable carrier. Generally, relative to 1 part by weight of the nucleic acid (or 1 part by weight of the targeted drug delivery system calculated as per the nucleic acid), the content of the pharmaceutically acceptable carrier can be 1-100,000 parts by weight (for example 1, 5, 10, 50, 100, 500, 1,000, 5,000, 10,000, 50,000, or 100,000 parts by weight, or any value between any two of the above values).

[0101] For the pharmaceutical composition according to the present disclosure, the pharmaceutically acceptable carrier may be any of the various carriers conventionally used in the art, including, for example, at least one of pH buffers, protective agents, and osmotic pressure regulators. The pH buffer may be a tris(hydroxymethyl)aminomethane hydrochloride buffer with a pH of 7.5-8.5 and/or a phosphate buffer with a pH of 5.5-8.5, or may be preferably a phosphate buffer with a pH of 5.5-8.5. The protective agent may be at least one of inositol, sorbitol, and sucrose. Based on the total weight of the pharmaceutical composition, the content of the protective agent may be 0.01 wt% to 30 wt% (e.g., 0.01 wt%, 0.05 wt%, 0.1 wt%, 0.5 wt%, 1 wt%, 5 wt%, 10 wt%, 15 wt%, 20 wt%, 25 wt%, or 30 wt%, or any value between any two of the above values). The osmotic pressure regulator may be sodium chloride and/or potassium chloride. The content of the osmotic pressure regulator should be sufficient to ensure that the osmotic pressure of the pharmaceutical composition is 200-700 milliosmoles per kilogram. Based on the required osmotic pressure, a person skilled in the art can determine the content of the osmotic pressure regulator.

[0102] According to a preferred embodiment of the present disclosure, the pharmaceutically acceptable carrier is a liposome. The liposome can be any type of liposome capable of encapsulating nucleic acids, and may have a diameter of 25 to 1,000 nm, and include, but not limited to, cholesterol and its analogs or derivatives.

[0103] The dosage of the pharmaceutical composition of the present disclosure may be conventional in the art, and may be determined based on various parameters, especially the age, weight, and gender of the subject. For example, for female mice aged 3-4 months and weighing 25g to 30g, based on the amount of the nucleic acid in the pharmaceutical composition, the dosage of the pharmaceutical composition may be 0.01-100 mg per kg of body weight, or may be preferably 1-10 mg per kg of body weight.

[0104] Method and Use

[0105] The present disclosure further provides a method of inhibiting expression of angiotensin gene in a cell, where the method includes: contacting the cell with the nucleic acid, targeted drug delivery system, or pharmaceutical composition as described above to inhibit the expression of the angiotensin gene in the cell.

[0106] In some embodiments, the cell is in a subject such as a human subject, for example, a subject with an angiotensinogen (AGT)-associated disease.

[0107] In some embodiments, the cell is located *in vitro*. The method is intended for research or used for constructing animal models.

[0108] In some embodiments, contact between the cell and the nucleic acid inhibits the expression of AGT by at least 50%, 60%, 70%, 80%, 90%, or 95% (e.g., compared to the expression level of AGT before the first contact between the cell and the nucleic acid; for example, before administration of a first dose of the nucleic acid to the subject). In some
5 embodiments, inhibiting the expression of AGT reduces the level of AGT protein in the serum sample of the subject by at least 50%, 60%, 70%, 80%, 90%, or 95%, compared to, for example, the expression level of AGT before the first contact between the cell and the nucleic acid.

[0109] The present disclosure further provides the use of the nucleic acid, targeted drug
10 delivery system, or pharmaceutical composition as described above in any one of the following aspects: (1) treatment and/or prevention of a disease associated with angiotensin; and (2) preparation of a medicament for treating and/or preventing a disease associated with angiotensin.

[0110] In some embodiments, the disease is: (i) a disease associated with enhancement or
15 elevation of an activity level of angiotensin; or (ii) a disease that benefits from reduced expression of angiotensinogen.

[0111] In some embodiments, the disease is a disease associated with excessive activation of the renin-angiotensin-aldosterone system (RAAS). In some specific embodiments, "disease associated with excessive activation of the RAAS system" may include hypertension, heart
20 failure, kidney diseases, presenile dementia, ophthalmic diseases, and the like.

[0112] In some embodiments, the disease is selected from one or more of hypertension, hypertensive disorder, borderline hypertensive disorder, primary hypertensive disorder, secondary hypertensive disorder, isolated systolic or diastolic hypertensive disorder, pregnancy-related hypertensive disorder, diabetic hypertensive disorder, resistant hypertensive
25 disorder, refractory hypertensive disorder, paroxysmal hypertensive disorder, renovascular hypertensive disorder, Goldblatt hypertension, hypertensive disorder associated with low plasma renin activity or plasma renin concentration, high intraocular pressure, glaucoma, pulmonary arterial hypertension, portal hypertension, systemic venous hypertension, systolic hypertensive disorder, unstable hypertensive disorder; hypertensive heart disease, hypertensive
30 nephropathy, atherosclerosis, arteriosclerosis, vascular disease, diabetic nephropathy, diabetic retinopathy, chronic heart failure, cardiomyopathy, diabetic cardiomyopathy, glomerulosclerosis, coarctation of the aorta, aortic aneurysm, ventricular fibrosis, heart failure, myocardial infarction, angina pectoris, stroke, nephropathy, renal failure, systemic sclerosis, intrauterine growth restriction (IUGR), fetal growth restriction, obesity, hepatic steatosis/fatty

liver, non-alcoholic steatohepatitis (NASH), non-alcoholic fatty liver disease (NAFLD), glucose intolerance, type 2 diabetes mellitus (non-insulin-dependent diabetes mellitus), and metabolic syndrome.

