【DESCRIPTION】

【Invention Title】

ARTIFICIALLY ENGINEERED ANGIOGENESIS REGULATORY SYSTEM

【Technical Field】

The present invention relates to an artificially manipulated neovascularization-associated factor for regulating neovascularization and a use thereof. More particularly, the present invention relates to a system capable of artificially regulating neovascularization, which includes an artificially manipulated neovascularization-associated factor for regulating neovascularization and/or a composition able to be used in artificial manipulation of the neovascularization-associated factor.

【Background Art】

Excessive neovascularization found in many cases of severe diseases occurs in diseases such as cancer, macular degeneration, diabetic retinopathy, arthritis and psoriasis. In such a state, new blood vessels are provided to tissue with a disease, resulting in destroyed normal tissue, and in the case of cancer, new blood vessels allow tumor cells to enter the circulation system and thus settle in another organ (tumor metastasis).

Particularly, since cancer cells receive nutrients through neovascularization and are metastasized to another organ, neovascularization is essential for growth and metastasis of cancer. It has been known that there is an actual close relationship between the density of capillaries generated in cancer tissue and probability of cancer metastasis in various types of cancer. In addition, rheumatoid arthritis, which is the representative disease among inflammatory diseases, is caused by an autoimmune disorder, however, during the development of the disease, chronic inflammation generated in the synovial cavity between

joints leads to neovascularization, resulting in destroyed cartilage. Various ophthalmologic diseases leading to blindness in several million people in the world every year are also caused by neovascularization. As a representative example, diabetic blindness is a diabetic complication, and refers to an invasion of capillaries generated in the retina to the vitreous body through neovascularization, ending up in blindness. Therefore, neovascularization inhibitory substances may be usefully employed as therapeutic agents and preventive agents for various diseases such as cancer, rheumatoid arthritis and diabetic blindness, in which continuous neovascularization occurs.

Meanwhile, conventionally, inhibition of signaling of vascular endothelial growth factors (VEGFs) in order to inhibit neovascularization had been actively studied. However, in the conventional art, initially, neovascularization seemed to be inhibited, and then there was a side effect in which cancer cells became more aggressive because the pathway of an anticancer agent to the cancer cells was also inhibited.

As such, while a variety of studies to treat diseases induced by neovascularization are progressing, there is almost no fundamental method for treating such a disease.

Particularly, there is no method for treating a severe disease such as cancer or cancer metastasis caused by neovascularization, blindness caused by retinal or corneal degeneration, and therefore, there is an urgent demand for developing such a fundamental method for treating a neovascularization-associated disease.

【Disclosure】

【Technical Problem】

To solve the above problems, the present invention relates to an artificially manipulated neovascularization system, which has an improved neovascularizing effect.More

particularly, the present invention relates to an artificially manipulated neovascularization-associated factor and a neovascularization system which has a function artificially modified by the factor.

The present invention also relates to a neovascularization-associated factor genetically manipulated or modified for a specific purpose.

As an exemplary embodiment, the present invention is directed to providing an artificially manipulated neovascularization-regulating system.

As an exemplary embodiment, the present invention is directed to providing an artificially manipulated neovascularization-associated factor and an expression product thereof.

As an exemplary embodiment, the present invention is directed to providing a composition for manipulating a gene to manipulate a neovascularization-associated factor and a method for utilizing the same.

As an exemplary embodiment, the present invention is directed to providing a method for regulating neovascularization.

As an exemplary embodiment, the present invention is directed to providing a pharmaceutical composition for treating a neovascularization-associated disease and various uses thereof.

As an exemplary embodiment, the present invention is directed to providing an artificially manipulated neovascularization-associated factor, for example, VEGFA, HIF1A, ANGPT2, EPAS1, ANGPTL4, etc., and/or expression products thereof.

As an exemplary embodiment, the present invention is directed to providing a composition for manipulating a gene to enable artificial manipulation of a neovascularization-associated factor, for example, VEGFA, HIF1A, ANGPT2, EPAS1, ANGPTL4, etc.

As an exemplary embodiment, the present invention is directed to providing a therapeutic use of an artificially manipulated neovascularization-associated factor, for example, VEGFA, HIF1A, ANGPT2, EPAS1, ANGPTL4, etc., and/or a composition for manipulating a gene to enable the artificial manipulation.

As an exemplary embodiment, the present invention is directed to providing an additional use of an artificially manipulated neovascularization-associated factor, for example, VEGFA, HIF1A, ANGPT2, EPAS1, ANGPTL4, etc., and/or a composition for manipulating a gene to enable the artificial manipulation.

【Technical Solution】

To solve these problems, the present invention relates to a system for artificially regulating neovascularization, which includes an artificially manipulated neovascularization-associated factor for regulating neovascularization and/or a composition for artificially manipulating the neovascularization-associated factor.

The present invention provides an artificially manipulated neovascularization-associated factor for a specific purpose.

The term “neovascularization-associated factor” encompasses a variety of non-natural, artificially manipulated substances capable of having a neovascularization regulating function, which directly participate in or indirectly affect neovascularization. The substances may be DNA, RNA, genes, peptides, polypeptides or proteins. For example, the substances may be genetically manipulated or modified genes or proteins expressed in an immune cells.The neovascularization-associated factor may promote or increase neovascularization, or conversely, suppress or inhibit neovascularization.

In addition, it may induce, activate or inactivate a neovascularization environment or a neovascularization-inhibiting environment.

In an exemplary embodiment of the present invention, the neovascularization-associated factor may be, for example, an artificially manipulated VEGFA gene, HIF1A gene, ANGPT2 gene, EPAS1 gene or ANGPTL4 gene.

In an exemplary embodiment of the present invention, the neovascularization-associated factor may include two or more artificially manipulated genes. For example, two or more genes selected from the group consisting of a VEGFA gene, an HIF1A gene, an ANGPT2 gene, an EPAS1 gene and an ANGPTL4 gene may be artificially manipulated.

Therefore, in an exemplary embodiment of the present invention, one or more artificially manipulated neovascularization-associated factors selected from the group consisting of a VEGFA gene, an HIF1A gene, an ANGPT2 gene, an EPAS1 gene and an ANGPTL4 gene, which have undergone modification in a nucleic acid sequence, are provided.

The modification in a nucleic acid sequence may be non-limitedly, artificially manipulated by a guide nucleic acid-editor protein complex.

The term “guide nucleic acid-editor protein complex” refers to a complex formed through the interaction between a guide nucleic acid and an editor protein, and the nucleic acid-protein complex includes the guide nucleic acid and the editor protein.

The guide nucleic acid-editor protein complex may serve to modify a subject. The subject may be a target nucleic acid, a gene, a chromosome or a protein.

For example, the gene may be a neovascularization-associated factor, artificially manipulated by a guide nucleic acid-editor protein complex,

wherein the neovascularization-associated factor artificially manipulated includes one or more modifications of nucleic acids which is

at least one of a deletion or insertion of one or more nucleotides, a substitution with one or more nucleotides different from a wild-type gene, and an insertion of one or more foreign nucleotide, in a proto-spacer-adjacent motif (PAM) sequence in a nucleic acid sequence constituting the neovascularization-associated factor or in a continuous 1 bp to 50 bp the base sequence region adjacent to the 5’ end and/or 3’ end thereof,

or

a chemical modification of one or more nucleotides in a nucleic acid sequence constituting the neovascularization-associated factor .

The modification of nucleic acids may occur in a promoter region of the gene.

The modification of nucleic acids may occur in an exon region of the gene. In one exemplary embodiment, 50% of the modifications may occur in the upstream section of the coding regions of the gene.

The modification of nucleic acids may occur in an intron region of the gene.

The modification of nucleic acids may occur in an enhancer region of the gene.

The PAM sequence may be, for example, one or more of the following sequences (described in the 5’ to 3’ direction):

NGG (N is A, T, C or G);

NNNNRYAC (each of N is independently A, T, C or G, R is A or G, and Y is C or T);

NNAGAAW (each of N is independently A, T, C or G, and W is A or T);

NNNNGATT (each of N is independently A, T, C or G);

NNGRR(T) (each of N is independently A, T, C or G, R is A or G, and Y is C or T); and

TTN (N is A, T, C or G).

The editor protein may be derived from *Streptococcus pyogenes*, *Streptococcus thermophilus*, *Streptococcus* sp., *Staphylococcus aureus*, *Nocardiopsis dassonvillei*,

Streptomyces pristinaespiralis, *Streptomyces viridochromogenes*, *Streptomyces viridochromogenes*, *Streptosporangium roseum*, *Streptosporangium roseum*, *AlicyclobacHlus acidocaldarius*, *Bacillus pseudomycoides*, *Bacillus selenitireducens*, *Exiguobacterium sibiricum*, *Lactobacillus delbrueckii*, *Lactobacillus salivarius*, *Microscilla marina*, *Burkholderiales bacterium*, *Polaromonas naphthalenivorans*, *Polaromonas* sp., *Crocosphaera watsonii*, *Cyanothece* sp., *Microcystis aeruginosa*, *Synechococcus* sp., *Acetohalobium arabaticum*, *Ammonifex degensii*, *Caldicelulosiruptor bescii*, *Candidatus Desulforudis*, *Clostridium botulinum*, *Clostridium difficile*, *Finegoldia magna*, *Natranaerobius thermophilus*, *Pelotomaculum thermopropionicum*, *Acidithiobacillus caldus*, *Acidithiobacillus ferrooxidans*, *Allochromatium vinosum*, *Marinobacter* sp., *Nitrosococcus halophilus*, *Nitrosococcus watsonii*, *Pseudoalteromonas haloplanktis*, *Ktedonobacter racemifer*, *Methanohalobium evestigatum*, *Anabaena variabilis*, *Nodularia spumigena*, *Nostoc* sp., Arthrospira maxima, Arthrospira platensis, *Arthrospira* sp., *Lyngbya* sp., *Microcoleus chthonoplastes*, *Oscillatoria* sp., *Petrotoga mobilis*, *Thermosipho africanus*, or *Acaryochloris marina*.

In one exemplary embodiment, the editor protein may be one or more selected from the group consisting of a *Streptococcus pyogenes*-derived Cas9 protein, a *Campylobacter jejuni*-derived Cas9 protein, a *Streptococcus thermophilus*-derived Cas9 protein, a *Streptocuccus aureus*-derived Cas9 protein, a *Neisseria meningitidis*-derived Cas9 protein, and a Cpf1 protein. As an example, the editor protein may be a *Streptococcus pyogenes*-derived Cas9 protein or a *Campylobacter jejuni*-derived Cas9 protein.

In addition, in another embodiment, the present invention provides a guide nucleic acid, which is capable of forming a complementary bond with respect to target sequences of SEQ ID NOs: 1 to 1522, for example, SEQ ID Nos: 1 to 79 in the nucleic acid sequences of

one or more genes selected from the group consisting of a VEGFA gene, an HIF1A gene, an ANGPT2 gene, an EPAS1 gene and an ANGPTL4 gene, respectively.

The guide nucleic acid may form a complementary bond with a part of nucleic acid sequences of one or more genes selected from the group consisting of a VEGFA gene, an HIF1A gene, an ANGPT2 gene, an EPAS1 gene and an ANGPTL4 gene. It may create 0 to 5, 0 to 4, 0 to 3, or 0 to 2 mismatches. As an exemplary example, the guide nucleic acid may be nucleotides forming a complementary bond with one or more of the target sequences of SEQ ID NOs: 1 to 1522, for example, SEQ ID NOs: 1 to 79, respectively.

For example, the present invention may provide one or more guide nucleic acids selected from the group as described below:

a guide nucleic acid capable of forming a complementary bond with respect to the target sequences of SEQ ID NOs: 3, 4, 7, 9, 10 and 11 in the nucleic acid sequence of the VEGFA gene, respectively;

a guide nucleic acid capable of forming a complementary bond with respect to the target sequences of SEQ ID NOs: 14, 18, 19, 20, 26, 29 and 31 in the nucleic acid sequence of the HIF1A gene, respectively;

a guide nucleic acid capable of forming a complementary bond with respect to the target sequences of SEQ ID NOs: 33, 34, 37, 38, 39 and 43 in the nucleic acid sequence of the ANGPT2 gene, respectively;

a guide nucleic acid capable of forming a complementary bond with respect to the target sequences of SEQ ID NOs: 47, 48, 49, 50, 53, 54 and 55 in the nucleic acid sequence of the EPAS1 gene, respectively; and

a guide nucleic acid capable of forming a complementary bond with respect to the target sequences of SEQ ID NOs: 64, 66, 67, 73, 76 and 79 in the nucleic acid sequence of the ANGPTL4 gene, respectively.

The guide nucleic acid may be non-limitedly 18 to 25 bp, 18 to 24 bp, 18 to 23 bp, 19 to 23 bp, or 20 to 23 bp nucleotides.

In addition, the present invention provides a composition for gene manipulation, which may be employed in artificial manipulation of a neovascularization-associated factor for a specific purpose.

The composition for gene manipulation may include a guide nucleic acid-editor protein complex or a nucleic acid sequence encoding the same.

The composition for gene manipulation may include:

(a) a guide nucleic acid capable of forming a complementary bond with respect to each of target sequences of SEQ ID NOs: 1 to 1522, for example, SEQ ID NOs: 1 to 79 in the nucleic acid sequences of one or more genes selected from the group consisting of a VEGFA gene, an HIF1A gene, an ANGPT2 gene, an EPAS1 gene and an ANGPTL4 gene, respectively or a nucleic acid sequence encoding the guide nucleic acid;

(b) an editor protein including one or more proteins selected from the group consisting of a *Streptococcus pyogenes*-derived Cas9 protein, a *Campylobacter jejuni*-derived Cas9 protein, a *Streptococcus thermophilus*-derived Cas9 protein, a *Streptocuccus aureus*-derived Cas9 protein, a *Neisseria meningitidis*-derived Cas9 protein, and a Cpf1 protein or a nucleic acid sequence encoding the same.

In one exemplary embodiment, the guide nucleic acid may be a nucleic acid sequence which forms a complementary bond with respect to one or more of the target sequences of SEQ ID NOs: 3, 4, 7, 9, 10 and 11 (VEGFA), SEQ ID NOs: 14, 18, 19, 20, 26, 29 and 31 (HIF1A), SEQ ID NOs: 33, 34, 37, 38, 39 and 43 (ANGPT2), SEQ ID NOs: 47, 48, 49, 50, 53, 54 and 55 (EPAS1), and SEQ ID NOs: 64, 66, 67, 73, 76 and 79 (ANGPTL4), respectively.

In one exemplary embodiment, the composition for gene manipulation may be a viral vector system.

The viral vector may include one or more selected from the group consisting of a retrovirus, a lentivirus, an adenovirus, an adeno-associated virus (AAV), a vaccinia virus, a poxvirus and a herpes simplex virus.

In an exemplary embodiment, the present invention provides a method for artificially manipulating cells, which includes: introducing (a) a guide nucleic acid which is capable of forming a complementary bond with respect to the target sequences of SEQ ID NOs: 1 to 1522, for example, SEQ ID NOs: 1 to 79 in the nucleic acid sequences of one or more genes selected from the group consisting of a VEGFA gene, an HIF1A gene, an ANGPT2 gene, an EPAS1 gene and an ANGPTL4 gene, respectively, or a nucleic acid sequence encoding the same; and

(b) an editor protein including one or more proteins selected from the group consisting of a *Streptococcus pyogenes*-derived Cas9 protein, a *Campylobacter jejuni*-derived Cas9 protein, a *Streptococcus thermophilus*-derived Cas9 protein, a *Streptocuccus aureus*-derived Cas9 protein, a *Neisseria meningitidis*-derived Cas9 protein, and a Cpf1 protein, respectively, or a nucleic acid sequence encoding the same to cells.

The guide nucleic acid and the editor protein may be present in one or more vectors in the form of a nucleic acid sequence, or may be present in a complex formed by coupling the guide nucleic acid with the editor protein.

The introduction may be performed *in vivo* or *ex vivo*.

The introduction may be performed by one or more methods selected from electroporation, liposomes, plasmids, viral vectors, nanoparticles and a protein translocation domain (PTD) fusion protein method.

The viral vector may be one or more selected from the group consisting of a retrovirus, a lentivirus, an adenovirus, an adeno-associated virus (AAV), a vaccinia virus, a poxvirus and a herpes simplex virus.

In another exemplary embodiment, the present invention provides a pharmaceutical composition for treating a neovascularization-associated disease.

The pharmaceutical composition may include a composition for gene manipulation which may be employed in artificial manipulation of a neovascularization-associated factor.

The formulation of the composition for gene manipulation is the same as described above.

In an exemplary embodiment, the present invention provides a method for obtaining information about the sequences of target sites that are artificially manipulated from a subject by sequencing one or more genes selected from the group consisting of a VEGFA gene, an HIF1A gene, an ANGPT2 gene, an EPAS1 gene and an ANGPTL4 gene.

In addition, the present invention provides a method for constructing libraries using the information obtained thereby.

In an exemplary embodiment, the present invention provides a kit for gene manipulation, which includes the following components:

(a) a guide nucleic acid capable of forming a complementary bond with respect to the target sequences of SEQ ID NOs: 1 to 1522, for example, SEQ ID NOs: 1 to 79 in the nucleic acid sequences of one or more genes selected from the group consisting of a VEGFA gene, an HIF1A gene, an ANGPT2 gene, an EPAS1 gene and an ANGPTL4 gene, respectively, or a nucleic acid sequence encoding the same; and

(b) an editor protein including one or more proteins selected from the group consisting of a *Streptococcus pyogenes*-derived Cas9 protein, a *Campylobacter jejuni*-derived Cas9 protein, a *Streptococcus thermophilus*-derived Cas9 protein, a *Streptocuccus aureus*-

derived Cas9 protein, a *Neisseria meningitidis*-derived Cas9 protein, and a Cpf1 protein, respectively, or a nucleic acid sequence encoding the same.

The gene of interest may be artificially manipulated using such a kit.

In one exemplary embodiment, the present invention may provide a composition for treating a neovascularization-related disorder, which includes:

a guide nucleic acid capable of forming a complementary bond with one or more target sequences in the nucleic acid sequences of one or more genes selected from the group consisting of a VEGFA gene, an HIF1A gene, an ANGPT2 gene, an EPAS1 gene and an ANGPTL4 gene, respectively, or a nucleic acid sequence encoding the same; and

an editor protein or a nucleic acid sequence encoding the same.

The target sequences may be one or more sequences of SEQ ID NOs: 1 to 1522, for example, SEQ ID NOs: 1 to 79.

In one exemplary embodiment, a *Campylobacter jejuni*-derived Cas9 protein may be employed as the editor protein.

In one exemplary embodiment, the neovascularization-related disorder may be ischemic retinopathy or retinopathy of prematurity.

In one exemplary embodiment, the present invention provides all aspects of uses of an artificially manipulated neovascularization-associated factor or a composition for gene manipulation which is employed in artificial manipulation of the neovascularization-associated factor for treating a disease in a target.

Targets for treatment may be mammals including primates such as humans, monkeys, etc., rodents such as mice, rats, etc., and the like.

【Advantageous Effects】

An artificially manipulated neovascularization-associated factor and a neovascularization system whose function is artificially modified thereby can be effectively used to treat a neovascularization-related disease, for example, a neovascularization-related ocular disease. The efficiency of the neovascularization system can be improved by modulation of a variety of *in vivo* mechanisms in which various neovascularization-associated factors are involved.

For example, one or more genes of a VEGFA gene, an HIF1A gene, an ANGPT2 gene, an EPAS1 gene and an ANGPTL4 gene can be utilized.

【Brief Description of Drawings】

FIG. 1 shows the reducing effect on a laser-induced choroidal neovascularization (CNV) area due to CjCas9 targeting Vegfa or Hif1a in mouse models with age-related macular degeneration (AMD): (A) CjCas9 target sequences in Vegfa and Hif1a/HIF1A genes (the PAM sequence and the target sequence of sgRNA are marked with a dotted line and solid line, respectively), (B) the all-in-one AAV vector encoding CjCas9 and an *in vivo* test schedule, (C) graphs of indel frequencies at Rosa26, Vegfa, and Hif1a target sites in RPE cells (Error bars=s.e.m. (a control not injected with AAV: n=4, AAV-CjCa9-injected test group: n=5), Student's t-tests, * $p < 0.05$, *** $p < 0.001$), (D) a graph of Vegfa expression levels measured by ELISA in RPE cells (Error bars=s.e.m. (a control not injected with AAV: n=4, AAV-CjCa9-injected test group: n=5), One-way ANOVA and Tukey post-hoc tests, * $p < 0.05$, *** $p < 0.001$), (E) a graph of indel frequencies at off-target sites (the mismatched base sequence is marked with a solid line, and the PAM sequence is marked with a dotted line), (F) laser-induced CNV areas of eyeballs of mice injected withAAV9-CjCa9 targeting Rosa26,

Vegfa, or Hif1a, stained with isolectin B4 (Scale bar=200 μm), and (G) a graph of the laser-induced CNV areas (%) (Error bars=s.e.m.(n=17-18), One-way ANOVA and Tukey post-hoc tests, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns: not significant).