[0113] In the present disclosure, the subject may be a mammal, including a primate (for example, a human and a non-human primate such as a monkey and a chimpanzee), a non-primate (for example, cattle, a pig, a horse, a goat, a rabbit, sheep, a hamster, a guinea pig, a cat, a dog, a rat, or a mouse), or a bird. In some embodiments, the subject is preferably a primate, more preferably, a human. Administration can be performed via multiple routes, depending on whether local treatment or systemic treatment is required. The administration routes may include, but not limited to, intravenous administration, intraarterial administration, subcutaneous administration, intraperitoneal administration, transdermal administration (for example, via an implanted device), and intra-soft tissue administration. The dosage may refer to the foregoing description. Details are not described again herein.

[0114] In some embodiments, the nucleic acid, the targeted drug delivery system, or the pharmaceutical composition is administered to a subject via subcutaneous administration, intravenous administration, and/or intramuscular administration.

[0115] Examples

[0116] The embodiments of the present disclosure are described in detail below with reference to examples. It should be understood that these examples are only used to illustrate the present disclosure and are not intended to limit the scope of the present disclosure. For experimental methods in the following examples where specific conditions are not indicated, reference should be made to the guidance provided in the present disclosure. Alternatively, standard laboratory manuals, conventional conditions known in the art, or manufacturer-recommended procedures may be adopted.

[0117] In the following specific examples, measurement parameters related to raw material components may exhibit slight deviations within the weighing accuracy range unless otherwise specified. Parameters involving temperature and time may allow acceptable deviations resulting from instrument testing accuracy or operational precision.

[0118] Example 1 Sequence Screening of siRNA

[0119] Double-stranded siRNAs can enter cells and be incorporated into the RISC complex. In the RISC complex, the double-stranded siRNA is unwound by the endonuclease Ago2, leaving only the antisense strand to recognize the target mRNA. A GC content of 35-55% is generally regarded as the optimal choice for siRNA. GC content is also correlated with the melting temperature of siRNA. However, siRNAs with lower GC content may unwind more

rapidly or efficiently due to their thermal stability. The siRNA sequences designed in the present disclosure (as shown in Table 2) include those with lower GC content, thereby facilitating the optimal selection of siRNA.

Table 2

Target gene loci	Sequence (5'-3')	GC%
778	CCACGCUCUCUGGACUUCACA	57.10%
977	UGGACAACAGCACCUCAGUGU	52.40%
1145	GUCUCACUUUCCAGCAAAACU	42.90%
1167	CCUCAACUGGAUGAAGAAACU	42.90%
1450	UUCCUGUUUGCUGUGUAUGAU	38.10%
1763	GCUGGGUUUAUUUUAGAGAAU	33.30%
1764	CUGGGUUUAUUUUAGAGAAUG	33.30%
1843	CCAACCGACCAGCUUGUUUGU	52.40%
1847	CCGACCAGCUUGUUUGUGAAA	47.60%
1849	GACCAGCUUGUUUGUGAAACA	42.90%
1849A	GACCAGCUUGUUUGUGAAACA	42.90%
1851	CCAGCUUGUUUGUGAAACAAA	38.10%
1854	GCUUGUUUGUGAAACAAAAAA	28.60%
1858	GUUUGUGAAACAAAAAAGUGU	28.60%
1868	CAAAAAAGUGUCCCCUUUCA	33.30%
1880	CCCUUUUCAAGUUGAGAACAA	38.10%
1893	GAGAACAAAAAUUGGGUUUUA	28.60%
1895	GAACAAAAAUUGGGUUUAAA	23.80%
1934	GCAUUGCCUUCGGUUUGUAUU	42.90%
1935	CAUUGCCUUCGGUUUGUAUUU	38.10%
1940	CCUUCGGUUUGUAUUUAGUGU	38.10%
1945	GGUUUGUAUUUAGUGUCUUGA	33.30%
1950	GUAUUUAGUGUCUUGAAUGUA	28.60%
1959	GUGUCUUGAAUGUAAGAACA	33.30%
1961	GUCUUGAAUGUAAGAACAUGA	33.30%
2085	CCAUAGCUGGUUAUUUCUCCU	42.90%

5 **[0120]** A preferred sequence is GUUUGUGAAACAAAAAAGUGU, with a GC content of 28.6%.

[0121] The sense strand and antisense strand sequences corresponding to the foregoing sequences are shown in Table 1.

10 **[0122]** In this example, each sense strand and antisense strand were further modified. The modified sequences are shown in Table 3.

Table 3

No.	Sense strand (5'-3')	No.	Antisense strand (5'-3')
SN-22158	cscsacgcUfcUfCfUfggacuucaca	SN-52158	usGfsuGfaAfguccaGfaGfagcguggsgsa

SN-22159	usgsgacaAfcAfGfCfaccucagugu	SN-52159	asCfsaCfuGfaggugcuGfuUfguccasusu
SN-22160	gsuscucaCfuUfUfCfcagcaaaacu	SN-52160	asGfsuUfuUfgcuggaaAfgUfgagacsusu
SN-22161	cscsucaaCfuGfGfAfugaagaaacu	SN-52161	asGfsuUfuCfuucauccAfgUfugaggsgsa
SN-22163	ususcugUfuUfGfCfugugaugau	SN-52163	asUfscAfuAfcacagcaAfaCfaggaasusg
SN-22164	gscsugggUfuUfAfUfuuuagagaau	SN-52164	asUfsuCfuCfuaaaaaAfaCfccagcsusu
SN-22165	csusggguUfuAfUfUfuagagaau	SN-52165	csAfsuUfcUfcuaaaaAfaAfccagsusu
SN-22166	cscsaaccGfaCfCfAfgcuuguuugu	SN-52166	asCfsaAfaCfaagcuggUfcGfguuggsasa
SN-22167	cscsgaccAfgCfUfUfguuugugaaa	SN-52167	usUfsuCfaCfaaacaagCfuGfgucggsusu
SN-22168	gsasccagCfuUfGfUfuugugaaaca	SN-52168	usGfsuUfuCfacaacaAfgCfuggucsgsg
SN-22169	gsasccagCfuUfGfUfuugugaaaca	SN-52169	usGfsuUfuCfacaacaAfgCfuggucsusu
SN-22170	cscsagcuUfgUfUfUfgugaaacaaa	SN-52170	usUfsuGfuUfucacaaaCfaAfgcuggsusc
SN-22171	gscsuuguUfuGfUfGfaacaaaaaa	SN-52171	usUfsuUfuUfguuucacAfaAfaagcsusg
SN-22172	gsusuuguGfaAfAfCfaaaaagugu	SN-52172	asCfsaCfuUfuuuuguUfcAfaaaacsasa
SN-22173	csaaaaAfgUfGfUfuccuuuuc	SN-52173	usGfsaAfaAfgggaacaCfuUfuuuugsusu
SN-22174	cscscuuUfcAfAfGfuugagaacaa	SN-52174	usUfsgUfuCfucaacuGfaAfaagggsusu
SN-22175	gsasgaacAfaAfAfAfuuggguuuua	SN-52175	usAfsaAfaCfcaauuuUfuGfuucacsasa
SN-22176	gsasacaaAfaAfUfUfggguuuuaaa	SN-52176	usUfsuAfaAfaaccaauUfuUfuguucsusc
SN-22177	gscsauugCfcUfUfCfguuuguaau	SN-52177	asAfsuAfcAfaaccgaaGfgCfaaugcsasa
SN-22178	csasuugcCfuUfCfGfguuuguaauu	SN-52178	asAfsaUfaCfaaaccgaAfgGfcaaugcsa
SN-22179	cscsuucgGfuUfUfGfuauuuagugu	SN-52179	asCfsaCfuAfaauacaaAfcCfgaaggcsa
SN-22180	gsgsuuugUfaUfUfUfagugucuuga	SN-52180	usCfsaAfgAfcacuaaaUfaCfaaaccsgsa
SN-22181	gsusauuuAfgUfGfUfcuugaauua	SN-52181	usAfsaAfuUfcaagacaCfuAfaauacsusu
SN-22182	gsusgucuUfgAfAfUfguaagaacau	SN-52182	asUfsgUfuCfuacauuUfaAfgacacsusu
SN-22183	gsuscuugAfaUfGfUfaagaacauga	SN-52183	usCfsaUfgUfucuuacaUfuCfaagacsasc
SN-22184	cscsauagCfuGfGfUfuauuucuccu	SN-52184	asGfsgAfgAfaauaaccAfgCfuauuggsusu

[0123] In the table, a/c/g/u = 2'-OMe nucleotide; Af/Cf/Gf/Uf = 2'-F nucleotide; and s = phosphorothioate diester bond.

[0124] The sense strands and antisense strands in Table 2 formed the modified double-stranded siRNAs in Table 4 via solid-phase synthesis.

5

Table 4

No.	Sense strand (5'-3')	Antisense strand (5'-3')
SN-252158	cscsacgcUfcUfCfUfggacuucaca	usGfsuGfaAfguccaGfaGfagcguggsgsa
SN-252159	usgsgacaAfcAfGfCfaccucagugu	asCfsaCfuGfaggugcuGfuUfguccasusu
SN-252160	gsuscucaCfuUfUfCfcagcaaaacu	asGfsuUfuUfgcuggaaAfgUfgagacsusu
SN-252161	cscsucaaCfuGfGfAfugaagaaacu	asGfsuUfuCfuucauccAfgUfugaggsgsa
SN-252163	ususcugUfuUfGfCfugugaugau	asUfscAfuAfcacagcaAfaCfaggaasusg
SN-252164	gscsugggUfuUfAfUfuuuagagaau	asUfsuCfuCfuaaaaaAfaCfccagcsusu
SN-252165	csusggguUfuAfUfUfuagagaau	csAfsuUfcUfcuaaaaAfaAfccagsusu
SN-252166	cscsaaccGfaCfCfAfgcuuguuugu	asCfsaAfaCfaagcuggUfcGfguuggsasa
SN-252167	cscsgaccAfgCfUfUfguuugugaaa	usUfsuCfaCfaaacaagCfuGfgucggsusu
SN-252168	gsasccagCfuUfGfUfuugugaaaca	usGfsuUfuCfacaacaAfgCfuggucsgsg
SN-252169	gsasccagCfuUfGfUfuugugaaaca	usGfsuUfuCfacaacaAfgCfuggucsusu
SN-252170	cscsagcuUfgUfUfUfgugaaacaaa	usUfsuGfuUfucacaaaCfaAfgcuggsusc
SN-252171	gscsuuguUfuGfUfGfaacaaaaaa	usUfsuUfuUfguuucacAfaAfaagcsusg

SN-252172	gsusuuguGfaAfAfCfaaaaaagugu	asCfsaCfuUfuuuuguuUfcAfaaacsasa
SN-252173	csasaaaaAfgUfGfUfuccuuuua	usGfsaAfaAfgggaacaCfuUfuuuugsusu
SN-252174	cscscuuuUfcAfAfGfuugagaacaa	usUfsgUfuCfucaacuGfaAfaaggsusu
SN-252175	gsasgaacAfaAfAfAfuuggguuuua	usAfsaAfaCfcaauuuUfuGfuucucsasa
SN-252176	gsasacaaAfaAfUfUfgguuuuuaa	usUfsuAfaAfaaccaauUfuUfuguucsusc
SN-252177	gscsauugCfcUfUfCfguuuguauu	asAfsuAfcAfaaccgaaGfgCfaaugcsasa
SN-252178	csasuugcCfuUfCfGfguuuguauuu	asAfsaUfaCfaaaccgaAfgGfcaaugcsa
SN-252179	cscsuucgGfuUfUfGfuauuuagugu	asCfsaCfuAfaauacaaAfcCfgaaggcsa
SN-252180	gsgsuuugUfaUfUfUfagugucuuga	usCfsaAfgAfcacuaaaUfaCfaaaccgsa
SN-252181	gsusauuuAfgUfGfUfcuugaaugua	usAfsaCfuUfcaagacaCfuAfaauacsusu
SN-252182	gsusgucuUfgAfAfUfguaagaacau	asUfsgUfuCfuuaacuUfaAfgacacsusu
SN-252183	gsuscuugAfaUfGfUfaagaacauga	usCfsaUfgUfucuuacaUfuCfaagacsasc
SN-252184	cscsauagCfuGfGfUfuauuucuccu	asGfsgAfgAfaaaccAfgCfuauuggsusu

[0125] In the table, a/c/g/u = 2'-OMe nucleotide; Af/Cf/Gf/Uf = 2'-F nucleotide; and s = phosphorothioate diester bond.