FIG. 2 shows images of *in vivo* expression of eGFP with CjCas9 in mouse RPE cells (n=6, anti-GFP antibody (green) and DAPI (blue), Scale bar=20 μm).

FIG. 3 shows opsin-positive areas in the retinas of AAV/CjCas9-injected mice: (A) images of opsin-positive areas corresponding to RPE cells expressing HA-tagged CjCas9 in Rosa26-, Vegfa-, or Hif1a-specific CjCas9-injected mice (Opsin: red, HA: green, and DAPI: blue, Scale bar=20 μm, ONL: outer nuclear layer, IS: inner segment of photoreceptor cells, OS: outer segment of photoreceptor cells); and (B) a graph of relative opsin-positive areas (%) (Error bars=s.e.m.(n=4), One-way ANOVA and Tukey post-hoc tests, * $p < 0.05$).

FIG. 4 shows reduced expression of VEGF due to CjCas9 targeting Vegfa or Hif1a in retinal tissue: (A) a graph of indel frequencies at target sites of Rosa26, Vegfa and Hif1a in retinal cells (Error bars=s.e.m. (a control not injected with AAV: n=4, AAV-CjCa9-injected test group: n=5), Student's t-tests, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$), (B) a graph of Vegfa expression levels in retinal cells, measured using ELISA (Error bars=s.e.m. (n=6-7), One-way ANOVA and Tukey post-hoc tests, * $p < 0.05$, *** $p < 0.001$).

FIG. 5 shows the reducing effect of CjCas9 targeting Vegfa on vascular leakage and blood leakage in retinas of diabetic retinopathy mouse models, (a) an *in vivo* test schedule, (b) images of reduced effects on vascular leakage and blood leakage in mouse retinas injected with AAV2-CjCa9 targeting Vegfa.

FIG. 6 shows the reducing effect of CjCas9 targeting Vegfa on vascular leakage and blood leakage in retinas of diabetic retinopathy mouse models: FIG. 6A is an *in vivo* test schedule (a), images of the reducing effect on vascular leakage and blood leakage in mouse

retinas injected with AAV2-CjCa9 targeting Vegfa (b), and FIG. 6B is a graph of relative vascular leakage (%) due to CjCas9 targeting Rosa26 or Vegfa.

FIG. 7 shows CjCas9 target site screening results and indel frequencies of human VEGFAs for gene manipulation.

FIG. 8 shows the result of CjCas9 target site screening of human HIF1As for gene manipulation: (A) CjCas9 target site screening results and indel frequencies of human HIF1As, and (B) target sites of HIF1As, conserved between various mammals.

FIG. 9 shows CjCas9 target site screening results and indel frequencies of human ANGPT2s for gene manipulation.

FIG. 10 shows CjCas9 target site screening results and indel frequencies of human EPAS1s for gene manipulation.

FIG. 11 shows CjCas9 target site screening results and indel frequencies of human ANGPTL4s for gene manipulation.

【Modes of the Invention】

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

One aspect of the present invention relates to an artificially manipulated neovascularization system, which has a regulated neovascularization effect.

Specifically, the present invention relates to combination of various aspects capable of regulating neovascularization or improving or treating a neovascularization-associated disease by artificially manipulating a neovascularization-associated factor. The present invention includes a neovascularization-associated factor whose function is artificially modified, a method of manufacturing the same, a composition including the same, and a therapeutic use thereof.

Another aspect of the present invention relates to an additional regulating system with a third in vivo mechanism, concomitant with various functions of a specific factor (e.g., a gene known as a neovascularization-associated factor, etc.) whose function is artificially modified.

Specifically, targeting of a third in vivo function as well as a neovascularization function in which artificially manipulated specific factors are involved may lead to the regulation of corresponding mechanisms. The present invention includes a neovascularization-associated factor whose function is artificially modified, a method for manufacturing the same, a composition including the same, and therapeutic uses thereof for improving or treating a disease associated with the third function.

[**Neovascularization**]

An exemplary embodiment of the present invention relates to improvement and modification of a neovascularization-associated system.

The term “neovascularization” refers to a process of tissue vascularization, including generation, development and/or differentiation of new blood vessels. Here, neovascularization encompasses angiogenesis and vasculogenesis. Neovascularization may be closely related to various factors to promote or inhibit proliferation of vascular endothelial cells.

Neovascularization encompasses all mechanisms for extension from existing blood vessels, new generation of blood vessels from precursor cells, and/or an increase in diameter of an existing small blood vessel.

In addition, neovascularization encompasses all mechanisms associated with formation of new vessels, which is involved in vascular leakage or repair of damaged blood vessels.

The vascularization includes mechanisms concomitant with excessive and/or abnormal neovascularization in various severe disease states.

For example, in diseases such as cancer, macular degeneration, diabetic retinopathy, arthritis and psoriasis, excessive neovascularization occurs. In such a disease state, new blood vessels are provided to tissue with a disease, and normal tissue is damaged. In the case of cancer, new blood vessels allow tumor cells to enter into the circulation system and thus enable them to settle in another organ (tumor metastasis).

In one exemplary embodiment, the neovascularization may be ocular vascularization.

For example, the neovascularization may be found in eye diseases such as AMD, diabetic retinopathy and the like. Particularly, AMD is the most common cause of legal irreversible blindness in older people over the age of 65 in the US, Canada, England, Wales, Scotland and Australia, and about 10% to 15% of the patients show exudative (wet) diseases. The exudative AMD is characterized by neovascularization and disease-causing angiogenesis.

For example, ocular neovascularization may include choroidal neovascularization (CNV), corneal neovascularization and/or rubeosis iridis.

CNV is the vascularization in the choroid layer, and rapidly occurs in people having a defect in the Bruch's membrane, which is the innermost layer of the choroid. In addition, CNV is associated with an excessive amount of vascular endothelial growth factor (VEGF).

CNV may cause excessive myopia, malignant myopia, or neovascular degenerative macular degeneration (e.g., wet macular degeneration).

Corneal neovascularization is the growth of new blood vessels from the pericorneal plexus in the periphery of the cornea into avascular corneal tissue due to oxygen deprivation, and may be mainly caused by congenital or inflammatory (e.g., rejection after corneal transplantation, grafted tissue or host diseases, atopic conjunctivitis, injections, ocular pemphigoid, Lyell's syndrome, and Stevens-Johnson syndrome), infectious (e.g., bacterial (chlamydia, syphilis, pseduomonas), viral (herpes simplex virus and herpes zoster virus), fungal (candida, aspergillus, fusarium), parasistic (onchocerca volvolus), degenerative, traumatic and iatrogenic (e.g., the wearing of contact lenses) diseases.

Rubeosis iridis is the vascularization on the surface of the iris, associated with diabetic retinopathy, and also known to be caused by central retinal vein occlusion, ocular ischemic syndrome, chronic retinal detachment, and the like.

In a certain embodiment, the neovascularization may be associated with survival, proliferation, persistency, cytotoxicity, and a cytokine-release function of vascular endothelial cells.

In a certain embodiment, neovascularization may be associated with an increase in the expression of an angiogenic cytokine.

In a certain embodiment, the neovascularization may be associated with functions of a receptor of vascular endothelial cells.

In a certain embodiment, the neovascularization may be associated with a migration ability of vascular endothelial cells.

In a certain embodiment, the neovascularization may be associated with an attachment ability of vascular endothelial cells.

[**Neovascularization-associated factor**]

Neovascularization-associated factor

One embodiment of the present invention relates to an artificially manipulated or modified neovascularization-associated factor.

The term "neovascularization-associated factor" includes all elements directly participating in or indirectly affecting vasculogenesis or angiogenesis. Here, the elements may include DNA, RNA, genes, peptides, polypeptides or proteins.

In an exemplary embodiment, the neovascularization-associated factor may include various substances which can have a non-natural, that is, artificially manipulated, neovascularization regulating function. For example, it may be a genetically manipulated or modified genes or proteins expressed in an immune cells.

The term "artificially manipulated" means an artificially modified state, which is not a naturally occurring state.

The term "genetically manipulated" means that a genetic modification is artificially introduced to biological or non-biological substances cited in the present invention, and may be, for example, genes and/or gene products (polypeptides, proteins, etc.) in which their genomes are artificially modified for a specific purpose.

As an exemplary example, the present invention provides a neovascularization-associated factor which is genetically manipulated or modified for a specific purpose.

Genes or proteins having the functions listed below may have multiple types of functions, not only one type of neovascularization-associated function. In addition, as needed, two or more neovascularization functions and factors may be provided.

The neovascularization-associated factor may promote or increase neovascularization.

The neovascularization-associated factor may suppress or inhibit neovascularization.

The neovascularization-associated factor may induce or activate a neovascularization environment.

The neovascularization-associated factor may induce a neovascularization-inhibited environment or inactivate a neovascularization environment.

The neovascularization-associated factor may regulate (promote, increase, suppress and/or inhibit etc.) neovascularization.

The neovascularization-associated factor may be utilized in improvement and treatment of a neovascularization-associated disease.

In an exemplary embodiment, the neovascularization-associated factor may be one or more selected from the group consisting of ABCA1, ACAT, ACC2, ADAMTS12, ADCY2, ADIPOQ, ADIPOR1, ADIPOR2, ADRB2, AGPAT5, AIP4, AKAP2, AKR1C2, AMPK, ANG2, ANGPT2, ANGPTL4, ANK1, ANXA1, APOA1, ARHGAP17, ATP10A, AUH, AUTOTAXIN, BAI3, BCAR1, BIN1, BMP3A, CA10, CAMK1D, CAMKK2, CD36, CD44, CDC42, CDH13, CHAT, CNTFR, COL4A2, CPT, CSH1, CTNN, CUBN, CYP7B1, CYSLTR1, CYSLTR2, DGKB, DGKH, DGKZ, DHCR7, DHFR, DRD2, DRD5, EDG1, EDG2, EDG3, EDG4, EDG5, EDG6, EDG7, EDG8, EDNRA, EHHADH, ENPP6, EPAS1, ERBB4, ERK1, ERK2, ESRRG, ETFA, F2, FDPS, FGF2, FLNA, FLT4, FOXO1, FOXO3A, FTO, GABBR2, GATA3, GH1, GNA12, GNA13, GRK2, GRK5, GRM5, HAPLN1, HAS1, HAS2, HAS3, HCRTR2, HIF1A, HSD11B1, HYAL1, HYAL2, HYAL3, IL20RA, IL20RB, IL6ST, IL8, ITGA6, ITGB1, KDR, LAMA1, LDLR, LEPR, LEPTIN, LIFR, LIPL2, LKB1, LRP, LTBP2, MAT2B, ME1, MEGALIN, MERLIN, MET, MGST2, MMP2, MMP9, MTOR, MTR, NCK2, NEDD9, NFKB1, NFKBIB, NOS2A, NOS3, NR1I2, NR3C2, NRG1, NRP1, NRP2, OPRS1, OSBPL10, OSBPL3, OSTEOPONTIN, P2RY1, P2RY12, PAI1, PAI2, PAK1, PAK6, PALLD, PAP1, PAR1, PAXILLIN, PC, PCTP, PDE11A, PDE1A, PDE3A, PDE4D,

PDE5, PDGFA, PDGFB, PDGFRA, PDGFRB, PI3K, PITPNC1, PKA, PKCD, PLA1A, PLA2, PLAT, PLAU, PLCB1, PLD1, PLD2, PLG, PLXDC2, PPARA, PPARG, PPARGC1B, PRKG1, PRL, PTGS2, PTN, PTPN11, PYK2, RAC1, RAS, RHEB, RHOA, ROCK1, ROCK2, RPS6KA1, RPS6KB2, SCARB1, SCHIP1, SGPP2, SLC25A21, SMAD3, SMAD4, SNCA, SORBS2, SPLA2, SPOCK1, SRD5A1, SREBF1, SREBF2, STAT3, TGFBR1, TGFBR2, TGFBR3, THBS1, THBS2, THEM2, THRB, TIAM1, TIMP2, TLL2, TSC1, TSC2, TSPO, VEGFA, VEGFR1, and YES1.

As an exemplary example of the present invention, the neovascularization-associated factor may be one or more selected from the group consisting of VEGFA, HIF1A, ANGPT2, EPAS1, and ANGPTL4.

In a certain embodiment, the neovascularization-associated factor may be VEGFA.

A vascular endothelial growth factor A (VEGFA) gene is a gene (full-length DNA, cDNA or mRNA) encoding a VEGFA protein also called MVCD1, VEGF or VPF. In one example, the VEGFA gene may be one or more selected from the group consisting of the following genes, but the present invention is not limited thereto: genes encoding human VEGFA (e.g., NCBI Accession No. NP_001020537.2 or NP_001020538.2), for example, VEGFA genes represented by NCBI Accession No. NM_001025366.2, NM_001025367.2, NM_003376, or NG_008732.1.

In a certain embodiment, the neovascularization-associated factor may be HIF1A.

A hypoxia-inducible factor 1-alpha (HIF-1-alpha; HIF1A) gene refers to a gene (full-length DNA, cDNA or mRNA) encoding a HIF1A protein also called HIF1, MOP1, PASD8 or bHLHe78. In an example, the HIF1A gene may be one or more selected from the group consisting of the following genes, but the present invention is not limited thereto: genes encoding human HIF1A (e.g., NCBI Accession No. NP_001230013.1 or NP_001521.1), for

example, HIF1A genes represented by NCBI Accession No. NM_001243084.1, NM_001530.3, NM_181054.2, or NG_029606.1.

In a certain embodiment, the neovascularization-associated factor may be ANGPT2.

An angiopoietin-2 (ANGPT2) gene refers to a gene (full-length DNA, cDNA or mRNA) encoding an ANGPT2 protein also called AGPT2 or ANG2. In an example, the ANGPT2 gene may be one or more selected from the group consisting of the following genes, but the present invention is not limited thereto: genes encoding human ANGPT2 (e.g., NCBI Accession No. NP_001112359.1, NP_001112360.1 or NP_001138.1), for example, ANGPT2 genes represented by NCBI Accession No. NM_001118887.1, NM_001118888.1, NM_001147.2 or NG_029483.1.

In a certain embodiment, the neovascularization-associated factor may be EPAS1.

An endothelial PAS domain-containing protein 1 (EPAS1) gene refers to a gene (full-length DNA, cDNA or mRNA) encoding an EPAS1 protein also called ECYT4, HIF2A, HLF, MOP2, PASD2 or bHLHe73. In an example, the EPAS1 gene may be one or more selected from the group consisting of the following genes, but the present invention is not limited thereto: genes encoding human EPAS1 (e.g., NCBI Accession No. NP_001421.2, etc.), for example, EPAS1 genes represented by NCBI Accession No. NM_001430.4 or NG_016000.1.

In a certain embodiment, the neovascularization-associated factor may be ANGPTL4.

An angiopoietin-like 4 (ANGPTL4) gene refers to a gene (full-length DNA, cDNA or mRNA) encoding an ANGPTL4 protein also called ARP4, FIAF, HARP, HFARP, NL2, PGAR, TGQTL or UNQ171. In an example, the ANGPTL4 gene may be one or more selected from the group consisting of the following genes, but the present invention is not limited thereto: genes encoding human ANGPTL4 (e.g., NCBI Accession No. NP_001034756.1 or NP_647475.1), for example, ANGPTL4 genes represented by NCBI Accession No. NM_001039667.2, NM_139314.2, or NG_012169.1.

The neovascularization-associated factor may be derived from mammals including primates such as human, monkeys and the like, rodents such as rats, mice and the like, etc.

Information about the genes may be obtained from a known database such as GeneBank of the National Center for Biotechnology Information (NCBI).

In an exemplary embodiment of the present invention, the neovascularization-associated factor, for example, VEGFA, HIF1A, ANGPT2, EPAS1 or ANGPTL4, may be an artificially manipulated neovascularization-associated factor.

In a certain embodiment, the artificially manipulated neovascularization-associated factor may be genetically manipulated.

The gene manipulation or modification may be achieved by artificial insertion, deletion, substitution or inversion occurring in a partial or entire region of the genomic sequence of a wild type gene. In addition, the gene manipulation or modification may be achieved by fusion of manipulation or modification of two or more genes.

For example, the gene is inactivated by such gene manipulation or modification, such that a protein encoded from the gene may not be expressed in the form of a protein having an innate function.

For example, the gene may be further activated by such gene manipulation or modification, such that a protein encoded from the gene is to be expressed in the form of a protein having an improved function, compared to the innate function. In an example, when a function of the protein encoded by a specific gene is A, a function of a protein expressed by a manipulated gene may be totally different from A or may have an additional function (A+B) including A.

For example, a fusion of two or more proteins may be expressed using two or more genes having different or complementary functions due to such gene manipulation or modification.

For example, two or more proteins may be expressed separately or independently in cells by using two or more genes having different or complementary functions due to such gene manipulation or modification.

The manipulated neovascularization-associated factor may promote or increase neovascularization.

The manipulated neovascularization-associated factor may suppress or inhibit neovascularization.

The manipulated neovascularization-associated factor may induce or activate a neovascularization environment.

The manipulated neovascularization-associated factor may induce a neovascularization inhibiting environment or inactivate a neovascularization environment.

The manipulated neovascularization-associated factor may regulate (promote, increase, suppress and/or inhibit) neovascularization.

The manipulated neovascularization-associated factor may be utilized in improvement and treatment of a neovascularization-associated disease.

The manipulation includes all types of structural or functional modifications of the neovascularization-associated factor.

The structural modification of the neovascularization-associated factor includes all types of modifications, which are not the same as those of a wild type existing in a natural state.

For example, when the neovascularization-associated factor is DNA, RNA or a gene, the structural modification may be the loss of one or more nucleotides.

The structural modification may be the insertion of one or more nucleotides.

Here, the inserted nucleotides include all of a subject including a neovascularization-associated factor and nucleotides entering from the outside of the subject.

The structural modification may be the substitution of one or more nucleotides.

The structural modification may include the chemical modification of one or more nucleotides.

Here, the chemical modification includes all of the addition, removal and substitution of chemical functional groups.

As another example, when the neovascularization-associated factor is a peptide, a polypeptide or a protein, the structural modification may be the loss of one or more amino acids.

The structural modification may be the insertion of one or more amino acids.

Here, the inserted amino acids include all of a subject including a neovascularization-associated factor and amino acids entering from the outside of the subject.

The structural modification may be the substitution of one or more amino acids.

The structural modification may include the chemical modification of one or more amino acids.

Here, the chemical modification includes all of the addition, removal and substitution of chemical functional groups.

The structural modification may be the partial or entire attachment of a different peptide, polypeptide or protein.

Here, the different peptide, polypeptide or protein may be a neovascularization-associated factor, or a peptide, polypeptide or protein having a different function.

The functional modification of the neovascularization-associated factor may include all types having an improved or reduced function, compared to that of a wild type existing in a natural state, and having a third different function.

For example, when the neovascularization-associated factor is a peptide, polypeptide or protein, the functional modification may be a mutation of the neovascularization-associated factor.

Here, the mutation may be a mutation that enhances or suppresses a function of the neovascularization-associated factor.

The functional modification may have an additional function of the neovascularization-associated factor.

Here, the additional function may be the same or a different function. In addition, the neovascularization-associated factor having the additional function may be fused with a different peptide, polypeptide or protein.

The functional modification may be the enhancement in functionality due to increased expression of the neovascularization-associated factor.

The functional modification may be the degradation in functionality due to decreased expression of the neovascularization-associated factor.

In an exemplary embodiment, the manipulated neovascularization-associated factor may be induced by one or more of the following mutations:

all or partial deletions of the neovascularization-associated factor, that is, a gene to be manipulated (hereinafter, referred to as a target gene), for example, deletion of 1 bp or longer nucleotides, for example, 1 to 30, 1 to 27, 1 to 25, 1 to 23, 1 to 20, 1 to 15, 1 to 10, 1 to 5, 1 to 3, or 1 nucleotide of the target gene,

substitution of 1 bp or longer nucleotides, for example, 1 to 30, 1 to 27, 1 to 25, 1 to 23, 1 to 20, 1 to 15, 1 to 10, 1 to 5, 1 to 3, or 1 nucleotide of the target gene with a nucleotide different from a wild type, and

insertion of one or more nucleotides, for example, 1 to 30, 1 to 27, 1 to 25, 1 to 23, 1 to 20, 1 to 15, 1 to 10, 1 to 5, 1 to 3, or 1 nucleotide (each independently selected from A, T, C and G) into a certain position of the target gene.