[0126] 0.5 mL of cell culture medium (DMEM, 10% fetal bovine serum, and 1% penicillin-streptomycin solution) containing 10^4 Hep3B cells (Procell, Cat# CL-0102) was added into a 96-well cell culture dish, and incubated overnight in a cell incubator with 5% CO₂ at 37°C. RNAiMAX (1.5 µL per well) and the small interfering nucleic acids (siRNAs) in Table 3 were added into Opti-MEM medium, and then the resulting mixture was added to the cell culture wells until a final concentration in each well reached 1 nM or 10 nM, and further cultured for 48 hours in a cell incubator with 5% CO₂ at 37°C. To extract RNA, the cell culture supernatant was aspirated completely, and the wells were rinsed with PBS. After removing residual liquid, 50 µL of prepared lysis buffer (as recommended in the Cells-to-CT Kit (Thermo Fisher Scientific, Cat#4391851c)) was added and mixed well. After 10 minutes of standing, 2.5 µL of Stop solution was added to terminate the reaction for 2 minutes. RT-PCR was performed as per the recommendation in the High Capacity cDNA Reverse Transcription Kits (Thermo Fisher, Catalog No.: 4368814), with 10 µL of lysed liquid in each reaction. Gene expression quantification was measured by using real-time fluorescent PCR. The TaqMan probe for human AGT was Hs00174854_m1, and the probe for the reference gene (human HPRT1) was Hs02800695_m1 (Thermo Fisher Scientific, Waltham, MA, USA). The PCR conditions were as follows: one cycle of 95°C for 20 seconds, followed by 40 cycles of 95°C for 1 second and 60°C for 20 seconds. The real-time fluorescent PCR instrument was the QuantStudio™ 6 Pro Real-Time PCR System (Thermo Fisher). The expression of the AGT gene was calculated using the $2^{-\Delta\Delta C_t}$ method, and the expression of the human HPRT1 gene was used as an internal reference. The expression level of the AGT gene is presented as a relative value

compared with the cell group treated with RNAiMAX alone. The results are shown in Table 5.

Table 5

Sample No.	1nM	Standard deviation	10nM	Standard deviation
SN-252158	1.82	0.09	0.22	0.09
SN-252159	2.67	0.07	0.78	0.21
SN-252160	0.41	0.06	0.29	0.09
SN-252161	0.90	0.04	0.22	0.02
SN-252163	0.90	0.03	0.15	0.07
SN-252164	0.35	0.02	0.19	0.05
SN-252165	0.89	0.06	0.15	0.07
SN-252166	1.63	0.01	0.14	0.01
SN-252167	0.91	0.12	0.18	0.03
SN-252168	0.90	0.25	0.61	0.06
SN-252169	0.92	0.06	0.60	0.01
SN-252170	0.31	0.02	0.22	0.02
SN-252171	2.43	0.46	0.49	0.01
SN-252172	0.25	0.00	0.12	0.03
SN-252173	0.40	0.03	0.16	0.02
SN-252174	0.31	0.12	0.08	0.01
SN-252175	0.38	0.09	0.23	0.03
SN-252176	0.26	0.14	0.29	0.01
SN-252177	1.67	0.84	1.11	0.07
SN-252178	1.22	0.39	1.05	0.01
SN-252179	1.49	0.44	1.53	0.07
SN-252180	1.48	0.55	1.27	0.03
SN-252181	1.11	0.05	0.69	0.12
SN-252182	1.26	0.12	0.35	0.10
SN-252183	1.68	0.05	0.94	0.24
SN-252184	0.97	0.00	0.49	0.11

[0127] To further determine the activity of the siRNAs, a dose-response experiment was conducted in human primary hepatocytes using the siRNAs with superior efficacy listed in Table 5, which were conjugated to Tri-GalNAc (its specific structure is shown in Formula I above; the conjugated sequences are provided in Table 6). The siRNA-GalNAc samples were separately dissolved in 100 μ L of enzyme-free and sterile water to prepare 100 μ M solutions. 30 μ L of the 100 μ M test substance solution was added to 70 μ L of inVitroGRO Plating Medium for dilution to prepare a 30 μ M solution for later use as the working solution for the 30 nM final concentration group. Subsequently, the 30 μ M test substance solution was diluted by 3 folds with inVitroGRO Plating Medium to achieve 8 concentration points, so that the final working solution concentrations were 4.6, 13.7, 41.2, 123.4, 370.4, 1111.1, 3333.3, and 10000 nM. Primary human hepatocytes were thawed from liquid nitrogen, recovered at 37°C, rinsed