A part of the modified target gene ("target region") may be a continuous 1 bp or more, 3 bp or more, 5 bp or more, 7 bp or more, 10 bp or more, 12 bp or more, 15 bp or more,17 bp or more, or 20 bp or more, for example, 1 bp to 30 bp, 3 bp to 30 bp, 5 bp to 30 bp,7 bp to 30 bp, 10 bp to 30 bp, 12 bp to 30 bp, 15 bp to 30 bp, 17 bp to 30 bp, 20 bp to 30 bp, 1 bp to 27 bp, 3 bp to 27 bp, 5 bp to 27 bp, 7 bp to 27 bp, 10 bp to 27 bp, 12 bp to 27 bp, 15 bp to 27 bp, 17 bp to 27 bp, 20 bp to 27 bp, 1 bp to 25 bp, 3 bp to 25 bp, 5 bp to 25 bp, 7 bp to 25 bp, 10 bp to 25 bp, 12 bp to 25 bp, 15 bp to 25 bp, 17 bp to 25 bp, 20 bp to 25 bp, 1 bp to 23 bp, 3 bp to 23 bp, 5 bp to 23 bp, 7 bp to 23 bp, 10 bp to 23 bp, 12 bp to 23 bp, 15 bp to 23 bp, 17 bp to 23 bp, 20 bp to 23 bp, 1 bp to 20 bp, 3 bp to 20 bp, 5 bp to 20 bp, 7 bp to 20 bp, 10 bp to 20 bp, 12 bp to 20 bp, 15 bp to 20 bp, 17 bp to 20 bp, 21 bp to 25 bp, 18 bp to 22 bp, or 21 bp to 23 bp region of the base sequence of the gene.

Meanwhile, a different embodiment of the present invention relates to an additional system for regulating a third *in vivo* mechanism, concomitant with various functions of the above-described neovascularization-associated factors whose functions are artificially modified.

In one exemplary embodiment, VEGF may be involved in regulation of the third *in vivo* mechanism.

Since an increase in vascular permeability by VEGF may be a cause of edema, as well as tumor growth, artificially manipulated VEGF may increase a survival rate in various types of tumors (e.g., brain tumor, uterine cancer, vestibular schwannomas, etc.) or recover hearing loss, for example, by manipulation to inactivate VEGF. In addition, a decrease in vascular permeability by artificially manipulated VEGF may impart therapeutic effects on renal failure, arthritis, psoriasis, coronary disease, etc.

In addition, the artificially manipulated VEGF may impart a therapeutic effect on an autoimmune disease. For example, inflammation-inducing activity may be artificially regulated by VEGF, thereby imparting therapeutic effects on uveitis, rheumatoid arthritis, systemic lupus erythematosus, an inflammatory bowel disease, psoriasis, systemic sclerosis, multiple sclerosis, etc.

In addition, the artificially manipulated VEGF may impart a therapeutic effect on a mental disease. For example, a therapeutic effect on depression may be imparted by artificially regulating the expression of a neurotransmission-associated factor by VEGF.

In another embodiment, the artificially manipulated VEGF may be involved in the regulation of a third *in vivo* mechanism of HIF. The HIF may be HIF1 or HIF2.

The artificially manipulated HIF may regulate inflammation-inducing activity, thereby imparting therapeutic effects on uveitis, rheumatoid arthritis, systemic lupus erythematosus, an inflammatory bowel disease, psoriasis, systemic sclerosis, multiple sclerosis, etc.

In addition, the artificially manipulated HIF may provide a therapeutic effect on an autoimmune disease.

Likewise, the illustrative factors of the present invention which are artificially manipulated may regulate corresponding mechanisms by targeting the third *in vivo* function as well as the neovascularization function. One exemplary embodiment of the present

invention includes such a neovascularization factor whose function is artificially modified and a method for manufacturing the same, a composition including the same, and uses of the factor and the composition for improving or treating a disease associated with a third function.

[**Neovascularization system**]

Neovascularization-regulating system

In one aspect of the present invention, a neovascularization-regulating system for regulating neovascularization by artificially manipulating a neovascularization-associated factor is provided.

The term “neovascularization-regulating system” used herein includes all phenomena affecting the promotion, increase, suppression and/or inhibition of neovascularization by change of a function of an artificially manipulated neovascularization-associated factor, and also includes all substances, compositions, methods, and uses which are directly or indirectly involved in such a neovascularization-regulating system.

Each factor constituting such a neovascularization-regulating system is also generally called “neovascularization-regulating factor.”

The system of the present invention includes a modified *in vivo* mechanism, associated with an artificially manipulated neovascularization-associated factor.

In a certain embodiment, the expression of hematopoietic stem cell surface antigens such as CD34, CD117, CD133, etc. and vascular endothelial cell antigens such as Flk-1/KDR, Tie-2, etc. may be regulated by the artificially manipulated neovascularization-associated factor.

In a certain embodiment, angiogenesis in which new vessels are formed by sprouting and the growth of cells constituting a blood vessel may be regulated.

In a certain embodiment, the activity of direct angiogenic factors (DAFs) directly stimulating endothelial cells may be regulated. The growth and/or migration of endothelial cells may be promoted or inhibited.

For example, the DAFs may include vascular endothelial growth factors (VEGFs), basic fibroblast growth factors (bFGFs), hepatocyte growth factors (HGFs), epidermal growth factors (EGFs), thymidine phosphorylase (PD-ECGF), placental growth factors (PlGFs), transforming growth factors (TGFs), proliferin, interleukin-8 of a cytokine, angiogenin (angiogenesis-inducing protein), fibrin, nicotinamide (vitamin B complex), angiopoietin (angiogenesis-promoting protein), platelet activating factors (PAFs), 12-hydroxy eicosatetraenoate (12-HETE; a toxic degradation product of arachidonic acid, which is an angiogenesis-promoting factor of epithelial cells), matrix metalloproteases (MMPs), sphingosine 1-phosphate (S1P), and leptin.

In a certain embodiment, two different intercellular signaling pathways operating in blood vessel cells, that is, PDGF and VEGF signaling pathways may be utilized.

In a certain embodiment, the activity of indirect angiogenic factors (IAFs) inducing angiogenesis by formation of DAFs may be regulated by stimulating vascular pericytes.

In a certain embodiment, vascular endothelial cells may be differentiated from endothelial progenitor cells (EPCs), and thus a mechanism of forming a primary vascular plexus may be regulated.

In a certain embodiment, the degradability of extracellular matrix components for the migration of endothelial cells may be regulated.

In a certain embodiment, a cell migration-associated signaling pathway may be regulated.

In a certain embodiment, the activity of VEGF receptors such as VEGFR-1 (flt-1; fmslike-tyrosine kinase-1), VEGFR-2 (flk-1/KDR), and VEGFR-3, and a platelet derived growth factor (PDGF) receptor, or neuropilin-1 (NP-1) may be regulated.

In an exemplary embodiment, the neovascularization-regulating system includes a composition for manipulating a neovascularization-associated factor.

The composition for manipulation may be a composition capable of artificially manipulating a neovascularization-associated factor, and preferably, a composition for gene manipulation.

Hereinafter, the composition for gene manipulation will be described.

[**Composition for manipulating neovascularization-associated factor**]

Manipulation or modification of substances involved in the neovascularization-associated factor and the neovascularization system of the present invention is preferably accomplished by genetic manipulation.

In one aspect, composition and method for manipulating a gene by targeting a partial or entire non-coding or coding region of the neovascularization-associated factor may be provided.

In an exemplary embodiment, the composition and method may be used in manipulation or modification of one or more neovascularization regulating genes involved in the formation of a desired neovascularization system. The manipulation or modification may be performed by modification of nucleic acids constituting a gene. As a result of the manipulation, all of knockdown, knockout, and knockin are included.

In an exemplary embodiment, the manipulation may be performed by targeting a promoter region, or a transcription sequence, for example, an intron or exon sequence. A coding sequence, for example, a coding region, specifically, an initial coding region may be targeted for the modification of expression and knockout.

In an exemplary embodiment, the modification of nucleic acids may be substitution, deletion, and/or insertion of one or more nucleotides, for example, 1 to 30 bp, 1 to 27 bp, 1 to 25 bp, 1 to 23 bp, 1 to 20 bp, 1 to 15 bp, 1 to 10 bp, 1 to 5 bp, 1 to 3 bp, or 1 bp nucleotides.

In an exemplary embodiment, for the knockout of one or more neovascularization-associated genes, elimination of expression of one or more of the genes, or one or more knockouts of one or two alleles, the above-mentioned region may be targeted such that one or more neovascularization-associated genes contain a deletion or mutation.

In an exemplary embodiment, the knockdown of a gene may be used to decrease the expression of undesired alleles or transcriptomes.

In an exemplary embodiment, non-coding sequences of a promoter, an enhancer, an intron, a 3'UTR, and/or a polyadenylation signal may be targeted to be used in modifying a neovascularization-associated gene affecting a neovascularization function.

In an exemplary embodiment, the activity of a neovascularization-associated gene may be regulated, for example, activated or inactivated by the modification of nucleic acids of the gene.

In an exemplary embodiment, the modification of nucleic acids of the gene may catalyze cleavage of a single strand or double strands, that is, breaks of nucleic acid strands in a specific region of the target gene by a guide nucleic acid-editor protein complex, resulting in inactivation of the target gene.

In an exemplary embodiment, the nucleic acid strand breaks may be repaired through a mechanism such as homologous recombination or non-homologous end joining (NHEJ).

In this case, when the NHEJ mechanism takes place, a change in DNA sequence is induced at the cleavage site, resulting in inactivation of the gene. The repair by NHEJ may induce substitution, insertion or deletion of a short gene fragment, and may be used in the induction of a corresponding gene knockout.

In another aspect, the present invention provides a composition for manipulating a neovascularization-associated factor.

The composition for manipulation is a composition that is able to artificially manipulate a neovascularization-associated factor, and preferably, a composition for gene manipulation.

The composition may be employed in gene manipulation for one or more neovascularization-associated factors involved in formation of a desired neovascularization-regulating system.

The gene manipulation may be performed in consideration of a gene expression regulating process.

In an exemplary embodiment, it may be performed by selecting a suitable manipulation means for each stage of transcription, RNA processing, RNA transporting, RNA degradation, translation, and protein modification regulating stages.

In an exemplary embodiment, small RNA (sRNA) interferes with mRNA or reduces stability thereof using RNA interference (RNAi) or RNA silencing, and in some cases, breaks up mRNA to interrupt the delivery of protein synthesis information, resulting in regulation of the expression of genetic information.

The gene manipulation may be performed by modification of nucleic acids constituting a neovascularization-associated factor. As manipulation results, all of knockdown, knockout, and knockin are included.

In a certain embodiment, the modification of nucleic acids may be substitution, deletion, and/or insertion of one or more nucleotides, for example, 1 to 30 bp, 1 to 27 bp, 1 to 25 bp, 1 to 23 bp, 1 to 20 bp, 1 to 15 bp, 1 to 10 bp, 1 to 5 bp, 1 to 3 bp, or 1 bp nucleotides.

In a certain embodiment, for knockout of one or more neovascularization-associated factors, elimination of the expression of one or more factors, or one or more knockouts of one or two alleles, the gene may be manipulated such that one or more neovascularization-associated factors contain a deletion or mutation.

In a certain embodiment, knockdown of the neovascularization-associated factor may be used to decrease expression of undesired alleles or transcriptomes.

In a certain embodiment, the modification of nucleic acids may be insertion of one or more nucleic acid fragments or genes. Here, the nucleic acid fragment may be a nucleic acid sequence consisting of one or more nucleotides, and a length of the nucleic acid fragment may be 1 to 40 bp, 1 to 50 bp, 1 to 60 bp, 1 to 70 bp, 1 to 80 bp, 1 to 90 bp, 1 to 100 bp, 1 to 500 bp or 1 to 1000 bp. Here, the inserted gene may be one of the neovascularization-associated factors, or a gene having a different function.

In an exemplary embodiment, the modification of nucleic acids may employ a wild type or variant enzyme which is capable of catalyzing hydrolysis (cleavage) of bonds between nucleic acids in a DNA or RNA molecule, preferably, a DNA molecule. It may also employ a guide nucleic acid-editor protein complex.

For example, the gene may be manipulated using one or more nucleases selected from the group consisting of a meganuclease, a zinc finger nuclease, CRISPR/Cas9 (Cas9 protein), CRISPR-Cpf1 (Cpf1 protein) and a TALE-nuclease, thereby regulating the expression of genetic information.

In a certain embodiment, non-limitedly, the gene manipulation may be mediated by NHEJ or homology-directed repair (HDR) using a guide nucleic acid-editor protein complex, for example, a CRISPR/Cas system.

In this case, when the NHEJ mechanism takes place, a change in DNA sequence may be induced at a cleavage site, thereby inactivating the gene. Repair by NHEJ may induce substitution, insertion or deletion of a short gene fragment, and may be used in the induction of the knockout of a corresponding gene.

In another aspect, the present invention may provide the gene manipulation site.

In an exemplary embodiment, when the gene is modified by NHEJ-mediated modification, the gene manipulation site may be a site in the gene, triggering the decrease or elimination of expression of a neovascularization regulating gene product.

For example, the site may be in an initial coding region,

a promoter sequence,

an enhancer sequence,

a specific intron sequence, or

a specific exon sequence.

In an exemplary embodiment, the composition for manipulating a neovascularization-associated factor may target

a neovascularization-associated factor affecting the regulation of neovascularization, such as a VEGFA gene, an HIF1A gene, an ANGPT2 gene, an EPAS1 gene, or an ANGPTL4 gene, as a manipulation subject.

Examples of target regions, that is, target sequences for regions in which gene manipulation occurs or which are recognized for gene manipulation are summarized in Table 1, Table 2, Table 3, Table 4 and Table5.

The target sequence may target one or more genes.

The target sequence may simultaneously target two or more genes. Here, the two or more genes may be homologous genes or heterologous genes.

The gene may contain one or more target sequences.

The gene may be simultaneously targeted at two or more target sequences.

The gene may be changed in the site and number of gene manipulations according to the number of target sequences.

The gene manipulation may be designed in various forms depending on the number and positions of the target sequences.

The gene manipulation may simultaneously occur in two or more target sequences. Here, the two or more target sequences may be present in the homologous gene or heterologous gene.

The gene manipulation may be simultaneously performed with respect to the two or more genes. Here, the two or more genes may be homologous genes or heterologous genes.

Hereinafter, examples of target sequences which are able to be used in embodiments of the present invention are shown in the following tables:

Table 1 Target sequences of VEGFA gene

Table 2 Target sequences of HIF1A gene

Table 3 Target sequences of ANGPT2 gene

Table 4 Target sequences of EPAS1 gene

Table 5 Target sequences of ANGPTL4 gene

【Table 1】

Gene	No.	Target sequence
VEGFA	1	GTAGAGCAGCAAGGCAAGGCTC (SEQ ID NO: 1)
VEGFA	2	CTTTCTGTCCTCAGTGGTCCCA (SEQ ID NO: 2)
VEGFA	3	GAGACCCTGGTGGACATCTTCC (SEQ ID NO: 3)
VEGFA	4	TTCCAGGAGTACCCTGATGAGA (SEQ ID NO: 4)
VEGFA	5	TTGAAGATGTACTCGATCTCAT (SEQ ID NO: 5)
VEGFA	6	AGGGGCACACAGGATGGCTTGA (SEQ ID NO: 6)
VEGFA	7	AGCAGCCCCCGCATCGCATCAG (SEQ ID NO: 7)
VEGFA	8	GCAGCAGCCCCCGCATCGCATC (SEQ ID NO: 8)
VEGFA	9	GTGATGTTGGACTCCTCAGTGG (SEQ ID NO: 9)
VEGFA	10	TGGTGATGTTGGACTCCTCAGT (SEQ ID NO: 10)
VEGFA	11	CATGGTGATGTTGGACTCCTCA (SEQ ID NO: 11)
VEGFA	12	ATGCGGATCAAACCTCACCAAG (SEQ ID NO: 12)
VEGFA	13	CACATAGGAGAGATGAGCTTCC (SEQ ID NO: 13)

【Table 2】

Gene	No.	Target sequence
HIF1A	1	ACTCACCAGCATCCAGAAGTTT (SEQ ID NO: 14)
HIF1A	2	ATTTGGATATTGAAGATGACAT (SEQ ID NO: 15)
HIF1A	3	ATTTACATTTCTGATAATGTGA (SEQ ID NO: 16)
HIF1A	4	ATGTGTTTACAGTTTGAACTAA (SEQ ID NO: 17)
HIF1A	5	CTGTGTCCAGTTAGTTCAAACT (SEQ ID NO: 18)
HIF1A	6	ATGGTCACATGGATGAGTAAAA (SEQ ID NO: 19)
HIF1A	7	CATGAGGAAATGAGAGAAATGC (SEQ ID NO: 20)
HIF1A	8	CCCAGTGAGAAAAGGGAAAGAA (SEQ ID NO: 21)
HIF1A	9	TTGTGAAAAAGGGTAAAGAACA (SEQ ID NO: 22)
HIF1A	10	ATAGTTCTTCCTCGGCTAGTTA (SEQ ID NO: 23)
HIF1A	11	TCATAGTTCTTCCTCGGCTAGT (SEQ ID NO: 24)
HIF1A	12	TGTTCTTCATACACAGGTATTG (SEQ ID NO: 25)
HIF1A	13	TACGTGAATGTGGCCTGTGCAG (SEQ ID NO: 26)
HIF1A	14	CTGCACAGGCCACATTCACGTA (SEQ ID NO: 27)
HIF1A	15	CTGAGGTTGGTTACTGTTGGTA (SEQ ID NO: 28)
HIF1A	16	CAGGTCATAGGTGGTTTCTTAT (SEQ ID NO: 29)
HIF1A	17	ACCAAGCAGGTCATAGGTGGTT (SEQ ID NO: 30)
HIF1A	18	TTAGATAGCAAGACTTTCCTCA (SEQ ID NO: 31)

【Table 3】

Gene	No.	Target sequence
ANGPT2	1	TCAGGTCCAGCATGGGTCCTGC (SEQ ID NO: 32)
ANGPT2	2	CGGCGCGTCCCTCTGCACAGCA (SEQ ID NO: 33)
ANGPT2	3	GCTGTGCAGAGGGACGCGCCGC (SEQ ID NO: 34)
ANGPT2	4	ATCGTATTCGAGCGGCGCGTCC (SEQ ID NO: 35)
ANGPT2	5	GATGTTCTCCAGCACTTGCAGC (SEQ ID NO: 36)
ANGPT2	6	AGTGCTGGAGAACATCATGGAA (SEQ ID NO: 37)
ANGPT2	7	ACAACATGAAGAAAGAAATGGT (SEQ ID NO: 38)
ANGPT2	8	AAATGGTAGAGATACAGCAGAA (SEQ ID NO: 39)
ANGPT2	9	TTCTATCATCACAGCCGTCTGG (SEQ ID NO: 40)
ANGPT2	10	AAGTTCAAGTCTCGTGGTCTGA (SEQ ID NO: 41)
ANGPT2	11	ACGAGACTTGAACTTCAGCTCT (SEQ ID NO: 42)
ANGPT2	12	AAGAAGGTGCTAGCTATGGAAG (SEQ ID NO: 43)
ANGPT2	13	GATGATGTGCTTGTCTTCCATA (SEQ ID NO: 44)

【Table 4】

Gene	No.	Target sequence
EPAS1	1	AACACCTCCGTCTCCTTGCTCC (SEQ ID NO: 45)
EPAS1	2	GAAGCTGACCAGCAGATGGACA (SEQ ID NO: 46)
EPAS1	3	GCAATGAAACCCTCCAAGGCTT (SEQ ID NO: 47)
EPAS1	4	AAAACATCAGCAAGTTCATGGG (SEQ ID NO: 48)
EPAS1	5	GCAAGTTCATGGGACTTACACA (SEQ ID NO: 49)
EPAS1	6	GGTCGCAGGGATGAGTGAAGTC (SEQ ID NO: 50)
EPAS1	7	GCGGGACTTCTTCATGAGGATG (SEQ ID NO: 51)
EPAS1	8	GAAGTGCACGGTCACCAACAGA (SEQ ID NO: 52)
EPAS1	9	ACAGTACGGCCTCTGTTGGTGA (SEQ ID NO: 53)
EPAS1	10	TCCAGGTGGCTGACTTGAGGTT (SEQ ID NO: 54)
EPAS1	11	CAGGACAGCAGGGGCTCCTTGT (SEQ ID NO: 55)
EPAS1	12	TAGCCCCCATGCTTTGCGAGCA (SEQ ID NO: 56)

【Table 5】

Gene	No.	Target sequence
ANGPTL4	1	GCATCAGGGCTGCCCCGGCCGT (SEQ ID NO: 57)
ANGPTL4	2	CACGGGTCCGCCCTGAGCGCTC (SEQ ID NO: 58)
ANGPTL4	3	GGACGCAAAGCGCGGCGACTTG (SEQ ID NO: 59)
ANGPTL4	4	TCCTGGGACGAGATGAATGTCC (SEQ ID NO: 60)
ANGPTL4	5	CTGCAGCTCGGCCAGGGGCTGC (SEQ ID NO: 61)
ANGPTL4	6	CCAGGGGCTGCGCGAACACGCG (SEQ ID NO: 62)
ANGPTL4	7	CCCTCGGTTCCCTGACAGGCGG (SEQ ID NO: 63)
ANGPTL4	8	ACCCTGAGGTCCTTCACAGCCT (SEQ ID NO: 64)
ANGPTL4	9	TTCCACAAGGTGGCCCAGCAGC (SEQ ID NO: 65)
ANGPTL4	10	CAGCAGCAGCGGCACCTGGAGA (SEQ ID NO: 66)
ANGPTL4	11	TCCTAGTTTGGCCTCCTGGACC (SEQ ID NO: 67)
ANGPTL4	12	GACCCGGCTCACAATGTCAGCC (SEQ ID NO: 68)
ANGPTL4	13	GCTGTTGCGGTCCCCCGTGATG (SEQ ID NO: 69)
ANGPTL4	14	GGCGTTGCCATCCCAGTCCCGC (SEQ ID NO: 70)
ANGPTL4	15	AACGCCGAGTTGCTGCAGTTCT (SEQ ID NO: 71)
ANGPTL4	16	ATAGGCCGTGTCCTCGCCACCC (SEQ ID NO: 72)
ANGPTL4	17	GTTCTCCGTGCACCTGGGTGGC (SEQ ID NO: 73)
ANGPTL4	18	ACACGGCCTATAGCCTGCAGCT (SEQ ID NO: 74)
ANGPTL4	19	CCACCGTCCCACCCAGCGGCCT (SEQ ID NO: 75)
ANGPTL4	20	GTGATCCTGGTCCCAAGTGGAG (SEQ ID NO: 76)
ANGPTL4	21	GACCCCGGCAGGAGGCTGGTGG (SEQ ID NO: 77)
ANGPTL4	22	TGCAGCCATTCCAACCTCAACG (SEQ ID NO: 78)
ANGPTL4	23	TGCCGCTGCTGTGGGATGGAGC (SEQ ID NO: 79)

Composition for manipulation-gene scissors system

The neovascularization-regulating system of the present invention may include a guide nucleic acid-editor protein complex as a composition for manipulating a neovascularization-associated factor.

Guide nucleic acid-editor protein complex

The term "guide nucleic acid-editor protein complex" refers to a complex formed through the interaction between a guide nucleic acid and an editor protein, and the nucleic acid-protein complex includes a guide nucleic acid and an editor protein.

The term "guide nucleic acid" refers to a nucleic acid capable of recognizing a target nucleic acid, gene, chromosome or protein.

The guide nucleic acid may be present in the form of DNA, RNA or a DNA/RNA hybrid, and may have a nucleic acid sequence of 5 to 150 bases.

The guide nucleic acid may include one or more domains.

The domains may be, but are not limited to, a guide domain, a first complementary domain, a linker domain, a second complementary domain, a proximal domain, or a tail domain.

The guide nucleic acid may include two or more domains, which may be the same domain repeats, or different domains.

The guide nucleic acid may have one continuous nucleic acid sequence.

For example, the one continuous nucleic acid sequence may be $(N)_m$, where N represents A, T, C or G, or A, U, C or G, and m is an integer of 1 to 150.

The guide nucleic acid may have two or more continuous nucleic acid sequences.

For example, the two or more continuous nucleic acid sequences may be $(N)_m$ and $(N)_o$, where N represents A, T, C or G, or A, U, C or G, m and o are an integer of 1 to 150, and m and o may be the same as or different from each other.

The term "editor protein" refers to a peptide, polypeptide or protein which is able to directly bind to or interact with, without direct binding to, a nucleic acid.

The editor protein may be an enzyme.

The editor protein may be a fusion protein.

Here, the "fusion protein" refers to a protein that is produced by fusing an enzyme with an additional domain, peptide, polypeptide or protein.

The term "enzyme" refers to a protein that contains a domain capable of cleaving a nucleic acid, gene, chromosome or protein.

The additional domain, peptide, polypeptide or protein may be a functional domain, peptide, polypeptide or protein, which has a function the same as or different from the enzyme.

The fusion protein may include an additional domain, peptide, polypeptide or protein at one or more regions of the amino terminus (N-terminus) of the enzyme or the vicinity thereof; the carboxyl terminus (C-terminus) or the vicinity thereof; the middle part of the enzyme; and a combination thereof.

The fusion protein may include a functional domain, peptide, polypeptide or protein at one or more regions of the N-terminus of the enzyme or the vicinity thereof; the C-terminus or the vicinity thereof; the middle part of the enzyme; and a combination thereof.

The guide nucleic acid-editor protein complex may serve to modify a subject.

The subject may be a target nucleic acid, gene, chromosome or protein.

For example, the guide nucleic acid-editor protein complex may result in final regulation (e.g., inhibition, suppression, reduction, increase or promotion) of the expression of a protein of interest, removal of the protein, or expression of a new protein.

Here, the guide nucleic acid-editor protein complex may act at a DNA, RNA, gene or chromosome level.

The guide nucleic acid-editor protein complex may act in gene transcription and translation stages.

The guide nucleic acid-editor protein complex may act at a protein level.

1. Guide nucleic acids

The guide nucleic acid is a nucleic acid that is capable of recognizing a target nucleic acid, gene, chromosome or protein, and forms a guide nucleic acid-protein complex.

Here, the guide nucleic acid is configured to recognize or target a nucleic acid, gene, chromosome or protein targeted by the guide nucleic acid-protein complex.

The guide nucleic acid may be present in the form of DNA, RNA or a DNA/RNA mixture, and have a 5 to 150-nucleic acid sequence.

The guide nucleic acid may be present in a linear or circular shape.

The guide nucleic acid may be one continuous nucleic acid sequence.

For example, the one continuous nucleic acid sequence may be $(N)_m$, where N is A, T, C or G, or A, U, C or G, and m is an integer of 1 to 150.

The guide nucleic acid may be two or more continuous nucleic acid sequences.

For example, the two or more continuous nucleic acid sequences may be $(N)_m$ and $(N)_o$, where N represents A, T, C or G, or A, U, C or G, m and o are an integer of 1 to 150, and may be the same as or different from each other.

The guide nucleic acid may include one or more domains.

Here, the domains may be, but are not limited to, a guide domain, a first complementary domain, a linker domain, a second complementary domain, a proximal domain, or a tail domain.

The guide nucleic acid may include two or more domains, which may be the same domain repeats, or different domains.

The domains will be described below.

i) Guide domain

The term "guide domain" is a domain having a complementary guide sequence which is able to form a complementary bond with a target sequence on a target gene or nucleic acid, and serves to specifically interact with the target gene or nucleic acid.

The guide sequence is a nucleic acid sequence complementary to the target sequence on a target gene or nucleic acid, which has, for example, at least 50% or more, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95% complementarity or complete complementarity.

The guide domain may be a sequence of 5 to 50 bases.

In an example, the guide domain may be a sequence of 5 to 50, 10 to 50, 15 to 50, 20 to 50, 25 to 50, 30 to 50, 35 to 50, 40 to 50 or 45 to 50 bases.

In another example, the guide domain may be a sequence of 1 to 5, 5 to 10, 10 to 15, 15 to 20, 20 to 25, 25 to 30, 30 to 35, 35 to 40, 40 to 45, or 45 to 50 bases.

The guide domain may have a guide sequence.

The guide sequence may be a complementary base sequence which is able to form a complementary bond with the target sequence on the target gene or nucleic acid.

The guide sequence may be a nucleic acid sequence complementary to the target sequence on the target gene or nucleic acid, which has, for example, at least 70%, 75%, 80%, 85%, 90% or 95% or more complementarity or complete complementarity.

The guide sequence may be a 5 to 50-base sequence.

In an example, the guide domain may be a 5 to 50, 10 to 50, 15 to 50, 20 to 50, 25 to 50, 30 to 50, 35 to 50, 40 to 50, or 45 to 50-base sequence.

In another example, the guide sequence may be a 1 to 5, 5 to 10, 10 to 15, 15 to 20, 20 to 25, 25 to 30, 30 to 35, 35 to 40, 40 to 45, or 45 to 50-base sequence.

In addition, the guide domain may include a guide sequence and an additional base sequence.

The additional base sequence may be utilized to improve or degrade the function of the guide domain.

The additional base sequence may be utilized to improve or degrade the function of the guide sequence.

The additional base sequence may be a 1 to 35-base sequence.

In one example, the additional base sequence may be a 5 to 35, 10 to 35, 15 to 35, 20 to 35, 25 to 35 or 30 to 35-base sequence.

In another example, the additional base sequence may be a 1 to 5, 5 to 10, 10 to 15, 15 to 20, 20 to 25, 25 to 30 or 30 to 35-base sequence.

The additional base sequence may be located at the 5’end of the guide sequence.

The additional base sequence may be located at the 3’end of the guide sequence.

ii) First complementary domain

The term “first complementary domain” is a nucleic acid sequence including a nucleic acid sequence complementary to a second complementary domain, and has enough complementarity so as to form a double strand with the second complementary domain.

The first complementary domain may be a 5 to 35-base sequence.

In an example, the first complementary domain may be a 5 to 35, 10 to 35, 15 to 35, 20 to 35, 25 to 35, or 30 to 35-base sequence.

In another example, the first complementary domain may be a 1 to 5, 5 to 10, 10 to 15, 15 to 20, 20 to 25, 25 to 30 or 30 to 35-base sequence.

iii) Linker domain

The term "linker domain" is a nucleic acid sequence connecting two or more domains, which are two or more identical or different domains. The linker domain may be connected with two or more domains by covalent bonding or non-covalent bonding, or may connect two or more domains by covalent bonding or non-covalent bonding.

The linker domain may be a 1 to 30-base sequence.

In one example, the linker domain may be a 1 to 5, 5 to 10, 10 to 15, 15 to 20, 20 to 25, or 25 to 30-base sequence.

In another example, the linker domain may be a 1 to 30, 5 to 30, 10 to 30, 15 to 30, 20 to 30, or 25 to 30-base sequence.

iv) Second complementary domain

The term "second complementary domain" is a nucleic acid sequence including a nucleic acid sequence complementary to the first complementary domain, and has enough complementarity so as to form a double strand with the first complementary domain.

The second complementary domain may have a base sequence complementary to the first complementary domain, and a base sequence having no complementarity to the first complementary domain, for example, a base sequence not forming a double strand with the first complementary domain, and may have a longer base sequence than the first complementary domain.

The second complementary domain may have a 5 to 35-base sequence.

In an example, the second complementary domain may be a 1 to 35, 5 to 35, 10 to 35, 15 to 35, 20 to 35, 25 to 35, or 30 to 35-base sequence.

In another example, the second complementary domain may be a 1 to 5, 5 to 10, 10 to 15, 15 to 20, 20 to 25, 25 to 30, or 30 to 35-base sequence.

v) Proximal domain

The term “proximal domain” is a nucleic acid sequence located adjacent to the second complementary domain.

The proximal domain may have a complementary base sequence therein, and may be formed in a double strand due to a complementary base sequence.

The proximal domain may be a 1 to 20-base sequence.

In one example, the proximal domain may be a 1 to 20, 5 to 20, 10 to 20 or 15 to 20-base sequence.

In another example, the proximal domain may be a 1 to 5, 5 to 10, 10 to 15 or 15 to 20-base sequence.

vi) Tail domain

The term “tail domain” is a nucleic acid sequence located at one or more ends of the both ends of the guide nucleic acid.

The tail domain may have a complementary base sequence therein, and may be formed in a double strand due to a complementary base sequence.

The tail domain may be a 1 to 50-base sequence.

In an example, the tail domain may be a 5 to 50, 10 to 50, 15 to 50, 20 to 50, 25 to 50, 30 to 50, 35 to 50, 40 to 50, or 45 to 50-base sequence.

In another example, the tail domain may be a 1 to 5, 5 to 10, 10 to 15, 15 to 20, 20 to 25, 25 to 30, 30 to 35, 35 to 40, 40 to 45, or 45 to 50-base sequence.

Meanwhile, a part or all of the nucleic acid sequences included in the domains, that is, the guide domain, the first complementary domain, the linker domain, the second complementary domain, the proximal domain and the tail domain may selectively or additionally include a chemical modification.

The chemical modification may be, but is not limited to, methylation, acetylation, phosphorylation, phosphorothioate linkage, a locked nucleic acid (LNA), 2'-O-methyl 3'phosphorothioate (MS) or 2'-O-methyl 3'thioPACE (MSP).

The guide nucleic acid includes one or more domains.

The guide nucleic acid may include a guide domain.

The guide nucleic acid may include a first complementary domain.

The guide nucleic acid may include a linker domain.

The guide nucleic acid may include a second complementary domain.

The guide nucleic acid may include a proximal domain.

The guide nucleic acid may include a tail domain.

Here, there may be 1, 2, 3, 4, 5, 6 or more domains.

The guide nucleic acid may include 1, 2, 3, 4, 5, 6 or more guide domains.

The guide nucleic acid may include 1, 2, 3, 4, 5, 6 or more first complementary domains.

The guide nucleic acid may include 1, 2, 3, 4, 5, 6 or more linker domains.

The guide nucleic acid may include 1, 2, 3, 4, 5, 6 or more second complementary domains.

The guide nucleic acid may include 1, 2, 3, 4, 5, 6 or more proximal domains.

The guide nucleic acid may include 1, 2, 3, 4, 5, 6 or more tail domains.

Here, in the guide nucleic acid, one type of domain may be duplicated.

The guide nucleic acid may include several domains with or without duplication.

The guide nucleic acid may include the same type of domain. Here, the same type of domain may have the same nucleic acid sequence or different nucleic acid sequences.

The guide nucleic acid may include two types of domains. Here, the two different types of domains may have different nucleic acid sequences or the same nucleic acid sequence.

The guide nucleic acid may include three types of domains. Here, the three different types of domains may have different nucleic acid sequences or the same nucleic acid sequence.

The guide nucleic acid may include four types of domains. Here, the four different types of domains may have different nucleic acid sequences, or the same nucleic acid sequence.

The guide nucleic acid may include five types of domains. Here, the five different types of domains may have different nucleic acid sequences, or the same nucleic acid sequence.

The guide nucleic acid may include six types of domains. Here, the six different types of domains may have different nucleic acid sequences, or the same nucleic acid sequence.

For example, the guide nucleic acid may consist of [guide domain]-[first complementary domain]-[linker domain]-[second complementary domain]-[linker domain]-[guide domain]-[first complementary domain]-[linker domain]-[second complementary domain]. Here, the two guide domains may include guide sequences for different or the same

targets, the two first complementary domains and the two second complementary domains may have the same or different nucleic acid sequences. When the guide domains include guide sequences for different targets, the guide nucleic acids may specifically bind to two different targets, and here, the specific bindings may be performed simultaneously or sequentially. In addition, the linker domains may be cleaved by specific enzymes, and the guide nucleic acids may be divided into two or three parts in the presence of specific enzymes.

As a specific example of the guide nucleic acid of the present invention, gRNA will be described below.

gRNA

The term "gRNA" refers to a nucleic acid capable of specifically targeting a gRNA-CRISPR enzyme complex, that is, a CRISPR complex, with respect to a target gene or nucleic acid. In addition, the gRNA is a nucleic acid-specific RNA which may bind to a CRISPR enzyme and guide the CRISPR enzyme to the target gene or nucleic acid.

The gRNA may include multiple domains. Due to each domain, interactions may occur in a three-dimensional structure or active form of a gRNA strand, or between these strands.

The gRNA may be called single-stranded gRNA (single RNA molecule); or double-stranded gRNA (including more than one, generally, two discrete RNA molecules).

In one exemplary embodiment, the single-stranded gRNA may include a guide domain, that is, a domain including a guide sequence capable of forming a complementary bond with a target gene or nucleic acid; a first complementary domain; a linker domain; a second complementary domain, a domain having a sequence complementary to the first complementary domain sequence, thereby forming a double-stranded nucleic acid with the

first complementary domain; a proximal domain; and optionally a tail domain in the 5’ to 3’ direction.

In another embodiment, the double-stranded gRNA may include a first strand which includes a guide domain, that is, a domain including a guide sequence capable of forming a complementary bond with a target gene or nucleic acid and a first complementary domain; and a second strand which includes a second complementary domain, a domain having a sequence complementary to the first complementary domain sequence, thereby forming a double-stranded nucleic acid with the first complementary domain, a proximal domain; and optionally a tail domain in the 5’ to 3’ direction.

Here, the first strand may be referred to as crRNA, and the second strand may be referred to as tracrRNA. The crRNA may include a guide domain and a first complementary domain, and the tracrRNA may include a second complementary domain, a proximal domain and optionally a tail domain.

In still another embodiment, the single-stranded gRNA may include a guide domain, that is, a domain including a guide sequence capable of forming a complementary bond with a target gene or nucleic acid; a first complementary domain; a second complementary domain, and a domain having a sequence complementary to the first complementary domain sequence, thereby forming a double-stranded nucleic acid with the first complementary domain in the 5’ to 3’ direction.

i) Guide domain

The guide domain includes a complementary guide sequence capable of forming a complementary bond with a target sequence on a target gene or nucleic acid. The guide sequence may be a nucleic acid sequence having complementarity to the target sequence on the target gene or nucleic acid, for example, at least 70%, 75%, 80%, 85%, 90% or 95% or

more complementarity or complete complementarity. The guide domain is considered to allow a gRNA-Cas complex, that is, a CRISPR complex to specifically interact with the target gene or nucleic acid.

The guide domain may be a 5 to 50-base sequence.

As an exemplary embodiment, the guide domain may be a 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25-base sequence.

As an exemplary embodiment, the guide domain may include a 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25-base sequence.

Here, the guide domain may include a guide sequence.

The guide sequence may be a complementary base sequence capable of forming a complementary bond with a target sequence on a target gene or nucleic acid.

The guide sequence may be a nucleic acid sequence complementary to the target sequence on the target gene or nucleic acid, which has, for example, at least 70%, 75%, 80%, 85%, 90% or 95% or more complementarity or complete complementarity.

In one exemplary embodiment, the guide sequence may be a nucleic acid sequence complementary to a target gene, that is, a target sequence of a neovascularization-associated factor such as a VEGFA gene, an HIF1A gene, an ANGPT2 gene, an EPAS1 gene or an ANGPTL4 gene, which has, for example, at least 70%, 75%, 80%, 85%, 90% or 95% or more complementarity or complete complementarity.

The guide sequence may be a 5 to 50-base sequence.

In an exemplary embodiment, the guide sequence may be a 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25-base sequence.

In one exemplary embodiment, the guide sequence may include a 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25-base sequence.

In one exemplary embodiment, the guide sequence may be a nucleic acid sequence complementary to a target sequence of the VEGFA gene, which is a 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25-base sequence.

In one exemplary embodiment, the guide sequence may be a nucleic acid sequence complementary to a target sequence of the HIF1A gene, which is a 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25-base sequence.

In one exemplary embodiment, the guide sequence may be a nucleic acid sequence complementary to a target sequence of the ANGPT2 gene, which is a 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25-base sequence.

In one exemplary embodiment, the guide sequence may be a nucleic acid sequence complementary to a target sequence of the EPAS1 gene, which is a 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25-base sequence.

In one exemplary embodiment, the guide sequence may be a nucleic acid sequence complementary to a target sequence of the ANGPTL4 gene, which is a 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25-base sequence.

Here, target sequences of the target genes, that is, the neovascularization-associated factors such as the VEGFA gene, the HIF1A gene, the ANGPT2 gene, the EPAS1 gene, and the ANGPTL4 gene for the guide sequence are listed above in Table 1, Table 2, Table 3, Table 4 and Table 5, respectively, but the present invention is not limited thereto.

Here, the guide domain may include a guide sequence and an additional base sequence.

The additional base sequence may be a 1 to 35-base sequence.

In one exemplary embodiment, the additional base sequence may be a 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10-base sequence.

For example, the additional base sequence may be a single base sequence, guanine (G), or a sequence of two bases, GG.

The additional base sequence may be located at the 5’ end of the guide sequence.

The additional base sequence may be located at the 3’ end of the guide sequence.

Selectively, a part or all of the base sequence of the guide domain may include a chemical modification. The chemical modification may be methylation, acetylation, phosphorylation, phosphorothioate linkage, a locked nucleic acid (LNA), 2’-O-methyl 3’ phosphorothioate (MS) or 2’-O-methyl 3’ thioPACE (MSP), but the present invention is not limited thereto.

ii) First complementary domain

The first complementary domain includes a nucleic acid sequence complementary to a second complementary domain, and has enough complementarity such that it is able to form a double strand with the second complementary domain.

Here, the first complementary domain may be a 5 to 35-base sequence. The first complementary domain may include a 5 to 35-base sequence.

In one exemplary embodiment, the first complementary domain may be a 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25-base sequence.

In another embodiment, the first complementary domain may include a 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25-base sequence.

The first complementary domain may have homology with a natural first complementary domain, or may be derived from a natural first complementary domain. In addition, the first complementary domain may have a difference in the base sequence of a first complementary domain depending on the species existing in nature, may be derived from a first complementary domain contained in the species existing in nature, or may have

partial or complete homology with the first complementary domain contained in the species existing in nature.

In one exemplary embodiment, the first complementary domain may have partial, that is, at least 50% or more, or complete homology with a first complementary domain of *Streptococcus pyogenes*, *Campylobacter jejuni*, *Streptococcus thermophilus*, *Streptocuccus aureus* or *Neisseria meningitides*, or a first complementary domain derived therefrom.

For example, when the first complementary domain is the first complementary domain of *Streptococcus pyogenes* or a first complementary domain derived therefrom, the first complementary domain may be 5'-GUUUUAGAGCUA-3' or a base sequence having partial, that is, at least 50% or more, or complete homology with 5'-GUUUUAGAGCUA-3'. Here, the first complementary domain may further include $(X)_n$, resulting in 5'-GUUUUAGAGCUA$(X)_n$-3'. The X may be selected from the group consisting of bases A, T, U and G, and the n may represent the number of bases, which is an integer of 5 to 15. Here, the $(X)_n$ may be n repeats of the same base, or a mixture of n bases of A, T, U and G.