with serum-containing inVtroGRO Plating Medium, counted, and centrifuged. After removing the supernatant, the cells were resuspended in fresh serum-containing inVtroGRO Plating Medium at a concentration of 300k cells/mL. Then, 90 μ L of the cell suspension was seeded into the wells of a 96-well cell culture plate, resulting in 30k cells per well. The prepared sample working solution was added to the cell suspension to achieve final concentrations of 0.46, 1.37, 4.12, 12.34, 37.04, 111.11, 333.33, and 1000 nM. Finally, the plate was placed in an incubator with 5% CO₂ and cultured at a constant temperature of 37 °C for 48 hours. 48 hours later, all the medium in the 96-well culture plate was aspirated, and the plate was washed with 1×PBS buffer. Then 50 μ L of the prepared Cells-to-CT lysis buffer (as per the manufacturer's recommendations) was added and mixed thoroughly. After standing for 10 minutes, 2.5 μ L of stop solution was added to terminate the reaction in 2 minutes. RT-PCR was performed as per the recommendation in the High Capacity cDNA Reverse Transcription Kits (Thermo Fisher, Catalog No.: 4368814), with 10 μ L of lysed liquid in each reaction. Gene expression quantification was measured by using real-time fluorescent PCR. The TaqMan probe for human AGT was Hs00174854_m1, and the probe for the reference gene (human HPRT1) was Hs02800695_m1 (Thermo Fisher Scientific, Waltham, MA, USA). The PCR conditions were as follows: one cycle of 95°C for 20 seconds, followed by 40 cycles of 95°C for 1 second and 60°C for 20 seconds. The real-time fluorescent PCR instrument was the QuantStudio™ 6 Pro Real-Time PCR System (Thermo Fisher). The expression of the AGT gene was calculated using the 2^{- $\Delta\Delta$ Ct} method, and the expression of the human HPRT1 gene was used as an internal reference. The expression level of the AGT gene was presented as a percentage relative to the control group treated with the culture medium alone, and the IC₅₀ value was calculated accordingly (see Table 7).

Table 6

No.	Sense strand (5'-3')	Antisense strand (5'-3')
SN-682160	gsuscucaCfuUfUfCfcagcaaaacu-TriGalNAc	asGfsuUfuUfgcuggaaAfgUfgagacsusu
SN-682161	cscsucaaCfuGfGfAfugaagaaacu-TriGalNAc	asGfsuUfuCfuucauccAfgUfugaggsgsa
SN-682163	ususccugUfuUfGfCfugugaugau-TriGalNAc	asUfscAfuAfcacagcaAfaCfaggaasusg
SN-682164	gscsugggUfuUfAfUfuuagagaau-TriGalNAc	asUfsuCfuCfuaaaauaAfaCfccagcsusu
SN-682165	csusggguUfuUfUfUfuuagagaug-TriGalNAc	csAfsuUfcUfcuaaaaauAfaAfcccagsusu
SN-682170	cscsagcuUfgUfUfUfgugaacaaa-TriGalNAc	usUfsuGfuUfucacaaaCfaAfgcuggsusc
SN-682172	gsusuuguGfaAfAfCfaaaaaagugu-TriGalNAc	asCfsaCfuUfuuuuguUfcAfcaaacsasa
SN-682173	csasaaaaAfgUfGfUfuccuuuuca-TriGalNAc	usGfsaAfaAfgggaacaCfuUfuuuugsusu
SN-682174	cscscuuUfcAfAfGfuugagaacaa-TriGalNAc	usUfsgUfuCfucaacuUgfaAfaaggggsusu
SN-682175	gsasgaacAfaAfAfAfuuuggguuuua-TriGalNAc	usAfsaAfaCfccaauuuUfuGfuucucsasa
SN-682176	gsasacaaAfaAfUfUfggguuuuaaa-TriGalNAc	usUfsuAfaAfacccaauUfuUfuguucsusc

[0128] In the table, a/c/g/u = 2'-OMe nucleotide; Af/Cf/Gf/Uf = 2'-F nucleotide; and s = phosphorothioate diester bond.

Table 7

No.	IC50 (nM)
SN-682160	296.8
SN-682161	380.3
SN-682163	505.8
SN-682164	467.2
SN-682165	701.0
SN-682170	345.9
SN-682172	253.4
SN-682173	79.4
SN-682174	344.5
SN-682175	8.7
SN-682176	72.2

[0129] To further verify the efficacy of siRNA, SN-682160, SN-682172, SN-682173, SN-682175, and SN-682176 were each subcutaneously injected into human AGT transgenic mice at a dose of 10 mg/kg. On day 10, blood was sampled to detect the residual AGT protein level in plasma. The results are shown in FIG. 1. As can be seen from FIG. 1, on Day 10, SN-682172 can knock down the AGT protein level by 90% or more.

[0130] Example 2 Optimization of siRNA Modification Methods

[0131] To further enhance the activity of siRNA, new modifications were made to the siRNA according to Table 8.

Table 8

Number of siRNA	Sense strand	Antisense strand
SN-682725	gsusuUfguGfaAfAfCfaaaaaagugu-TrigalNac	AfsCfsacuUfuuUfuguUfcAfcaacsasa
SN-682726	gsusuUfguGfaAfAfCfaaaaaagugu-TrigalNac	AfsCfsacuuUfuUfuguUfcAfcaacsasa
SN-682727	gsUfsuUfguGfaAfAfCfaaaaaagugu-TrigalNac	AfsCfsacuUfuuUfuguUfcAfcaacsasa

SN-682728	gsUfsuUfguGfaAfAfCfaaaaaagugu-TrigalNac	AfsCfsacuuUfuUfuguuUfcAfcaaacsasa
SN-682172	gsusuuguGfaAfAfCfaaaaaagugu-TrigalNac	asCfsaCfuUfuuuuguuUfcAfcaaacsasa

[0132] In the table, a/c/g/u = 2'-OMe nucleotide; Af/Cf/Gf/Uf = 2'-F nucleotide; and s = phosphorothioate diester bond.

[0133] To further determine the activity of the siRNAs, a dose-response experiment was conducted with the siRNAs listed in Table 8 in human primary hepatocytes, following the same method as described above. The absolute IC₅₀ values for reducing AGT expression are provided in Table 9.

Table 9	
Sample No.	IC ₅₀ (nM)
SN-682725	70.20
SN-682726	8.60
SN-682727	44.10
SN-682728	21.80
SN-682172	375.40

[0134] Example 3 *In Vitro* Efficacy Verification of siRNA

[0135] To further verify the efficacy, the preferred siRNA drugs were compared with SN-2073 in human primary hepatocytes, and the involved siRNAs are listed in Table 10. Among them, SN-2073 used as the control was prepared in accordance with the disclosure of US11015201B2. The experimental procedures in human primary hepatocytes were similar to the aforementioned experimental methods. The expression level of the AGT gene is presented as a percentage relative to the control group of cells treated with vehicle alone, and the results are shown in Table 11.