In another embodiment, when the first complementary domain is the first complementary domain of *Campylobacter jejuni* or a first complementary domain derived therefrom, the first complementary domain may be 5'-GUUUUAGUCCCUUUUUAAAUUUCUU-3', or a base sequence having partial, that is, at least 50% or more, or complete homology with 5'-GUUUUAGUCCCUUUUUAAAUUUCUU-3'. Here, the first complementary domain may further include $(X)_n$, resulting in 5'-GUUUUAGUCCCUUUUUAAAUUUCUU$(X)_n$-3'. The X may be selected from the group consisting of bases A, T, U and G, and the n may represent the number of bases, which is an integer of 5 to 15. Here, the $(X)_n$ may represent n repeats of the same base, or a mixture of n bases of A, T, U and G.

In another embodiment, the first complementary domain may have partial, that is, at least 50% or more, or complete homology with a first complementary domain of *Parcubacteria bacterium* (GWC2011_GWC2_44_17), *Lachnospiraceae bacterium* (MC2017), *Butyrivibrio proteoclasiicus*, *Peregrinibacteria bacterium* (GW2011_GWA_33_10), *Acidaminococcus sp.* (BV3L6), *Porphyromonas macacae*, *Lachnospiraceae bacterium* (ND2006), *Porphyromonas crevioricanis*, *Prevotella disiens*, *Moraxella bovoculi* (237), *Smiihella sp.* (SC_KO8D17), *Leptospira inadai*, *Lachnospiraceae bacterium* (MA2020), *Francisella novicida* (U112), *Candidatus Methanoplasma termitum* or *Eubacterium eligens*, or a first complementary domain derived therefrom.

For example, when the first complementary domain is the first complementary domain of *Parcubacteria bacterium* or a first complementary domain derived therefrom, the first complementary domain may be 5'-UUUGUAGAU-3', or a base sequence having partial, that is, at least 50% or more homology with 5'-UUUGUAGAU-3'. Here, the first complementary domain may further include $(X)_n$, resulting in 5'-$(X)_n$UUUGUAGAU-3'. The X may be selected from the group consisting of bases A, T, U and G, and the n may represent the number of bases, which is an integer of 1 to 5. Here, the $(X)_n$ may represent n repeats of the same base, or a mixture of n bases of A, T, U and G.

Selectively, a part or all of the base sequence of the first complementary domain may have a chemical modification. The chemical modification may be methylation, acetylation, phosphorylation, phosphorothioate linkage, a locked nucleic acid (LNA), 2'-O-methyl 3' phosphorothioate (MS) or 2'-O-methyl 3' thioPACE (MSP), but the present invention is not limited thereto.

iii) Linker domain

The linker domain is a nucleic acid sequence connecting two or more domains, and connects two or more identical or different domains. The linker domain may be connected with two or more domains, or may connect two or more domains by covalent or non-covalent bonding.

The linker domain may be a nucleic acid sequence connecting a first complementary domain with a second complementary domain to produce single-stranded gRNA.

The linker domain may be connected with the first complementary domain and the second complementary domain by covalent or non-covalent bonding.

The linker domain may connect the first complementary domain with the second complementary domain by covalent or non-covalent bonding

The linker domain may be a 1 to 30-base sequence. The linker domain may include a 1 to 30-base sequence.

In an exemplary embodiment, the linker domain may be a 1 to 5, 5 to 10, 10 to 15, 15 to 20, 20 to 25 or 25 to 30-base sequence.

In an exemplary embodiment, the linker domain may include a 1 to 5, 5 to 10, 10 to 15, 15 to 20, 20 to 25, or 25 to 30-base sequence.

The linker domain is suitable to be used in a single-stranded gRNA molecule, and may be used to produce single-stranded gRNA by being connected with a first strand and a second strand of double-stranded gRNA or connecting the first strand with the second strand by covalent or non-covalent bonding. The linker domain may be used to produce single-stranded gRNA by being connected with crRNA and tracrRNA of double-stranded gRNA or connecting the crRNA with the tracrRNA by covalent or non-covalent bonding.

The linker domain may have homology with a natural sequence, for example, a partial sequence of tracrRNA, or may be derived therefrom.

Selectively, a part or all of the base sequence of the linker domain may have a chemical modification. The chemical modification may be methylation, acetylation, phosphorylation, phosphorothioate linkage, a locked nucleic acid (LNA), 2'-O-methyl 3' phosphorothioate (MS) or 2'-O-methyl 3' thioPACE (MSP), but the present invention is not limited thereto.

iv) Second complementary domain

The second complementary domain includes a nucleic acid sequence complementary to the first complementary domain, and has enough complementarity so as to form a double strand with the first complementary domain. The second complementary domain may include a base sequence complementary to the first complementary domain, and a base sequence having no complementarity with the first complementary domain, for example, a base sequence not forming a double strand with the first complementary domain, and may have a longer base sequence than the first complementary domain.

Here, the second complementary domain may be a 5 to 35-base sequence. The first complementary domain may include a 5 to 35-base sequence.

In an exemplary embodiment, the second complementary domain may be a 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25-base sequence.

In an exemplary embodiment, the second complementary domain may include a 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25-base sequence.

In addition, the second complementary domain may have homology with a natural second complementary domain, or may be derived from the natural second complementary domain. In addition, the second complementary domain may have a difference in base sequence of a second complementary domain according to a species existing in nature, and may be derived from a second complementary domain contained in the species existing in

nature, or may have partial or complete homology with the second complementary domain contained in the species existing in nature.

In an exemplary embodiment, the second complementary domain may have partial, that is, at least 50% or more, or complete homology with a second complementary domain of *Streptococcus pyogenes*, *Campylobacter jejuni*, *Streptococcus thermophilus*, *Streptocuccus aureus* or *Neisseria meningitides*, or a second complementary domain derived therefrom.

For example, when the second complementary domain is a second complementary domain of *Streptococcus pyogenes* or a second complementary domain derived therefrom, the second complementary domain may be 5'-UAGCAAGUUAAAAU-3', or a base sequence having partial, that is, at least 50% or more homology with 5'-UAGCAAGUUAAAAU-3' (a base sequence forming a double strand with the first complementary domain is underlined). Here, the second complementary domain may further include $(X)_n$ and/or $(X)_m$, resulting in 5'-$(X)_n$ UAGCAAGUUAAAAU$(X)_m$-3'. The X may be selected from the group consisting of bases A, T, U and G, and each of the n and m may represent the number of bases, in which the n may be an integer of 1 to 15, and the m may be an integer of 1 to 6. Here, the $(X)_n$ may represent n repeats of the same base, or a mixture of n bases of A, T, U and G. In addition, $(X)_m$ may represent m repeats of the same base, or a mixture of m bases of A, T, U and G.

In another example, when the second complementary domain is the second complementary domain of *Campylobacter jejuni* or a second complementary domain derived therefrom, the second complementary domain may be 5'-AAGAAAUUUAAAAAGGGACUAAAAU-3', or a base sequence having partial, that is, at least 50% or more homology with 5'-AAGAAAUUUAAAAAGGGACUAAAAU -3' (a base sequence forming a double strand with the first complementary domain is underlined). Here, the second complementary domain may further include $(X)_n$ and/or $(X)_m$, resulting in 5'-$(X)_n$AAGAAAUUUAAAAAGGGACUAAAAU$(X)_m$-3'. The X may be selected from the

group consisting of bases A, T, U and G, and each of the n and m may represent the number of bases, in which the n may be an integer of 1 to 15, and the m may be an integer of 1 to 6. Here, $(X)_n$ may represent n repeats of the same base, or a mixture of n bases of A, T, U and G. In addition, $(X)_m$ may represent m repeats of the same base, or a mixture of m bases of A, T, U and G.

In another embodiment, the first complementary domain may have partial, that is, at least 50% or more, or complete homology with a first complementary domain of *Parcubacteria bacterium* (GWC2011_GWC2_44_17), *Lachnospiraceae bacterium* (MC2017), *Butyrivibrio proteoclasiicus*, *Peregrinibacteria bacterium* (GW2011_GWA_33_10), *Acidaminococcus sp.* (BV3L6), *Porphyromonas macacae*, *Lachnospiraceae bacterium* (ND2006), *Porphyromonas crevioricanis*, *Prevotella disiens*, *Moraxella bovoculi* (237), *Smiihella sp.* (SC_KO8D17), *Leptospira inadai*, *Lachnospiraceae bacterium* (MA2020), *Francisella novicida* (U112), *Candidatus Methanoplasma termitum* or *Eubacterium eligens*, or a first complementary domain derived therefrom.

For example, when the second complementary domain is a second complementary domain of *Parcubacteria bacterium* or a second complementary domain derived therefrom, the second complementary domain may be 5'-AAAUUUCUACU-3', or a base sequence having partial, that is, at least 50% or more homology with 5'-AAAUUUCUACU-3' (a base sequence forming a double strand with the first complementary domain is underlined). Here, the second complementary domain may further include $(X)_n$ and/or $(X)_m$, resulting in 5'-$(X)_n$AAAUUUCUACU$(X)_m$-3'. The X may be selected from the group consisting of bases A, T, U and G, and each of the n and m may represent the number of bases, in which the n may be an integer of 1 to 10, and the m may be an integer of 1 to 6. Here, the $(X)_n$ may represent n repeats of the same base, or a mixture of n bases of A, T, U and G. In addition, the $(X)_m$ may represent m repeats of the same base, or a mixture of m bases of A, T, U and G.

Selectively, a part or all of the base sequence of the second complementary domain may have a chemical modification. The chemical modification may be methylation, acetylation, phosphorylation, phosphorothioate linkage, a locked nucleic acid (LNA), 2'-O-methyl 3'phosphorothioate (MS) or 2'-O-methyl 3'thioPACE (MSP), but the present invention is not limited thereto.

v) Proximal domain

The proximal domain is a sequence of 1 to 20 bases located adjacent to the second complementary domain, and a domain located at the 3'end direction of the second complementary domain. Here, the proximal domain may be used to form a double strand between complementary base sequences therein.

In one exemplary embodiment, the proximal domain may be a 5, 6, 7, 8, 8, 9, 10, 11, 12, 13, 14 or 15-base sequence.

In another embodiment, the proximal domain may include a 5, 6, 7, 8, 8, 9, 10, 11, 12, 13, 14 or 15-base sequence.

In addition, the proximal domain may have homology with a natural proximal domain, or may be derived from the natural proximal domain. In addition, the proximal domain may have a difference in base sequence according to a species existing in nature, may be derived from a proximal domain contained in the species existing in nature, or may have partial or complete homology with the proximal domain contained in the species existing in nature.

In an exemplary embodiment, the proximal domain may have partial, that is, at least 50% or more, or complete homology with a proximal domain of *Streptococcus pyogenes*, *Campylobacter jejuni*, *Streptococcus thermophilus*, *Streptocuccus aureus* or *Neisseria meningitides*, or a proximal domain derived therefrom.

For example, when the proximal domain is a proximal domain of *Streptococcus pyogenes* or a proximal domain derived therefrom, the proximal domain may be 5'-AAGGCUAGUCCG-3', or a base sequence having partial, that is, at least 50% or more homology with 5'-AAGGCUAGUCCG-3'. Here, the proximal domain may further include $(X)_n$, resulting in 5'-AAGGCUAGUCCG$(X)_n$-3'. The X may be selected from the group consisting of bases A, T, U and G, and the n may represent the number of bases, which is an integer of 1 to 15. Here, the $(X)_n$ may represent n repeats of the same base, or a mixture of n bases of A, T, U and G.

In yet another example, when the proximal domain is a proximal domain of *Campylobacter jejuni* or a proximal domain derived therefrom, the proximal domain may be 5'-AAAGAGUUUGC-3', or a base sequence having at least 50% or more homology with 5'-AAAGAGUUUGC-3'. Here, the proximal domain may further include $(X)_n$, resulting in 5'-AAAGAGUUUGC$(X)_n$-3'. The X may be selected from the group consisting of bases A, T, U and G, and the n may represent the number of bases, which is an integer of 1 to 40. Here, the $(X)_n$ may represent n repeats of the same base, or a mixture of n bases of A, T, U and G.

Selectively, a part or all of the base sequence of the proximal domain may have a chemical modification. The chemical modification may be methylation, acetylation, phosphorylation, phosphorothioate linkage, a locked nucleic acid (LNA), 2'-O-methyl 3'phosphorothioate (MS) or 2'-O-methyl 3'thioPACE (MSP), but the present invention is not limited thereto.

vi) Tail domain

The tail domain is a domain which is able to be selectively added to the 3' end of single-stranded gRNA or double-stranded gRNA. The tail domain may be a 1 to 50-base

sequence, or include a 1 to 50-base sequence. Here, the tail domain may be used to form a double strand between complementary base sequences therein.

In an exemplary embodiment, the tail domain may be a 1 to 5, 5 to 10, 10 to 15, 15 to 20, 20 to 25, 25 to 30, 30 to 35, 35 to 40, 40 to 45, or 45 to 50-base sequence.

In an exemplary embodiment, the tail domain may include a 1 to 5, 5 to 10, 10 to 15, 15 to 20, 20 to 25, 25 to 30, 30 to 35, 35 to 40, 40 to 45, or 45 to 50-base sequence.

In addition, the tail domain may have homology with a natural tail domain, or may be derived from the natural tail domain. In addition, the tail domain may have a difference in base sequence according to a species existing in nature, may be derived from a tail domain contained in a species existing in nature, or may have partial or complete homology with a tail domain contained in a species existing in nature.

In one exemplary embodiment, the tail domain may have partial, that is, at least 50% or more, or complete homology with a tail domain of *Streptococcus pyogenes*, *Campylobacter jejuni*, *Streptococcus thermophilus*, *Streptocuccus aureus* or *Neisseria meningitides* or a tail domain derived therefrom.

For example, when the tail domain is a tail domain of *Streptococcus pyogenes* or a tail domain derived therefrom, the tail domain may be 5'-UUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC-3', or a base sequence having partial, that is, at least 50% or more homology with 5'-UUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC-3'. Here, the tail domain may further include $(X)_n$, resulting in 5'-UUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC$(X)_n$-3'. The X may be selected from the group consisting of bases A, T, U and G, and the n may represent the number of bases, which is an integer of 1 to 15. Here, the $(X)_n$ may represent n repeats of the same base, or a mixture of n bases such as A, T, U and G.

In another example, when the tail domain is a tail domain of *Campylobacter jejuni* or a tail domain derived therefrom, the tail domain may be 5'-GGGACUCUGCGGGGUUACAAUCCCCUAAAACCGCUUUU-3', or a base sequence having partial, that is, at least 50% or more homology with 5'-GGGACUCUGCGGGGUUACAAUCCCCUAAAACCGCUUUU-3'. Here, the tail domain may further include $(X)_n$, resulting in 5'-GGGACUCUGCGGGGUUACAAUCCCCUAAAACCGCUUUU$(X)_n$-3'. The X may be selected from the group consisting of bases A, T, U and G, and the n may represent the number of bases, which is an integer of 1 to 15. Here, the $(X)_n$ may represent n repeats of the same base, or a mixture of n bases of A, T, U and G.

In another embodiment, the tail domain may include a 1 to 10-base sequence at the 3' end involved in an *in vitro* or *in vivo* transcription method.

For example, when a T7 promoter is used in *in vitro* transcription of gRNA, the tail domain may be an arbitrary base sequence present at the 3' end of a DNA template. In addition, when a U6 promoter is used in *in vivo* transcription, the tail domain may be UUUUUU, when an H1 promoter is used in transcription, the tail domain may be UUUU, and when a pol-III promoter is used, the tail domain may include several uracil bases or alternative bases.

Selectively, a part or all of the base sequence of the tail domain may have a chemical modification. The chemical modification may be methylation, acetylation, phosphorylation, phosphorothioate linkage, a locked nucleic acid (LNA), 2'-O-methyl 3'phosphorothioate (MS) or 2'-O-methyl 3'thioPACE (MSP), but the present invention is not limited thereto.

The gRNA may include a plurality of domains as described above, and therefore, the length of the nucleic acid sequence may be regulated according to a domain contained in the

gRNA, and interactions may occur in strands in a three-dimensional structure or active form of gRNA or between theses strands due to each domain.

The gRNA may be referred to as single-stranded gRNA (single RNA molecule); or double-stranded gRNA (including more than one, generally two discrete RNA molecules).

Double-stranded gRNA

The double-stranded gRNA consists of a first strand and a second strand.

Here, the first strand may consist of

5'-[guide domain]-[first complementary domain]-3', and

the second strand may consist of

5'-[second complementary domain]-[proximal domain]-3' or

5'-[second complementary domain]-[proximal domain]-[tail domain]-3'.

Here, the first strand may be referred to as crRNA, and the second strand may be referred to as tracrRNA.

First strand

Guide domain

In the first strand, the guide domain includes a complementary guide sequence which is able to form a complementary bond with a target sequence on a target gene or nucleic acid. The guide sequence is a nucleic acid sequence complementary to the target sequence on the target gene or nucleic acid, which has, for example, at least 70%, 75%, 80%, 85%, 90% or 95% or more complementarity or complete complementarity. The guide domain is considered to allow a gRNA-Cas complex, that is, a CRISPR complex to specifically interact with the target gene or nucleic acid.

Here, the guide domain may be a 5 to 50-base sequence, or includes a 5 to 50-base sequence. For example, the guide domain may be or include a 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25-base sequence.

In addition, the guide domain may include a guide sequence.

Here, the guide sequence may be a complementary base sequence which is able to form a complementary bond with a target sequence on a target gene or nucleic acid, which has, for example, at least 70%, 75%, 80%, 85%, 90% or 95% or more complementarity or complete complementarity.

In an exemplary embodiment, the guide sequence may be a nucleic acid sequence complementary to a target gene, that is, a target sequence of a neovascularization-associated factor such as a VEGFA gene, an HIF1A gene, an ANGPT2 gene, an EPAS1 gene, or an ANGPTL4 gene, which has, for example, at least 70%, 75%, 80%, 85%, 90% or 95% or more complementarity or complete complementarity.

Here, the guide sequence may be a 5 to 50-base sequence or include a 5 to 50-base sequence. For example, the guide sequence may be or include a 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25-base sequence.

In one exemplary embodiment, the guide sequence is a nucleic acid sequence complementary to a target sequence of the VEGFA gene. The guide sequence may be or include a 5 to 50-base sequence. For example, the guide sequence may be or include a 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25-base sequence.

In one exemplary embodiment, the guide sequence may be a nucleic acid sequence complementary to a target sequence of the HIF1A gene. The guide sequence may be or include a 5 to 50-base sequence. For example, the guide sequence may be or include a 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25-base sequence.

In one exemplary embodiment, the guide sequence may be a nucleic acid sequence complementary to a target sequence of the ANGPT2 gene. The guide sequence may be or include a 5 to 50-base sequence. For example, the guide sequence may be or include a 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25-base sequence.

In one exemplary embodiment, the guide sequence is a nucleic acid sequence complementary to a target sequence of the EPAS1 gene. The guide sequence may be or include a 5 to 50-base sequence. For example, the guide sequence may be or include a 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25-base sequence.

In one exemplary embodiment, the guide sequence is a nucleic acid sequence complementary to a target sequence of the ANGPTL4 gene. The guide sequence may be or include a 5 to 50-base sequence. For example, the guide sequence may be or include a 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25-base sequence.

Here, for the guide sequence, target genes, that is, target sequences of neovascularization-associated factors such as a VEGFA gene, an HIF1A gene, an ANGPT2 gene, an EPAS1 gene, and an ANGPTL4 gene are listed above in Table 1, Table 2, Table 3, Table 4 and Table 5, but the present invention is not limited thereto.

Selectively, the guide domain may include a guide sequence and an additional base sequence.

Here, the additional base sequence may be a 1 to 35-base sequence. For example, the additional base sequence may be a 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10-base sequence.

In one exemplary embodiment, the additional base sequence may include one base, guanine (G), or two bases, GG.

Here, the additional base sequence may be located at the 5’ end of the guide domain, or at the 5’ end of the guide sequence.

The additional base sequence may be located at the 3’ end of the guide domain, or at the 3’ end of the guide sequence.

First complementary domain

The first complementary domain includes a nucleic acid sequence complementary to a second complementary domain of the second strand, and is a domain having enough complementarity so as to form a double strand with the second complementary domain.

Here, the first complementary domain may be or include a 5 to 35-base sequence. For example, the first complementary domain may be or include a 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25-base sequence.

The first complementary domain may have homology with a natural first complementary domain, or may be derived from a natural first complementary domain. In addition, the first complementary domain may have a difference in base sequence according to a species existing in nature, may be derived from the first complementary domain contained in the species existing in nature, or may have partial or complete homology with the first complementary domain contained in the species existing in nature.

In one exemplary embodiment, the first complementary domain may have partial, that is, at least 50% or more, or complete homology with a first complementary domain of *Streptococcus pyogenes*, *Campylobacter jejuni*, *Streptococcus thermophilus*, *Streptocuccus aureus* or *Neisseria meningitides*, or a first complementary domain derived therefrom.

Selectively, the first complementary domain may include an additional base sequence which does not undergo complementary bonding with the second complementary domain of the second strand.

Here, the additional base sequence may be a sequence of 1 to 15 bases. For example, the additional base sequence may be a sequence of 1 to 5, 5 to 10, or 10 to 15 bases.

Selectively, a part or all of the base sequence of the guide domain and/or first complementary domain may have a chemical modification. The chemical modification may be methylation, acetylation, phosphorylation, phosphorothioate linkage, a locked nucleic acid (LNA), 2'-O-methyl 3' phosphorothioate (MS) or 2'-O-methyl 3' thioPACE (MSP), but the present invention is not limited thereto.

Therefore, the first strand may consist of 5'-[guide domain]-[first complementary domain]-3' as described above.

In addition, the first strand may optionally include an additional base sequence.

In one example, the first strand may be

5'-(N_{target})-$(Q)_m$-3'; or

5'-$(X)_a$-(N_{target})-$(X)_b$-$(Q)_m$-$(X)_c$-3'.

Here, the N_{target} is a base sequence capable of forming a complementary bond with a target sequence on a target gene or nucleic acid, and a base sequence region which may be changed according to a target sequence on a target gene or nucleic acid.

In one exemplary embodiment, N_{target} may be a base sequence capable of forming a complementary bond with a target gene, that is, a target sequence of a neovascularization-associated factor such as a VEGFA gene, an HIF1A gene, an ANGPT2 gene, an EPAS1 gene or an ANGPTL4 gene.

Here, the $(Q)_m$ is a base sequence including the first complementary domain, which is able to form a complementary bond with the second complementary domain of the second strand. The $(Q)_m$ may be a sequence having partial or complete homology with the first complementary domain of a species existing in nature, and the base sequence of the first complementary domain may be changed according to the species of origin. The Q may be

each independently selected from the group consisting of A, U, C and G, and the m may be the number of bases, which is an integer of 5 to 35.

For example, when the first complementary domain has partial or complete homology with a first complementary domain of *Streptococcus pyogenes* or a *Streptococcus pyogenes*-derived first complementary domain, the $(Q)_m$ may be 5'-GUUUUAGAGCUA-3', or a base sequence having at least 50% or more homology with 5'-GUUUUAGAGCUA-3'.

In another example, when the first complementary domain has partial or complete homology with a first complementary domain of *Campylobacter jejuni* or a *Campylobacter jejuni*-derived first complementary domain, the $(Q)_m$ may be 5'-GUUUUAGUCCCUUUUUAAAUUUCUU-3', or a base sequence having at least 50% or more homology with 5'-GUUUUAGUCCCUUUUUAAAUUUCUU-3'.

In still another example, when the first complementary domain has partial or complete homology with a first complementary domain of *Streptococcus thermophilus* or a *Streptococcus thermophilus*-derived first complementary domain, the $(Q)_m$ may be 5'-GUUUUAGAGCUGUGUUGUUUCG-3', or a base sequence having at least 50% or more homology with 5'-GUUUUAGAGCUGUGUUGUUUCG-3'.

In addition, each of the $(X)_a$, $(X)_b$ and $(X)_c$ is selectively an additional base sequence, where the X may be each independently selected from the group consisting of A, U, C and G, and each of the a, b and c may be the number of bases, which is 0 or an integer of 1 to 20.

Second strand

The second strand may consist of a second complementary domain and a proximal domain, and selectively include a tail domain.

<u>Second complementary domain</u>

In the second strand, the second complementary domain includes a nucleic acid sequence complementary to the first complementary domain of the first strand, and has enough complementarity so as to form a double strand with the first complementary domain. The second complementary domain may include a base sequence complementary to the first complementary domain and a base sequence not complementary to the first complementary domain, for example, a base sequence not forming a double strand with the first complementary domain, and may have a longer base sequence than the first complementary domain.

Here, the second complementary domain may be a 5 to 35-base sequence, or include a 5 to 35-base sequence. For example, the second complementary domain may be or include a 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25-base sequence, but the present invention is not limited thereto.

The second complementary domain may have homology with a natural second complementary domain, or may be derived from a natural second complementary domain. In addition, the second complementary domain may have a difference in base sequence thereof according to a species existing in nature, may be derived from a second complementary domain contained in the species existing in nature, or may have partial or complete homology with the second complementary domain contained in the species existing in nature.

In one exemplary embodiment, the second complementary domain may have partial, that is, at least 50% or more, or complete homology with a second complementary domain of *Streptococcus pyogenes*, *Campylobacter jejuni*, *Streptococcus thermophilus*, *Streptocuccus aureus* or *Neisseria meningitides*, or a second complementary domain derived therefrom.

Selectively, the second complementary domain may further include an additional base sequence which does not undergo complementary bonding with the first complementary domain of the first strand.

Here, the additional base sequence may be a 1 to 25-base sequence. For example, the additional base sequence may be a 1 to 5, 5 to 10, 10 to 15, 15 to 20 or 20 to 25-base sequence.

Proximal domain

In the second strand, the proximal domain is a sequence of 1 to 20 bases, and a domain located at the 3' end direction of the second complementary domain. For example, the proximal domain may be or include a sequence of 5, 6, 7, 8, 8, 9, 10, 11, 12, 13, 14 or 15 bases.

Here, the proximal domain may have a double strand bond between complementary base sequences therein.

In addition, the proximal domain may have homology with a natural proximal domain, or may be derived from a natural proximal domain. In addition, the proximal domain may have a difference in base sequence according to a species existing in nature, may be derived from a proximal domain of a species existing in nature, or may have partial or complete homology with the proximal domain of a species existing in nature.

In one exemplary embodiment, the proximal domain may have partial, that is, at least 50% or more, or complete homology with a proximal domain of *Streptococcus pyogenes*, *Campylobacter jejuni*, *Streptococcus thermophilus*, *Streptocuccus aureus* or *Neisseria meningitides*, or a proximal domain derived therefrom.

Tail domain

Selectively, in the second strand, the tail domain may be a domain selectively added to the 3’ end of the second strand, and the tail domain may be or include a 1 to 50-base sequence. For example, the tail domain may be or include a 1 to 5, 5 to 10, 10 to 15, 15 to 20, 20 to 25, 25 to 30, 30 to 35, 35 to 40, 40 to 45 or 45 to 50-base sequence.

Here, the tail domain may have a double strand bond between complementary base sequences therein.

In addition, the tail domain may have homology with a natural tail domain, or may be derived from a natural tail domain. In addition, the tail domain may have a difference in base sequence according to a species existing in nature, may be derived from a tail domain contained in the species existing in nature, or may have partial or complete homology with the tail domain contained in the species existing in nature.

In one exemplary embodiment, the tail domain may have partial, that is, at least 50% or more, or complete homology with a tail domain of *Streptococcus pyogenes*, *Campylobacter jejuni*, *Streptococcus thermophilus*, *Streptocuccus aureus* or *Neisseria meningitides*, or a tail domain derived therefrom.

In another embodiment, the tail domain may include a sequence of 1 to 10 bases at the 3’ end involved in an *in vitro* or *in vivo* transcription method.

For example, when a T7 promoter is used in *in vitro* transcription of gRNA, the tail domain may be an arbitrary base sequence present at the 3’ end of a DNA template. In addition, when a U6 promoter is used in *in vivo* transcription, the tail domain may be UUUUUU, when an H1 promoter is used in transcription, the tail domain may be UUUU, and when a pol-III promoter is used, the tail domain may include several uracil bases or alternative bases.

Selectively, a part or all of each of the base sequence of the second complementary domain, the proximal domain and/or the tail domain may have a chemical modification. The chemical modification may be methylation, acetylation, phosphorylation, phosphorothioate linkage, a locked nucleic acid (LNA), 2'-O-methyl 3'phosphorothioate (MS) or 2'-O-methyl 3'thioPACE (MSP), but the present invention is not limited thereto.

Therefore, the second strand may consist of 5'-[second complementary domain]-[proximal domain]-3' or 5'-[second complementary domain]-[proximal domain]-[tail domain]-3' as described above.

In addition, the second strand may selectively include an additional base sequence.

In one exemplary embodiment, the second strand may be 5'-$(Z)_h$-$(P)_k$-3'; or 5'-$(X)_d$-$(Z)_h$-$(X)_e$-$(P)_k$-$(X)_f$-3'.

In another embodiment, the second strand may be 5'-$(Z)_h$-$(P)_k$-$(F)_i$-3'; or 5'-$(X)_d$-$(Z)_h$-$(X)_e$-$(P)_k$-$(X)_f$-$(F)_i$-3'.

Here, the $(Z)_h$ is a base sequence including a second complementary domain, which is able to form a complementary bond with the first complementary domain of the first strand. The $(Z)_h$ may be a sequence having partial or complete homology with the second complementary domain of a species existing in nature, and the base sequence of the second complementary domain may be modified according to the species of origin. The Z may be each independently selected from the group consisting of A, U, C and G, and the h may be the number of bases, which is an integer of 5 to 50.

For example, when the second complementary domain has partial or complete homology with a second complementary domain of *Streptococcus pyogenes* or a second complementary domain derived therefrom, the $(Z)_h$ may be 5'-UAGCAAGUUAAAAU-3', or a base sequence having at least 50% or more homology with 5'-UAGCAAGUUAAAAU-3'.

In another example, when the second complementary domain has partial or complete homology with a second complementary domain of *Campylobacter jejuni* or a second complementary domain derived therefrom, the $(Z)_h$ may be 5'-AAGAAAUUUAAAAAGGGACUAAAAU-3', or a base sequence having at least 50% or more homology with 5'-AAGAAAUUUAAAAAGGGACUAAAAU-3'.

In still another example, when the second complementary domain has partial or complete homology with a second complementary domain of *Streptococcus thermophilus* or a second complementary domain derived therefrom, the $(Z)_h$ may be 5'-CGAAACAACACAGCGAGUUAAAAU-3', or a base sequence having at least 50% or more homology with 5'-CGAAACAACACAGCGAGUUAAAAU-3'.

The $(P)_k$ is a base sequence including a proximal domain, which may have partial or complete homology with a proximal domain of a species existing in nature, and the base sequence of the proximal domain may be modified according to the species of origin. The P may be each independently selected from the group consisting of A, U, C and G, and the k may be the number of bases, which is an integer of 1 to 20.

For example, when the proximal domain has partial or complete homology with a proximal domain of *Streptococcus pyogenes* or a proximal domain derived therefrom, the $(P)_k$ may be 5'-AAGGCUAGUCCG-3', or a base sequence having at least 50% or more homology with 5'-AAGGCUAGUCCG-3'.

In another example, when the proximal domain has partial or complete homology with a proximal domain of *Campylobacter jejuni* or a proximal domain derived therefrom, the $(P)_k$ may be 5'-AAAGAGUUUGC-3', or a base sequence having at least 50% or more homology with 5'-AAAGAGUUUGC-3'.

In still another example, when the proximal domain has partial or complete homology with a proximal domain of *Streptococcus thermophilus* or a proximal domain derived

therefrom, the $(P)_k$ may be 5'-AAGGCUUAGUCCG-3', or a base sequence having at least 50% or more homology with 5'-AAGGCUUAGUCCG-3'.

The $(F)_i$ may be a base sequence including a tail domain, and having partial or complete homology with a tail domain of a species existing in nature, and the base sequence of the tail domain may be modified according to the species of origin. The F may be each independently selected from the group consisting of A, U, C and G, and the i may be the number of bases, which is an integer of 1 to 50.

For example, when the tail domain has partial or complete homology with a tail domain of *Streptococcus pyogenes* or a tail domain derived therefrom, the $(F)_i$ may be 5'-UUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC-3', or a base sequence having at least 50% or more homology with 5'-UUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC-3'.

In another example, when the tail domain has partial or complete homology with a tail domain of *Campylobacter jejuni* or a tail domain derived therefrom, the $(F)_i$ may be 5'-GGGACUCUGCGGGGUUACAAUCCCCUAAAACCGCUUUU-3', or a base sequence having at least 50% or more homology with 5'-GGGACUCUGCGGGGUUACAAUCCCCUAAAACCGCUUUU-3'.

In still another example, when the tail domain has partial or complete homology with a tail domain of *Streptococcus thermophilus* or a tail domain derived therefrom, the $(F)_i$ may be 5'-UACUCAACUUGAAAAGGUGGCACCGAUUCGGUGUUUUU-3', or a base sequence having at least 50% or more homology with 5'-UACUCAACUUGAAAAGGUGGCACCGAUUCGGUGUUUUU-3'.

In addition, the $(F)_i$ may include a sequence of 1 to 10 bases at the 3' end involved in an *in vitro* or *in vivo* transcription method.

For example, when a T7 promoter is used in *in vitro* transcription of gRNA, the tail domain may be an arbitrary base sequence present at the 3' end of a DNA template. In addition, when a U6 promoter is used in *in vivo* transcription, the tail domain may be UUUUUU, when an H1 promoter is used in transcription, the tail domain may be UUUU, and when a pol-III promoter is used, the tail domain may include several uracil bases or alternative bases.

In addition, the $(X)_d$, $(X)_e$ and $(X)_f$ may be base sequences selectively added, where the X may be each independently selected from the group consisting of A, U, C and G, and each of the d, e and f may be the number of bases, which is 0 or an integer of 1 to 20.

Single-stranded gRNA

Single-stranded gRNA may be classified into two types.

i) Single-stranded gRNA

First, there is single-stranded gRNA in which a first strand or a second strand of the double-stranded gRNA is linked by a linker domain, and here, the single-stranded gRNA consists of 5'-[first strand]-[linker domain]-[second strand]-3'.

Specifically, the single-stranded gRNA may consist of

5'-[guide domain]-[first complementary domain]-[linker domain]-[second complementary domain]-[proximal domain]-3' or

5'-[guide domain]-[first complementary domain]-[linker domain]-[second complementary domain]-[proximal domain]-[tail domain]-3'.

Each domain except the linker domain is the same as the description of each domain of the first and second strands of the double-stranded gRNA.

Linker domain

In the single-stranded gRNA, the linker domain is a domain connecting a first strand and a second strand, and specifically, is a nucleic acid sequence which connects a first complementary domain with a second complementary domain to produce single-stranded gRNA. Here, the linker domain may be connected with the first complementary domain and the second complementary domain or connect the first complementary domain with the second complementary domain by covalent or non-covalent bonding.

The linker domain may be or include a 1 to 30-base sequence. For example, the linker domain may be or include a 1 to 5, 5 to 10, 10 to 15, 15 to 20, 20 to 25 or 25 to 30-base sequence.

The linker domain is suitable to be used in a single-stranded gRNA molecule, and may be connected with the first strand and the second strand of the double-stranded gRNA, or connect the first strand with the second strand by covalent or non-covalent bonding to be used in production of the single-stranded gRNA. The linker domain may be connected with crRNA and tracrRNA of the double-stranded gRNA, or connect crRNA with tracrRNA by covalent or non-covalent bonding to be used in production of the single-stranded gRNA.

The linker domain may have homology with a natural sequence, for example, a partial sequence of tracrRNA, or may be derived therefrom.

Selectively, a part or all of the base sequence of the linker domain may have a chemical modification. The chemical modification may be methylation, acetylation, phosphorylation, phosphorothioate linkage, a locked nucleic acid (LNA), 2'-O-methyl 3'phosphorothioate (MS) or 2'-O-methyl 3'thioPACE (MSP), but the present invention is not limited thereto.

Therefore, the single-stranded gRNA may consist of 5'-[guide domain]-[first complementary domain]-[linker domain]-[second complementary domain]-[proximal domain]-3' or 5'-[guide domain]-[first complementary domain]-[linker domain]-[second complementary domain]-[proximal domain]-[tail domain]-3' as described above.

In addition, the single-stranded gRNA may selectively include an additional base sequence.

In one exemplary embodiment, the single-stranded gRNA may be

$5'-(N_{target})-(Q)_m-(L)_j-(Z)_h-(P)_k-3'$; or

$5'-(N_{target})-(Q)_m-(L)_j-(Z)_h-(P)_k-(F)_i-3'$.

In another embodiment, the single-stranded gRNA may be

$5'-(X)_a-(N_{target})-(X)_b-(Q)_m-(X)_c-(L)_j-(X)_d-(Z)_h-(X)_e-(P)_k-(X)_f-3'$; or

$5'-(X)_a-(N_{target})-(X)_b-(Q)_m-(X)_c-(L)_j-(X)_d-(Z)_h-(X)_e-(P)_k-(X)_f-(F)_i-3'$.

Here, the N_{target} is a base sequence capable of forming a complementary bond with a target sequence on a target gene or nucleic acid, and a base sequence region capable of being changed according to a target sequence on a target gene or nucleic acid.

In one exemplary embodiment, N_{target} is a base sequence capable of forming a complementary bond with a target gene, that is, a target sequence of a neovascularization-associated factor such as a VEGFA gene, an HIF1A gene, an ANGPT2 gene, an EPAS1 gene, or an ANGPTL4 gene.

The $(Q)_m$ includes a base sequence including the first complementary domain, which is able to form a complementary bond with a second complementary domain. The $(Q)_m$ may be a sequence having partial or complete homology with a first complementary domain of a species existing in nature, and the base sequence of the first complementary domain may be changed according to the species of origin. The Q may be each independently selected from

the group consisting of A, U, C and G, and the m may be the number of bases, which is an integer of 5 to 35.

For example, when the first complementary domain has partial or complete homology with a first complementary domain of *Streptococcus pyogenes* or a first complementary domain derived therefrom, the $(Q)_m$ may be 5'-GUUUUAGAGCUA-3', or a base sequence having at least 50% or more homology with 5'-GUUUUAGAGCUA-3'.

In another example, when the first complementary domain has partial or complete homology with a first complementary domain of *Campylobacter jejuni* or a first complementary domain derived therefrom, the $(Q)_m$ may be 5'-GUUUUAGUCCCUUUUUAAAUUUCUU-3', or a base sequence having at least 50% or more homology with 5'-GUUUUAGUCCCUUUUUAAAUUUCUU-3'.

In still another example, when the first complementary domain has partial or complete homology with a first complementary domain of *Streptococcus thermophilus* or a first complementary domain derived therefrom, the $(Q)_m$ may be 5'-GUUUUAGAGCUGUGUUGUUUCG-3', or a base sequence having at least 50% or more homology with 5'-GUUUUAGAGCUGUGUUGUUUCG-3'.

In addition, the $(L)_j$ is a base sequence including the linker domain, and connecting the first complementary domain with the second complementary domain, thereby producing single-stranded gRNA. Here, the L may be each independently selected from the group consisting of A, U, C and G, and the j may be the number of bases, which is an integer of 1 to 30.

The $(Z)_h$ is a base sequence including the second complementary domain, which is able to have a complementary bond with the first complementary domain. The $(Z)_h$ may be a sequence having partial or complete homology with the second complementary domain of a species existing in nature, and the base sequence of the second complementary domain may

be changed according to the species of origin. The Z may be each independently selected from the group consisting of A, U, C and G, and the h is the number of bases, which may be an integer of 5 to 50.

For example, when the second complementary domain has partial or complete homology with a second complementary domain of *Streptococcus pyogenes* or a second complementary domain derived therefrom, the $(Z)_h$ may be 5'-UAGCAAGUUAAAAU-3', or a base sequence having at least 50% or more homology with 5'-UAGCAAGUUAAAAU-3'.

In another example, when the second complementary domain has partial or complete homology with a second complementary domain of *Campylobacter jejuni* or a second complementary domain derived therefrom, the $(Z)_h$ may be 5'-AAGAAAUUUAAAAAGGGACUAAAAU-3', or a base sequence having at least 50% or more homology with 5'-AAGAAAUUUAAAAAGGGACUAAAAU-3'.

In still another example, when the second complementary domain has partial or complete homology with a second complementary domain of *Streptococcus thermophilus* or a second complementary domain derived therefrom, the $(Z)_h$ may be 5'-CGAAACAACACAGCGAGUUAAAAU-3', or a base sequence having at least 50% or more homology with 5'-CGAAACAACACAGCGAGUUAAAAU-3'.

The $(P)_k$ is a base sequence including a proximal domain, which may have partial or complete homology with a proximal domain of a species existing in nature, and the base sequence of the proximal domain may be modified according to the species of origin. The P may be each independently selected from the group consisting of A, U, C and G, and the k may be the number of bases, which is an integer of 1 to 20.

For example, when the proximal domain has partial or complete homology with a proximal domain of *Streptococcus pyogenes* or a proximal domain derived therefrom, the

$(P)_k$ may be 5'-AAGGCUAGUCCG-3', or a base sequence having at least 50% or more homology with 5'-AAGGCUAGUCCG-3'.

In another example, when the proximal domain has partial or complete homology with a proximal domain of *Campylobacter jejuni* or a proximal domain derived therefrom, the $(P)_k$ may be 5'-AAAGAGUUUGC-3', or a base sequence having at least 50% or more homology with 5'-AAAGAGUUUGC-3'.

In still another example, when the proximal domain has partial or complete homology with a proximal domain of *Streptococcus thermophilus* or a proximal domain derived therefrom, the $(P)_k$ may be 5'-AAGGCUUAGUCCG-3', or a base sequence having at least 50% or more homology with 5'-AAGGCUUAGUCCG-3'.