Table 10

Number of siRNA	Sense strand	Antisense strand
SN-682726	gsusuUfguGfaAfAfCfaaaaaagugu-TrigalNac	AfsCfsacuuUfuUfuguuUfcAfcaaacsasa
SN-682728	gsUfsuUfguGfaAfAfCfaaaaaagugu-TrigalNac	AfsCfsacuuUfuUfuguuUfcAfcaaacsasa
SN-2073	gsuscaucCfaCfAfAfugagaguaca- TrigalNac	usGfsuacTgncucauugUfgGfaugacsgsa

[0136] In the table: a/c/g/u = 2'-O-methyl nucleotide; Af/Cf/Gf/Uf = 2'-fluoro nucleotide. Tgn = thymidine glycol; and s = phosphorothioate diester bond. Structures used for SN-682726 and SN-682728 are shown in Formula I, and Tri-GalNac used for SN-2073 is Alnylam Tri-

GalNAc (L96). Both the structure shown in Formula I and L96 are classified as triantennary N-acetylgalactosamine. Therefore, based on the following efficacy data, the advantages of the modified sequences in the present disclosure can also be confirmed.

Table 11

Sample No.	1000 nM Mean	Standard deviation	100 nM	Standard deviation
SN-682726	16.25	0.99	25.27	2.77
SN-682728	21.80	0.74	33.30	4.61
SN-2073	36.00	0.62	33.63	3.07

[0137] It can be learned from the free uptake experiment in primary human hepatocytes that SN-682726 and SN-682728 in the present disclosure exhibit better activity than SN-2073.

[0138] Example 4 *In Vivo* Efficacy Verification of siRNA

[0139] To further verify the activity of siRNA, human AGT transgenic mice were subcutaneously injected with 1, 3, and 10 mg/kg of SN-682726 or SN-682728 on Day 0, respectively, and PBS was used as the blank control group. The human AGT protein in the blood was then monitored. The results are shown in FIG. 2.

[0140] As can be seen from FIG. 2, both SN-682726 and SN-682728 can significantly reduce the level of AGT protein and exhibit efficacy for a longer time. SN-682726 shows better efficacy.

[0141] To further verify the effect of modification on efficacy, SN-682726 was compared with SN-2073 in human AGT transgenic mice. The mice were subcutaneously injected with 1, 3, and 10 mg/kg of compound SN-682726 or SN-2073 on Day 0, respectively, and PBS was used as the blank control group. The human AGT protein in the blood was then monitored. The results are shown in FIG. 3.

[0142] As can be seen from FIG. 3, SN-682726 can maintain efficacy for a longer time than the reference compound SN-2073 in all dose groups, and still exhibited a 40% knockdown effect on Day 130 after injection, while the AGT protein level in the control group receiving SN-2073 had returned to the baseline on Day 110 after injection, demonstrating the superiority of SN-682726.

[0143] In addition, it should be noted that the efficacy advantages of the siRNA drug in the present disclosure do not depend on the selection of the targeting carrier. After the TriGalNAc is replaced with other applicable carriers (for example, L96 as described above), the resulting siRNA drugs still exhibit the foregoing significant efficacy advantages compared with SN-2073 in both *in vitro* cell experiments and *in vivo* experiments. For example, after the TriGalNAc of SN-682726 is replaced with L96, the drug still exhibits a better AGT expression silencing effect than SN-2073 in primary monkey hepatocytes, as shown in Table 12.

Table 12

siRNA	1000 nM	Standard deviation	250 nM	Standard deviation
SN-682726-L96	74.5%	0.5%	74.7%	4.0%
SN-2073-L96	66.2%	6.0%	69.8%	4.4%

[0144] The modification of SN-682726 was also made to SN-2073, to obtain SN-683015 shown in Table 13. It can be seen that SN-683015 exhibited better efficacy than SN-2073 in primary monkey hepatocytes, and the results are shown in Table 14.

5

Table 13

No.	Sense strand	Antisense strand
SN-683015	gsuscAfucCfaCfAfAfugagaguaca-L96	UfsGfsuacuCfuCfauugUfgGfaugacsgsa
SN-2073	gsuscauCfaCfAfAfugagaguaca-L96	usGfsuacTgncucauugUfgGfaugacsgsa

Table 14

siRNA	1000 nM	Standard deviation	250 nM	Standard deviation
SN-683015	82.2%	3.6%	83.0%	2.8%
SN-2073	66.2%	6.0%	69.8%	4.4%

[0145] In addition, an unmodified sequence (SN-122726) of SN-682726 and an unmodified sequence (SN-122073 in Table 15) of SN-2073 at three concentrations (0.1, 1, and 10 nM) were also compared in Hep3B cells. The results are shown in Table 16. It can be seen that the unmodified SN-122726 in the present disclosure can more effectively reduce the AGT expression level.

10

Table 15

No.	Sense strand	Antisense strand
SN-122726	GUUUGUGAAACAAAAAAGUGU	ACACUUUUUUGUUUCACAAACAA
SN-122073	GUCAUCCACAAUGAGAGUACA	UGUACUCUCAUUGUGGAUGACGA

Table 16

siRNA	0.1nM	Standard deviation	1nM	Standard deviation	10nM	Standard deviation
SN-122726	92.7%	4.3%	97.3%	0.1%	98.5%	0.2%

SN-122073	82.8%	0.5%	91.6%	1.1%	96.5%	0.6%
-----------	-------	------	-------	------	-------	------

[0146] The above examples represent only a few embodiments of the present disclosure. Although described in detail, they should not be construed as limiting the scope of the present disclosure. It should be noted that those skilled in the art may make various modifications and improvements without departing from the spirit of the present disclosure, and such variations

5 are within the scope of protection of the present disclosure.

CLAIMS

1. A nucleic acid comprising a sense strand and an antisense strand, wherein the sense strand comprises a sequence having at least 80% sequence identity with a sequence as set forth in any one of SEQ ID NOs: 1 to 26, and the antisense strand comprises a sequence having at least 80% sequence identity with a sequence as set forth in any one of SEQ ID NOs: 27 to 52.
2. The nucleic acid according to claim 1, wherein the antisense strand comprises at least 15 consecutive nucleotides that differ from a sequence as set forth in any one of SEQ ID NOs: 27 to 52 by no more than 3 nucleotides, and the sense strand comprises a nucleotide sequence that is at least partially complementary to the antisense strand; and optionally, the sense strand comprises at least 15 consecutive nucleotides that differ from a sequence as set forth in any one of SEQ ID NOs: 1 to 26 by no more than 3 nucleotides.
3. The nucleic acid according to claim 1, wherein the antisense strand comprises a nucleotide sequence that differs from a sequence as set forth in any one of SEQ ID NO: 29, SEQ ID NO: 32, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, and SEQ ID NO: 44 by 0, 1, or 2 nucleotides, and the sense strand comprises a nucleotide sequence that is at least partially complementary to the antisense strand; and optionally, the sense strand comprises a nucleotide sequence that differs from a sequence as set forth in any one of SEQ ID NO: 3, SEQ ID NO: 6, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, and SEQ ID NO: 18 by 0, 1, or 2 nucleotides.
4. The nucleic acid according to claim 1, wherein the antisense strand comprises a nucleotide sequence that differs from the sequence as set forth in any one of SEQ ID NO: 29, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 43, and SEQ ID NO: 44 by 0, 1, or 2 nucleotides, and the sense strand comprises a nucleotide sequence that is at least partially complementary to the antisense strand; and optionally, the sense strand comprises a nucleotide sequence that differs from the sequence as set forth in any one of SEQ ID NO: 3, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 17, and SEQ ID NO: 18 by 0, 1, or 2 nucleotides.
5. The nucleic acid according to claim 1, wherein the antisense strand comprises a nucleotide sequence that differs from the sequence as set forth in SEQ ID NO: 40 by 0, 1, or 2 nucleotides, and the sense strand comprises a nucleotide sequence that is at least partially complementary to the antisense strand; and optionally, the sense strand comprises a nucleotide sequence that differs from the sequence as set forth in SEQ ID NO: 14 by 0, 1, or 2 nucleotides.
6. The nucleic acid according to claim 1, wherein a content of GC in the nucleic acid is 20%-

45%, preferably 20%-35%.

7. The nucleic acid according to claim 1, wherein at least one nucleotide in the nucleic acid is a modified nucleotide or comprises a modified internucleotide linkage;

the modified nucleotide is preferably one or more selected from the group consisting of 2'-O-methyl nucleotide, 2'-fluoro nucleotide, 2'-deoxy nucleotide, 2',3'-seco nucleotide mimetic, locked nucleotide, 2'-F-arabinonucleotide, 2'-methoxyethyl nucleotide, abasic nucleotide, ribitol, inverted nucleotide, inverted 2'-O-methyl nucleotide, inverted 2'-deoxy nucleotide, 2'-amino modified nucleotide, 2'-alkyl modified nucleotide, morpholino nucleotide, nucleotide containing vinyl phosphonate, nucleotide containing cyclopropyl phosphonate, and 3'-O-methyl nucleotide; and the modified nucleotide is more preferably one or more selected from the group consisting of 2'-O-methyl nucleotide and 2'-fluoro nucleotide; and

the modified internucleotide linkage is preferably one or more selected from the group consisting of phosphorothioate internucleotide linkage and methylphosphonate internucleotide linkage; and the modified internucleotide linkage is more preferably one or more selected from the group consisting of phosphorothioate monoester internucleotide linkage and phosphorothioate diester internucleotide linkage.

8. The nucleic acid according to claim 7, wherein, counted from the 5' end of the antisense strand, nucleotides at positions 5 and 6 are 2'-O-methyl nucleotides, and the nucleotide at position 7 is a 2'-fluoro nucleotide; and

preferably, each of the 5' end and 3' end of the antisense strand contains two phosphorothioate internucleotide linkages, nucleotides at positions 1, 2, 7, 9, 14, and 16, counted from the 5' end, are 2'-fluoro nucleotides, and all of the remaining nucleotides are 2'-O-methyl nucleotides.

9. The nucleic acid according to claim 7 or 8, wherein the nucleotide at position 2, counted from the 5' end of the sense strand, is a 2'-O-methyl nucleotide or a 2'-fluoro nucleotide, preferably a 2'-O-methyl nucleotide; and

preferably, the 5' end of the sense strand contains two phosphorothioate internucleotide linkages, nucleotides at positions 4, 7, 9, 10, and 11, counted from the 5' end, are 2'-fluoro nucleotides, and all of the remaining nucleotides are 2'-O-methyl nucleotides.

10. The nucleic acid according to claim 1, wherein the antisense strand has at least 15 consecutive nucleotides that differ from any one of antisense strand sequences shown in Table 3 or Table 8 by no more than 3 nucleotides, the sense strand has at least 15 consecutive

nucleotides that differ from any one of sense strand sequences shown in Table 3 or Table 8 by no more than 3 nucleotides; and preferably, the sense strand and the antisense strand form any one of siRNAs shown in Table 4 or Table 8.