The $(F)_i$ may be a base sequence including a tail domain, and having partial or complete homology with a tail domain of a species existing in nature, and the base sequence of the tail domain may be modified according to the species of origin. The F may be each independently selected from the group consisting of A, U, C and G, and the i may be the number of bases, which is an integer of 1 to 50.

For example, when the tail domain has partial or complete homology with a tail domain of *Streptococcus pyogenes* or a tail domain derived therefrom, the $(F)_i$ may be 5'-UUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC-3', or a base sequence having at least 50% or more homology with 5'-UUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC-3'

In another example, when the tail domain has partial or complete homology with a tail domain of *Campylobacter jejuni* or a tail domain derived therefrom, the $(F)_i$ may be 5'-GGGACUCUGCGGGGUUACAAUCCCCUAAAACCGCUUUU-3', or a base sequence having at least 50% or more homology with 5'-GGGACUCUGCGGGGUUACAAUCCCCUAAAACCGCUUUU-3'.

In still another example, when the tail domain has partial or complete homology with a tail domain of *Streptococcus thermophilus* or a tail domain derived therefrom, the $(F)_i$ may be 5'-UACUCAACUUGAAAAGGUGGCACCGAUUCGGUGUUUUU-3', or a base sequence having at least 50% or more homology with 5'-UACUCAACUUGAAAAGGUGGCACCGAUUCGGUGUUUUU-3'.

In addition, the $(F)_i$ may include a sequence of 1 to 10 bases at the 3' end involved in an *in vitro* or *in vivo* transcription method.

For example, when a T7 promoter is used in *in vitro* transcription of gRNA, the tail domain may be an arbitrary base sequence present at the 3' end of a DNA template. In addition, when a U6 promoter is used in *in vivo* transcription, the tail domain may be UUUUUU, when an H1 promoter is used in transcription, the tail domain may be UUUU, and when a pol-III promoter is used, the tail domain may include several uracil bases or alternative bases.

In addition, the $(X)_a$, $(X)_b$, $(X)_c$, $(X)_d$, $(X)_e$ and $(X)_f$ may be base sequences selectively added, where the X may be each independently selected from the group consisting of A, U, C and G, and each of the a, b, c, d, e and f may be the number of bases, which is 0 or an integer of 1 to 20.

ii) Single-stranded gRNA

Second, the single-stranded gRNA may be single-stranded gRNA consisting of a guide domain, a first complementary domain and a second complementary domain, and here, the single-stranded gRNA may consist of:

5'-[second complementary domain]-[first complementary domain]-[guide domain]-3';

or

5’-[second complementary domain]-[linker domain]-[first complementary domain]-[guide domain]-3’.

Guide domain

In the single-stranded gRNA, the guide domain includes a complementary guide sequence capable of forming a complementary bond with a target sequence on a target gene or nucleic acid. The guide sequence may be a nucleic acid sequence having complementarity to the target sequence on the target gene or nucleic acid, which has, for example, at least 70%, 75%, 80%, 85%, 90% or 95% or more complementarity or complete complementarity. The guide domain is considered to allow a gRNA-Cas complex, that is, a CRISPR complex to specifically interact with the target gene or nucleic acid.

Here, the guide domain may be or include a 5 to 50-base sequence. For example, the guide domain may be or include a 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25-base sequence.

In addition, the guide domain may include a guide sequence.

Here, the guide sequence may be a complementary base sequence capable of forming a complementary bond with a target sequence on a target gene or nucleic acid, which has, for example, at least 70%, 75%, 80%, 85%, 90% or 95% or more complementarity or complete complementarity.

In one exemplary embodiment, the guide sequence may be a nucleic acid sequence complementary to a target gene, that is, a target sequence of a neovascularization-associated factor such as a VEGFA gene, an HIF1A gene, an ANGPT2 gene, an EPAS1 gene or an ANGPTL4 gene, which has, for example, at least 70%, 75%, 80%, 85%, 90% or 95% or more complementarity or complete complementarity.

Here, the guide sequence may be or include a 5 to 50-base sequence. For example, the guide sequence may be or include a 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25-base sequence.

In one exemplary embodiment, the guide sequence may be a nucleic acid sequence complementary to a target sequence of the VEGFA gene. The guide sequence may be or include a 5 to 50-base sequence. For example, the guide sequence may be or include a 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25-base sequence.

In one exemplary embodiment, the guide sequence may be a nucleic acid sequence complementary to a target sequence of the HIF1A gene. The guide sequence may be or include a 5 to 50-base sequence. For example, the guide sequence may be or include a 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25-base sequence.

In one exemplary embodiment, the guide sequence may be a nucleic acid sequence complementary to a target sequence of the ANGPT2 gene. The guide sequence may be or include a 5 to 50-base sequence. For example, the guide sequence may be or include a 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25-base sequence.

In one exemplary embodiment, the guide sequence may be a nucleic acid sequence complementary to a target sequence of the EPAS1 gene. The guide sequence may be or include a 5 to 50-base sequence. For example, the guide sequence may be or include a 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25-base sequence.

In one exemplary embodiment, the guide sequence may be a nucleic acid sequence complementary to a target sequence of the ANGPTL4 gene. The guide sequence may be or include a 5 to 50-base sequence. For example, the guide sequence may be or include a 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25-base sequence.

Here, target sequences of the target genes, that is, the neovascularization-associated factors such as the VEGFA gene, the HIF1A gene, the ANGPT2 gene, the EPAS1 gene, and the ANGPTL4 gene for the guide sequence are listed above in Table 1, Table 2, Table 3, Table 4 and Table 5, respectively, but the present invention is not limited thereto.

Selectively, the guide domain may include a guide sequence and an additional base sequence.

Here, the additional base sequence may be a 1 to 35-base sequence. For example, the additional base sequence may be a 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10-base sequence.

In one exemplary embodiment, the additional base sequence may be a single base sequence, guanine (G), or a sequence of two bases, GG.

Here, the additional base sequence may be located at the 5' end of the guide domain, or at the 5' end of the guide sequence.

The additional base sequence may be located at the 3' end of the guide domain, or at the 3' end of the guide sequence.

First complementary domain

The first complementary domain is a domain including a nucleic acid sequence complementary to the second complementary domain, and having enough complementarity so as to form a double strand with the second complementary domain.

Here, the first complementary domain may be or include a 5 to 35-base sequence. For example, the first complementary domain may be or include a 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25-base sequence.

The first complementary domain may have homology with a natural first complementary domain, or may be derived from a natural first complementary domain. In addition, the first complementary domain may have a difference in the base sequence of a first complementary domain depending on the species existing in nature, may be derived from a first complementary domain contained in the species existing in nature, or may have partial or complete homology with the first complementary domain contained in the species existing in nature.

In one exemplary embodiment, the first complementary domain may have partial, that is, at least 50% or more, or complete homology with a first complementary domain of *Parcubacteria bacterium* (GWC2011_GWC2_44_17), *Lachnospiraceae bacterium* (MC2017), *Butyrivibrio proteoclasiicus*, *Peregrinibacteria bacterium* (GW2011_GWA_33_10), *Acidaminococcus sp.* (BV3L6), *Porphyromonas macacae*, *Lachnospiraceae bacterium* (ND2006), *Porphyromonas crevioricanis*, *Prevotella disiens*, *Moraxella bovoculi* (237), *Smiihella sp.* (SC_KO8D17), *Leptospira inadai*, *Lachnospiraceae bacterium* (MA2020), *Francisella novicida* (U112), *Candidatus Methanoplasma termitum* or *Eubacterium eligens*, or a first complementary domain derived therefrom.

Selectively, the first complementary domain may include an additional base sequence which does not undergo complementary bonding with the second complementary domain.

Here, the additional base sequence may be a 1 to 15-base sequence. For example, the additional base sequence may be a 1 to 5, 5 to 10, or 10 to 15-base sequence.

Second complementary domain

The second complementary domain includes a nucleic acid sequence complementary to the first complementary domain, and has enough complementarity so as to form a double strand with the first complementary domain. The second complementary domain may include a base sequence complementary to the first complementary domain, and a base sequence having no complementarity with the first complementary domain, for example, a base sequence not forming a double strand with the first complementary domain, and may have a longer base sequence than the first complementary domain.

Here, the second complementary domain may be or include a 5 to 35-base sequence. For example, the second complementary domain may be a 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25-base sequence.

The second complementary domain may have homology with a natural second complementary domain, or may be derived from the natural second complementary domain. In addition, the second complementary domain may have a difference in base sequence of the second complementary domain according to a species existing in nature, and may be derived from second complementary domain contained in the species existing in nature, or may have partial or complete homology with the second complementary domain contained in the species existing in nature.

In one exemplary embodiment, the second complementary domain may have partial, that is, at least 50% or more, or complete homology with a second complementary domain of *Parcubacteria bacterium* (GWC2011_GWC2_44_17), *Lachnospiraceae bacterium* (MC2017), *Butyrivibrio proteoclasiicus*, *Peregrinibacteria bacterium* (GW2011_GWA_33_10), *Acidaminococcus sp.* (BV3L6), *Porphyromonas macacae*, *Lachnospiraceae bacterium* (ND2006), *Porphyromonas crevioricanis*, *Prevotella disiens*, *Moraxella bovoculi* (237), *Smiihella sp.* (SC_KO8D17), *Leptospira inadai*, *Lachnospiraceae bacterium* (MA2020), *Francisella novicida* (U112), *Candidatus Methanoplasma termitum* or *Eubacterium eligens*, or a second complementary domain derived therefrom.

Selectively, the second complementary domain may include an additional base sequence which does not undergo complementary bonding with the first complementary domain.

Here, the additional base sequence may be a 1 to 15-base sequence. For example, the additional base sequence may be a 1 to 5, 5 to 10, or 10 to 15-base sequence.

Linker domain

Selectively, the linker domain is a nucleic acid sequence connecting a first complementary domain with a second complementary domain to produce single-stranded gRNA. Here, the linker domain may be connected with the first complementary domain and the second complementary domain, or may connect the first and second complementary domains by covalent or non-covalent bonding.

The linker domain may be or include a 1 to 30-base sequence. For example, the linker domain may be or include a 1 to 5, 5 to 10, 10 to 15, 15 to 20, 20 to 25 or 25 to 30-base sequence.

Selectively, a part or all of the base sequence of the guide domain, the first complementary domain, the second complementary domain and the linker domain may have a chemical modification. The chemical modification may be methylation, acetylation, phosphorylation, phosphorothioate linkage, a locked nucleic acid (LNA), 2'-O-methyl 3'phosphorothioate (MS) or 2'-O-methyl 3'thioPACE (MSP), but the present invention is not limited thereto.

Therefore, the single-stranded gRNA may consist of 5'-[second complementary domain]-[first complementary domain]-[guide domain]-3' or 5'-[second complementary domain]-[linker domain]-[first complementary domain]-[guide domain]-3' as described above.

In addition, the single-stranded gRNA may selectively include an additional base sequence.

In one exemplary embodiment, the single-stranded gRNA may be

$5'-(Z)_h-(Q)_m-(N_{target})-3'$; or

$5'-(X)_a-(Z)_h-(X)_b-(Q)_m-(X)_c-(N_{target})-3'$.

In another embodiment, the single-stranded gRNA may be

5'-$(Z)_h$-$(L)_j$-$(Q)_m$-(N_{target})-3'; or

5'-$(X)_a$-$(Z)_h$-$(L)_j$-$(Q)_m$-$(X)_c$-(N_{target})-3'.

Here, the N_{target} is a base sequence capable of forming a complementary bond with a target sequence on a target gene or nucleic acid, and a base sequence region which may be changed according to a target sequence on a target gene or nucleic acid.

In one exemplary embodiment, N_{target} may be a base sequence capable of forming a complementary bond with a target gene, that is, a target sequence of a neovascularization-associated factor such as a VEGFA gene, an HIF1A gene, an ANGPT2 gene, an EPAS1 gene or an ANGPTL4 gene.

The $(Q)_m$ is a base sequence including the first complementary domain, which is able to form a complementary bond with the second complementary domain of the second strand. The $(Q)_m$ may be a sequence having partial or complete homology with the first complementary domain of a species existing in nature, and the base sequence of the first complementary domain may be changed according to the species of origin. The Q may be each independently selected from the group consisting of A, U, C and G, and the m may be the number of bases, which is an integer of 5 to 35.

For example, when the first complementary domain has partial or complete homology with a first complementary domain of *Parcubacteria bacterium* or a first complementary domain derived therefrom, the $(Q)_m$ may be 5'-UUUGUAGAU-3', or a base sequence having at least 50% or more homology with 5'-UUUGUAGAU-3'.

The $(Z)_h$ is a base sequence including a second complementary domain, which is able to form a complementary bond with the first complementary domain of the first strand. The $(Z)_h$ may be a sequence having partial or complete homology with the second complementary domain of a species existing in nature, and the base sequence of the second complementary

domain may be modified according to the species of origin. The Z may be each independently selected from the group consisting of A, U, C and G, and the h may be the number of bases, which is an integer of 5 to 50.

For example, when the second complementary domain has partial or complete homology with a second complementary domain of *Parcubacteria bacterium* or a *Parcubacteria bacterium*-derived second complementary domain, the $(Z)_h$ may be 5'-AAAUUUCUACU-3', or a base sequence having at least 50% or more homology with 5'-AAAUUUCUACU-3'.

In addition, the $(L)_j$ is a base sequence including the linker domain, which connects the first complementary domain with the second complementary domain. Here, the L may be each independently selected from the group consisting of A, U, C and G, and the j may be the number of bases, which is an integer of 1 to 30.

In addition, each of the $(X)_a$, $(X)_b$ and $(X)_c$ is selectively an additional base sequence, where the X may be each independently selected from the group consisting of A, U, C and G, and the a, b and c may be the number of bases, which is 0 or an integer of 1 to 20.

2. Editor protein

An editor protein refers to a peptide, polypeptide or protein which is able to directly bind to or interact with, without direct binding to, a nucleic acid.

The nucleic acid may be a nucleic acid contained in a target nucleic acid, gene or chromosome.

The nucleic acid may be a guide nucleic acid.

The editor protein may be an enzyme.

The editor protein may be a fusion protein.

Here, the fusion protein refers to a protein produced by fusing an enzyme with an additional domain, peptide, polypeptide or protein.

The enzyme refers to a protein including a domain which is able to cleave a nucleic acid, gene, chromosome or protein.

The enzyme may be a nuclease, protease or restriction enzyme.

The additional domain, peptide, polypeptide or protein may be a functional domain, peptide, polypeptide or protein, which has a function the same as or different from the enzyme.

The fusion protein may include an additional domain, peptide, polypeptide or protein at one or more of an N-terminus of an enzyme or the proximity thereof; a C-terminus of the enzyme or the proximity thereof; the middle region of an enzyme; and a combination thereof.

The fusion protein may include a functional domain, peptide, polypeptide or protein at one or more of an N-terminus of an enzyme or the proximity thereof; a C-terminus of the enzyme or the proximity thereof; the middle region of an enzyme; and a combination thereof.

Here, the functional domain, peptide, polypeptide or protein may be a domain, peptide, polypeptide or protein having methylase activity, demethylase activity, transcription activation activity, transcription repression activity, transcription release factor activity, histone modification activity, RNA cleavage activity or nucleic acid binding activity, or a tag or reporter gene for isolation and purification of a protein (including a peptide), but the present invention is not limited thereto.

The functional domain, peptide, polypeptide or protein may be a deaminase.

The tag includes a histidine (His) tag, a V5 tag, a FLAG tag, an influenza hemagglutinin (HA) tag, a Myc tag, a VSV-G tag and a thioredoxin (Trx) tag, and the reporter gene includes glutathione-S-transferase (GST), horseradish peroxidase (HRP), chloramphenicol acetyltransferase (CAT) β-galactosidase, β-glucoronidase, luciferase,

autofluorescent proteins including the green fluorescent protein (GFP), HcRed, DsRed, cyan fluorescent protein (CFP), yellow fluorescent protein (YFP) and blue fluorescent protein (BFP), but the present invention is not limited thereto.

In addition, the functional domain, peptide, polypeptide or protein may be a nuclear localization sequence or signal (NLS) or a nuclear export sequence or signal (NES).

The NLS may be NLS of SV40 virus large T-antigen with an amino acid sequence PKKKRKV; NLS derived from nucleoplasmin (e.g., nucleoplasmin bipartite NLS with a sequence KRPAATKKAGQAKKKK); c-myc NLS with an amino acid sequence PAAKRVKLD or RQRRNELKRSP; hRNPA1 M9 NLS with a sequence NQSSNFGPMKGGNFGGRSSGPYGGGGQYFAKPRNQGGY; an importin-α-derived IBB domain sequence RMRIZFKNKGKDTAELRRRRVEVSVELRKAKKDEQILKRRNV; myoma T protein sequences VSRKRPRP and PPKKARED; human p53 sequence POPKKKPL; a mouse c-abl IV sequence SALIKKKKKMAP; influenza virus NS1 sequences DRLRR and PKQKKRK; a hepatitis virus-δ antigen sequence RKLKKKIKKL; a mouse Mx1 protein sequence REKKKFLKRR; a human poly(ADP-ribose) polymerase sequence KRKGDEVDGVDEVAKKKSKK; or steroid hormone receptor (human) glucocorticoid sequence RKCLQAGMNLEARKTKK, but the present invention is not limited thereto.

The editor protein may include a complete active enzyme.

Here, the "complete active enzyme" refers to an enzyme having the same function as a function of a wild-type enzyme, and for example, the wild-type enzyme cleaving the double strand of DNA has complete enzyme activity of entirely cleaving the double strand of DNA.

In addition, the complete active enzyme includes an enzyme having an improved function compared to the function of the wild-type enzyme, and for example, a specific modification or manipulation type of the wild-type enzyme cleaving the double strand of

DNA has full enzyme activity which is improved compared to the wild-type enzyme, that is, activity of cleaving the double strand of DNA.

The editor protein may include an incomplete or partially active enzyme.

Here, the "incomplete or partially active enzyme" refers to an enzyme having some of the functions of the wild-type enzyme, and for example, a specific modification or manipulation type of the wild-type enzyme cleaving the double strand of DNA has incomplete or partial enzyme activity of cleaving a part of the double strand, that is, a single strand of DNA.

The editor protein may include an inactive enzyme.

Here, the "inactive enzyme" refers to an enzyme in which the function of a wild-type enzyme is completely inactivated. For example, a specific modification or manipulation type of the wild-type enzyme cleaving the double strand of DNA has inactivity so as not to completely cleave the DNA double strand.

The editor protein may be a natural enzyme or fusion protein.

The editor protein may be present in the form of a partially modified natural enzyme or fusion protein.

The editor protein may be an artificially produced enzyme or fusion protein, which does not exist in nature.

The editor protein may be present in the form of a partially modified artificial enzyme or fusion protein, which does not exist in nature.

Here, the modification may be substitution, removal, addition of amino acids contained in the editor protein, or a combination thereof.

In addition, the modification may be substitution, removal, addition of some bases in the base sequence encoding the editor protein, or a combination thereof.

As one exemplary embodiment of the editor protein of the present invention, a CRISPR enzyme will be described below.

CRISPR enzyme

The term "CRISPR enzyme" is a main protein component of a CRISPR-Cas system, and forms a complex with gRNA, resulting in the CRISPR-Cas system.

The CRISPR enzyme is a nucleic acid or polypeptide (or a protein) having a sequence encoding the CRISPR enzyme, and representatively, a Type II CRISPR enzyme or Type V CRISPR enzyme is widely used.

The Type II CRISPR enzyme is Cas9, which may be derived from various microorganisms such as *Streptococcus pyogenes*, *Streptococcus thermophilus*, *Streptococcus* sp., *Staphylococcus aureus*, *Nocardiopsis dassonvillei*, *Streptomyces pristinaespiralis*, *Streptomyces viridochromogenes*, *Streptomyces viridochromogenes*, *Streptosporangium roseum*, *Streptosporangium roseum*, *AlicyclobacHlus acidocaldarius*, *Bacillus pseudomycoides*, *Bacillus selenitireducens*, *Exiguobacterium sibiricum*, *Lactobacillus delbrueckii*, *Lactobacillus salivarius*, *Microscilla marina*, *Burkholderiales bacterium*, *Polaromonas naphthalenivorans*, *Polaromonas* sp., *Crocosphaera watsonii*, *Cyanothece* sp., *Microcystis aeruginosa*, *Synechococcus* sp., *Acetohalobium arabaticum*, *Ammonifex degensii*, *Caldicelulosiruptor bescii*, *Candidatus Desulforudis*, *Clostridium botulinum*, *Clostridium difficile*, *Finegoldia magna*, *Natranaerobius thermophilus*, *Pelotomaculum thermopropionicum*, *Acidithiobacillus caldus*, *Acidithiobacillus ferrooxidans*, *Allochromatium vinosum*, *Marinobacter* sp., *Nitrosococcus halophilus*, *Nitrosococcus watsoni*, *Pseudoalteromonas haloplanktis*, *Ktedonobacter racemifer*, *Methanohalobium evestigatum*, *Anabaena variabilis*, *Nodularia spumigena*, *Nostoc* sp., *Arthrospira maxima*,

Arthrospira platensis, *Arthrospira* sp., *Lyngbya* sp., *Microcoleus chthonoplastes*, *Oscillatoria* sp., *Petrotoga mobilis*, *Thermosipho africanus* and *Acaryochloris marina*.