11. The nucleic acid according to claim 1, wherein the antisense strand comprises, from the 5' end to the 3' end, a nucleotide sequence that differs from the following nucleotide sequence by 0, 1, or 2 nucleotides:

AfsCfsacuuUfuUfuguuUfcAfcaaacsasa;

the sense strand comprises a nucleotide sequence that is at least partially complementary to the antisense strand; and

preferably, the sense strand comprises, from the 5' end to the 3' end, a nucleotide sequence that differs from any one of the following nucleotide sequences by 0, 1, or 2 nucleotides:

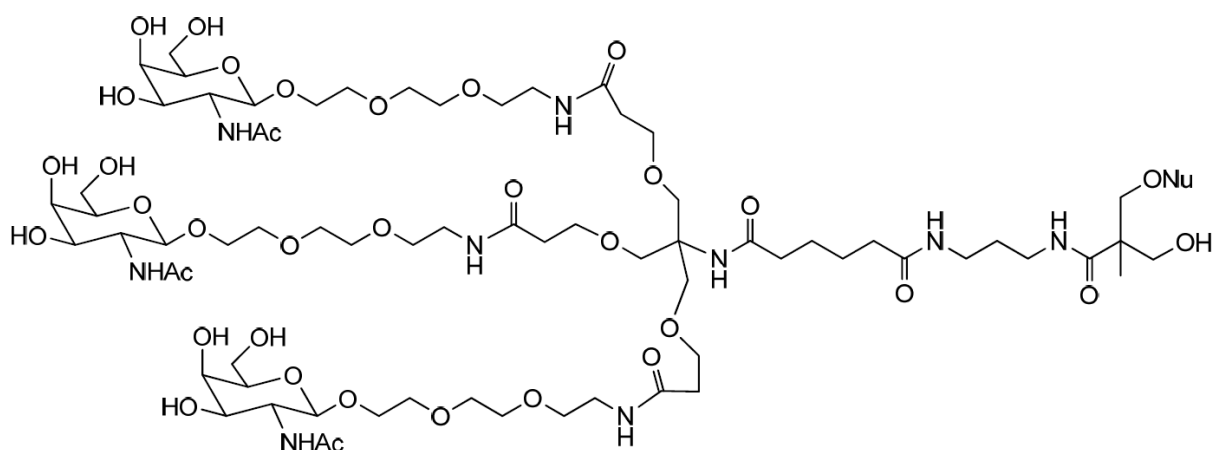
gsusuUfguGfaAfAfCfaaaaaagugu; or

gsUfsuUfguGfaAfAfCfaaaaaagugu;

wherein in each sequence, a nucleotide represented by a lowercase letter is 2'-O-methyl nucleotide; "f" indicates that an adjacent nucleotide on the left side is a nucleotide modified by 2'-fluoro; and "s" indicates that two adjacent nucleotides on the left and right sides are linked by a phosphorothioate diester bond.

12. A targeted drug delivery system, wherein the targeted drug delivery system comprises a targeting moiety, a linking moiety, and the nucleic acid according to any one of claims 1 to 11 that is linked to the targeting moiety through the linking moiety.

13. The targeted drug delivery system according to claim 12, wherein the targeted drug delivery system comprises a ligand and the nucleic acid linked to the ligand; preferably, the ligand is a GalNAc derivative; more preferably, the ligand is one or more GalNAc derivatives linked via a single-stranded, double-stranded or triple-stranded branched linker; and even more preferably, a structure of the targeted drug delivery system is represented by a formula of



in which Nu represents the nucleic acid.

14. A pharmaceutical composition, wherein the pharmaceutical composition comprises the nucleic acid according to any one of claims 1 to 11, or the targeted drug delivery system
5 according to any one of claims 12 to 13, and a pharmaceutically acceptable carrier.

15. A method of inhibiting expression of angiotensin gene in a cell, the method comprising: contacting the cell with the nucleic acid according to any one of claims 1 to 11, the targeted drug delivery system according to any one of claims 12 to 13, or the pharmaceutical composition according to claim 14, to inhibit the expression of the angiotensin gene in the cell.

16. Use of the nucleic acid according to any one of claims 1 to 11, the targeted drug delivery system according to any one of claims 12 to 13, or the pharmaceutical composition according to claim 14 in any one of the following aspects:

1) treatment and/or prevention of a disease associated with angiotensin; and

2) preparation of a medicament for treating and/or preventing a disease associated with
15 angiotensin.

17. The use according to claim 16, wherein the disease is:

i) a disease associated with enhancement or elevation of an activity level of angiotensin; or

ii) a disease that benefits from reduced expression of angiotensinogen.

18. The use according to claim 16, wherein the disease is a disease associated with excessive
20 activation of a renin-angiotensin-aldosterone system.

19. The use according to claim 16, wherein the disease is one or more selected from the group consisting of hypertension, hypertensive disorder, borderline hypertensive disorder, primary hypertensive disorder, secondary hypertensive disorder, isolated systolic or diastolic

hypertensive disorder, pregnancy-related hypertensive disorder, diabetic hypertensive disorder, resistant hypertensive disorder, refractory hypertensive disorder, paroxysmal hypertensive disorder, renovascular hypertensive disorder, Goldblatt hypertension, hypertensive disorder associated with low plasma renin activity or plasma renin concentration, high intraocular pressure, glaucoma, pulmonary arterial hypertension, portal hypertension, systemic venous hypertension, systolic hypertensive disorder, unstable hypertensive disorder; hypertensive heart disease, hypertensive nephropathy, atherosclerosis, arteriosclerosis, vascular disease, diabetic nephropathy, diabetic retinopathy, chronic heart failure, cardiomyopathy, diabetic cardiomyopathy, glomerulosclerosis, coarctation of the aorta, aortic aneurysm, ventricular fibrosis, heart failure, myocardial infarction, angina pectoris, stroke, nephropathy, renal failure, systemic sclerosis, intrauterine growth restriction, fetal growth restriction, obesity, hepatic steatosis/fatty liver, non-alcoholic steatohepatitis, non-alcoholic fatty liver disease, glucose intolerance, type 2 diabetes mellitus, and metabolic syndrome.

20. The use according to claim 16, wherein the nucleic acid, the targeted drug delivery system, or the pharmaceutical composition is administered to a subject via subcutaneous administration, intravenous administration, and/or intramuscular administration.