The term "Cas9" is an enzyme which binds to gRNA so as to cleave or modify a target sequence or position on a target gene or nucleic acid, and may consist of an HNH domain capable of cleaving a nucleic acid strand forming a complementary bond with gRNA, an RuvC domain capable of cleaving a nucleic acid strand forming a complementary bond with gRNA, an REC domain recognizing a target and a PI domain recognizing PAM. Hiroshi Nishimasu et al. (2014) Cell 156:935-949 may be referenced for specific structural characteristics of Cas9.

In addition, the Type V CRISPR enzyme may be Cpf1, which may be derived from *Streptococcus*, *Campylobacter*, *Nitratifractor*, *Staphylococcus*, *Parvibaculum*, *Roseburia*, *Neisseria*, *Gluconacetobacter*, *Azospirillum*, *Sphaerochaeta*, *Lactobacillus*, *Eubacterium*, *Corynebacter*, *Carnobacterium*, *Rhodobacter*, *Listeria*, *Paludibacter*, *Clostridium*, *Lachnospiraceae*, *Clostridiaridium*, *Leptotrichia*, *Francisella*, *Legionella*, *Alicyclobacillus*, *Methanomethyophilus*, *Porphyromonas*, *Prevotella*, *Bacteroidetes*, *Helcococcus*, *Letospira*, *Desulfovibrio*, *Desulfonatronum*, *Opitutaceae*, *Tuberibacillus*, *Bacillus*, *Brevibacillus*, *Methylobacterium* or *Acidaminococcus*.

The Cpf1 may consist of an RuvC domain similar and corresponding to the RuvC domain of Cas9, an Nuc domain without the HNH domain of Cas9, an REC domain recognizing a target, a WED domain and a PI domain recognizing PAM. For specific structural characteristics of Cpf1, Takashi Yamano et al. (2016) Cell 165:949-962 may be referenced.

The CRISPR enzyme of the Cas9 or Cpf1 protein may be isolated from a microorganism existing in nature or non-naturally produced by a recombinant or synthetic method.

Type II CRISPR enzyme

The crystal structure of the type II CRISPR enzyme was determined according to studies on two or more types of natural microbial type II CRISPR enzyme molecules (Jinek et al., Science, 343(6176):1247997, 2014) and studies on *Streptococcus pyogenes* Cas9 (SpCas9) complexed with gRNA (Nishimasu et al., Cell, 156:935-949, 2014; and Anders et al., Nature, 2014, doi: 10.1038/nature13579).

The type II CRISPR enzyme includes two lobes, that is, recognition (REC) and nuclease (NUC) lobes, and each lobe includes several domains.

The REC lobe includes an arginine-rich bridge helix (BH) domain, an REC1 domain and an REC2 domain.

Here, the BH domain is a long α-helix and arginine-rich region, and the REC1 and REC2 domains play an important role in recognizing a double strand formed in gRNA, for example, single-stranded gRNA, double-stranded gRNA or tracrRNA.

The NUC lobe includes an RuvC domain, an HNH domain and a PAM-interaction (PI) domain. Here, the RuvC domain encompasses RuvC-like domains, or the HNH domain is used to include HNH-like domains.

Here, the RuvC domain shares structural similarity with members of the microorganism family existing in nature having the type II CRISPR enzyme, and cleaves a single strand, for example, a non-complementary strand of a target gene or nucleic acid, that is, a strand not forming a complementary bond with gRNA. The RuvC domain is sometimes referred to as an RuvCI domain, RuvCII domain or RuvCIII domain in the art, and generally called an RuvC I, RuvCII or RuvCIII. For example, in the case of SpCas9, the RuvC domain is assembled from each of three divided RuvC domains (RuvC I, RuvCII and RuvCIII) located at the sequences ofamino acids 1 to 59, 718 to 769 and 909 to 1098 of SpCas9, respectively.

The HNH domain shares structural similarity with the HNH endonuclease, and cleaves a single strand, for example, a complementary strand of a target nucleic acid molecule, that is, a strand forming a complementary bond with gRNA. The HNH domain is located between RuvC II and III motifs. For example, in the case of SpCas9, the HNH domain is located at amino acid sequence 775 to 908 of SpCas9.

The PI domain recognizes a specific base sequence in a target gene or nucleic acid, that is, a protospacer adjacent motif (PAM) or interacts with PAM. For example, in the case of SpCas9, the PI domain is located at the sequence of amino acids1099 to 1368 of SpCas9.

Here, the PAM may vary according to the origin of the type II CRISPR enzyme. For example, when the CRISPR enzyme is SpCas9, PAM may be 5'-NGG-3', when the CRISPR enzyme is *Streptococcus thermophilus* Cas9 (StCas9), PAM may be 5'-NNAGAAW-3'(W = A or T), when the CRISPR enzyme is *Neisseria meningitides* Cas9 (NmCas9), PAM may be 5'-NNNNGATT-3', and when the CRISPR enzyme is *Campylobacter jejuni* Cas9 (CjCas9), PAM may be 5'-NNNVRYAC-3' (V = G or C or A, R = A or G, Y = C or T), where the N may be A, T, G or C; or A, U, G or C.

Type V CRISPR enzyme

Type V CRISPR enzyme includes similar RuvC domains corresponding to the RuvC domains of the type II CRISPR enzyme, and may consist of an Nuc domain, instead of the HNH domain of the type II CRISPR enzyme, REC and WED domains, which recognize a target, and a PI domain recognizing PAM. For specific structural characteristics of the type V CRISPR enzyme, Takashi Yamano et al. (2016) Cell 165:949-962 may be referenced.

The type V CRISPR enzyme may interact with gRNA, thereby forming a gRNA-CRISPR enzyme complex, that is, a CRISPR complex, and may allow a guide sequence to approach a target sequence including a PAM sequence in cooperation with gRNA. Here, the

ability of the type V CRISPR enzyme for interaction with a target gene or nucleic acid is dependent on the PAM sequence.

The PAM sequence is a sequence present in a target gene or nucleic acid, and may be recognized by the PI domain of the type V CRISPR enzyme. The PAM sequence may vary according to the origin of the type V CRISPR enzyme. That is, there are different PAM sequences which are able to be specifically recognized depending on a species.

In one example, the PAM sequence recognized by Cpf1 may be 5'-TTN-3' (N is A, T, C or G).

CRISPR enzyme activity

A CRISPR enzyme cleaves a double or single strand of a target gene or nucleic acid, and has nuclease activity causing breakage or deletion of the double or single strand. Generally, the wild-type type II CRISPR enzyme or type V CRISPR enzyme cleaves the double strand of the target gene or nucleic acid.

To manipulate or modify the above-described nuclease activity of the CRISPR enzyme, the CRISPR enzyme may be manipulated or modified, such a manipulated or modified CRISPR enzyme may be modified into an incompletely or partially active or inactive enzyme.

Incompletely or partially active enzyme

A CRISPR enzyme modified to change enzyme activity, thereby exhibiting incomplete or partial activity is called a nickase.

The term "nickase" refers to a CRISPR enzyme manipulated or modified to cleave only one strand of the double strand of the target gene or nucleic acid, and the nickase has nuclease activity of cleaving a single strand, for example, a strand that is not complementary

or complementary to gRNA of the target gene or nucleic acid. Therefore, to cleave the double strand, nuclease activity of the two nickases is needed.

For example, the nickase may have nuclease activity by the RuvC domain. That is, the nickase may include nuclease activity of the HNH domain, and to this end, the HNH domain may be manipulated or modified.

In one example, provided that the CRISPR enzyme is the type II CRISPR enzyme, when the residue 840 in the amino acid sequence of SpCas9 is mutated from histidine to alanine, the nuclease activity of the HNH domain is inactivated to be used as a nickase. Since the nickase produced thereby has nuclease activity of the RuvC domain, it is able to cleave a strand which does not form a complementary bond with a non-complementary strand of the target gene or nucleic acid, that is, gRNA.

In another exemplary embodiment, when the residue 559 in the amino acid sequence of CjCas9 is mutated from histidine to alanine, the nuclease activity of the HNH domain is inactivated to be used as a nickase. The nickase produced thereby has nuclease activity by the RuvC domain, and thus is able to cleave a non-complementary strand of the target gene or nucleic acid, that is, a strand that does not form a complementary bond with gRNA.

For example, the nickase may have nuclease activity by the HNH domain. That is, the nickase may include the nuclease activity of the RuvC domain, and to this end, the RuvC domain may be manipulated or modified.

In one example, provided that the CRISPR enzyme is the type II CRISPR enzyme, in one exemplary embodiment, when the residue 10 in the amino acid sequence of SpCas9 is mutated from aspartic acid to alanine, the nuclease activity of the RuvC domain is inactivated to be used as a nickase. The nickase produced thereby has the nuclease activity of the HNH domain, and thus is able to cleave a complementary strand of the target gene or nucleic acid, that is, a strand that forms a complementary bond with gRNA.

In another exemplary embodiment, when the residue 8 in the amino acid sequence of CjCas9 is mutated from aspartic acid to alanine, the nuclease activity of the RuvC domain is inactivated to be used as a nickase. The nickase produced thereby has the nuclease activity of the HNH domain, and thus is able to cleave a complementary strand of the target gene or nucleic acid, that is, a strand that forms a complementary bond with gRNA.

Inactive enzyme

A CRISPR enzyme which is modified to make enzyme activity completely inactive is called an inactive CRISPR enzyme.

The term "inactive CRISPR enzyme" refers to a CRISPR enzyme which is modified not to completely cleave the double strand of the target gene or nucleic acid, and the inactive CRISPR enzyme has nuclease inactivity due to the mutation in the domain with nuclease activity of the wild-type CRISPR enzyme. The inactive CRISPR enzyme may be one in which the nuclease activities of the RuvC domain and the HNH domain are inactivated.

For example, the inactive CRISPR enzyme may be manipulated or modified in the RuvC domain and the HNH domain so as to inactive nuclease activity.

In one example, provided that the CRISPR enzyme is the type II CRISPR enzyme, in one exemplary embodiment, when the residues 10 and 840 in the amino acid sequence of SpCas9 are mutated from aspartic acid and histidine to alanine, respectively, nuclease activities by the RuvC domain and the HNH domain are inactivated, such that the double strand may not cleave completely the double strand of the target gene or nucleic acid.

In another exemplary embodiment, when the residues 8 and 559 in the amino acid sequence of CjCas9 are mutated from aspartic acid and histidine to alanine, the nuclease activities by the RuvC domain and the HNH domain are inactivated, such that the double strand may not cleave completely the double strand of the target gene or nucleic acid.

Other activities

The CRISPR enzyme may have endonuclease activity, exonuclease activity or helicase activity, that is, an ability to anneal the helix structure of the double-stranded nucleic acid, in addition to the above-described nuclease activity.

In addition, the CRISPR enzyme may be modified to completely, incompletely, or partially activate the endonuclease activity, exonuclease activity or helicase activity.

Targeting of CRISPR enzyme

The CRISPR enzyme may interact with gRNA, thereby forming a gRNA-CRISPR enzyme complex, that is, a CRISPR complex, and lead a guide sequence to approach a target sequence including a PAM sequence in cooperation with gRNA. Here, the ability of the CRISPR enzyme to interact with the target gene or nucleic acid is dependent on the PAM sequence.

The PAM sequence is a sequence present in the target gene or nucleic acid, which may be recognized by the PI domain of the CRISPR enzyme. The PAM sequence may vary depending on the origin of the CRISPR enzyme. That is, there are various PAM sequences which are able to be specifically recognized according to species.

In one example, provided that the CRISPR enzyme is the type II CRISPR enzyme,

in the case of SpCas9, the PAM sequence may be 5'-NGG-3', 5'-NAG-3' and/or 5'-NGA-3',

in the case of StCas9, the PAM sequence may be 5'-NGGNG-3' and/or 5'-NNAGAAW-3' (W = A or T),

in the case of NmCas9, the PAM sequence may be 5'-NNNNGATT-3' and/or 5'-NNNGCTT-3',

in the case of CjCas9, the PAM sequence may be 5'-NNNVRYAC-3' (V = G, C or A; R = A or G; Y = C or T),

in the case of *Streptococcus mutans* Cas9 (SmCas9), the PAM sequence may be 5'-NGG-3' and/or 5'-NAAR-3' (R = A or G), and

in the case of *Staphylococcus aureus* Cas9 (SaCas9), the PAM sequence may be 5'-NNGRR-3', 5'-NNGRRT-3' and/or 5'-NNGRRV-3' (R = A or G; V = G, C or A).

In another example, provided that the CRISPR enzyme is the type V CRISPR enzyme, in the case of Cpf1, the PAM sequence may be 5'-TTN-3'.

Here, the N may be A, T, G or C; or A, U, G or C.

The CRISPR enzyme capable of recognizing a specific PAM sequence may be manipulated or modified using the PAM sequence capable of being specifically recognized according to species. For example, the PI domain of SpCas9 may be replaced with the PI domain of CjCas9 so as to have the nuclease activity of SpCas9 and recognize a CjCas9-specific PAM sequence, thereby producing SpCas9 recognizing the CjCas9-specific PAM sequence. A specifically recognized PAM sequence may be changed by substitution or replacement of the PI domain.

CRISPR enzyme mutant

The CRISPR enzyme may be modified to improve or inhibit various characteristics such as nuclease activity, helicase activity, an ability to interact with gRNA, and an ability to approach the target gene or nucleic acid, for example, PAM recognizing ability of the CRISPR enzyme.

In addition, the CRISPR enzyme mutant may be a CRISPR enzyme which interacts with gRNA to form a gRNA-CRISPR enzyme complex, that is, a CRISPR complex, and is modified or manipulated to improve target specificity, when approaching or localized to the

target gene or nucleic acid, such that only a double or single strand of the target gene or nucleic acid is cleaved without cleavage of a double or single strand of a non-target gene or nucleic acid which partially forms a complementary bond with gRNA and a non-target gene or nucleic acid which does not form a complementary bond therewith.

Here, an effect of cleaving the double or single strand of the non-target gene or nucleic acid partially forming a complementary bond with gRNA and the non-target gene or nucleic acid not forming a complementary bond therewith is referred to as an off-target effect, a position or base sequence of the non-target gene or nucleic acid partially forming a complementary bond with gRNA and the non-target gene or nucleic acid not forming a complementary bond therewith is referred to as an off-target. Here, there may be one or more off-targets. One the other hand, the cleavage effect of the double or single strand of the target gene or nucleic acid is referred to as an on-target effect, and a location or target sequence of the target gene or nucleic acid is referred to as an on-target.

The CRISPR enzyme mutant is modified in at least one of the amino acids of a naturally-occurring CRISPR enzyme, and may be modified, for example, improved or inhibited in one or more of the various characteristics such as nuclease activity, helicase activity, an ability to interact with gRNA, an ability to approach the target gene or nucleic acid and target specificity, compared to the unmodified CRISPR enzyme. Here, the modification may be substitution, removal, addition of an amino acid, or a mixture thereof.

In the CRISPR enzyme mutant,

the modification may be a modification of one or two or more amino acids located in a region consisting of amino acids having positive charges, present in the naturally-occurring CRISPR enzyme.

For example, the modification may be a modification of one or two or more amino acids of the positively-charged amino acids such as lysine (K), arginine (R) and histidine (H), present in the naturally-occurring CRISPR enzyme.

The modification may be a modification of one or two or more amino acids located in a region composed of non-positively-charged amino acids present in the naturally-occurring CRISPR enzyme.

For example, the modification may be a modification of one or two or more amino acids of the non-positively-charged amino acids, that is, aspartic acid (D), glutamic acid (E), serine (S), threonine (T), asparagine (N), glutamine (Q), cysteine (C), proline (P), glycine (G), alanine (A), valine (V), isoleucine (I), leucine (L), methionine (M), phenylalanine (F), tyrosine (Y) and tryptophan (W), present in the naturally-occurring CRISPR enzyme.

In another example, the modification may be a modification of one or two or more amino acids of non-charged amino acids, that is, serine (S), threonine (T), asparagine (N), glutamine (Q), cysteine (C), proline (P), glycine (G), alanine (A), valine (V), isoleucine (I), leucine (L), methionine (M), phenylalanine (F), tyrosine (Y) and tryptophan (W), present in the naturally-occurring CRISPR enzyme.

In addition, the modification may be a modification of one or two or more of the amino acids having hydrophobic residues present in the naturally-occurring CRISPR enzyme.

For example, the modification may be a modification of one or two or more amino acids of glycine (G), alanine (A), valine (V), isoleucine (I), leucine (L), methionine (M), phenylalanine (F), tyrosine (Y) and tryptophan (W), present in the naturally-occurring CRISPR enzyme.

The modification may be a modification of one or two or more of the amino acids having polar residues, present in the naturally-occurring CRISPR enzyme.

For example, the modification may be a modification of one or two or more amino acids of serine (S), threonine (T), asparagine (N), glutamine (Q), cysteine (C), proline (P), lysine (K), arginine (R), histidine (H), aspartic acid (D) and glutamic acid (E), present in the naturally-occurring CRISPR enzyme.

In addition, the modification may be a modification of one or two or more of the amino acids including lysine (K), arginine (R) and histidine (H), present in the naturally-occurring CRISPR enzyme.

For example, the modification may be a substitution of one or two or more of the amino acids including lysine (K), arginine (R) and histidine (H), present in the naturally-occurring CRISPR enzyme.

The modification may be a modification of one or two or more of the amino acids including aspartic acid (D) and glutamic acid (E), present in the naturally-occurring CRISPR enzyme.

For example, the modification may be a substitution of one or two or more of the amino acids including aspartic acid (D) and glutamic acid (E), present in the naturally-occurring CRISPR enzyme.

The modification may be a modification of one or two or more of the amino acids including serine (S), threonine (T), asparagine (N), glutamine (Q), cysteine (C), proline (P), glycine (G), alanine (A), valine (V), isoleucine (I), leucine (L), methionine (M), phenylalanine (F), tyrosine (Y) and tryptophan (W), present in the naturally-occurring CRISPR enzyme.

For example, the modification may be a substitution of one or two or more of the amino acid including serine (S), threonine (T), asparagine (N), glutamine (Q), cysteine (C), proline (P), glycine (G), alanine (A), valine (V), isoleucine (I), leucine (L), methionine (M),

phenylalanine (F), tyrosine (Y) and tryptophan (W), present in the naturally-occurring CRISPR enzyme.

In addition, the modification may be a modification of one, two, three, four, five, six, seven or more of the amino acids present in the naturally-occurring CRISPR enzyme.

In addition, in the CRISPR enzyme mutant,

the modification may be a modification of one or two or more of the amino acids present in the RuvC domain of the CRISPR enzyme. Here, the RuvC domain may be an RuvCI, RuvCII or RuvCIII domain.

The modification may be a modification of one or two or more of the amino acids present in the HNH domain of the CRISPR enzyme.

The modification may be a modification of one or two or more of the amino acids present in the REC domain of the CRISPR enzyme.

The modification may be one or two or more of the amino acids present in the PI domain of the CRISPR enzyme.

The modification may be a modification of two or more of the amino acids contained in at least two or more domains of the REC, RuvC, HNH and PI domains of the CRISPR enzyme.

In one example, the modification may be a modification of two or more of the amino acids contained in the REC and RuvC domains of the CRISPR enzyme.

In one exemplary embodiment, in the SpCas9 mutant, the modification may be a modification of at least two or more of the A203, H277, G366, F539, I601, M763, D965 and F1038 amino acids contained in the REC and RuvC domains of SpCas9.

In another example, the modification may be a modification of two or more of the amino acids contained in the REC and HNH domains of the CRISPR enzyme.

In one exemplary embodiment, in the SpCas9 mutant, the modification may be a modification of at least two or more of the A203, H277, G366, F539, I601 and K890 amino acids contained in the REC and HNH domains of SpCas9.

In one example, the modification may be a modification of two or more of the amino acids contained in the REC and PI domains of the CRISPR enzyme.

In one exemplary embodiment, in the SpCas9 mutant, the modification may be a modification of at least two or more of the A203, H277, G366, F539, I601, T1102 and D1127 amino acids contained in the REC and PI domains of SpCas9.

In another example, the modification may be a modification of three or more of the amino acids contained in the REC, RuvC and HNH domains of the CRISPR enzyme.

In one exemplary embodiment, in the SpCas9 mutant, the modification may be a modification of at least three or more of the A203, H277, G366, F539, I601, M763, K890, D965 and F1038 amino acids contained in the REC, RuvC and HNH domains of SpCas9.

In one example, the modification may be a modification of three or more of the amino acids contained in the REC, RuvC and PI domains contained in the CRISPR enzyme.

In one exemplary embodiment, in the SpCas9 mutant, the modification may be a modification of at least three or more of the A203, H277, G366, F539, I601, M763, D965, F1038, T1102 and D1127 amino acids contained in the REC, RuvC and PI domains of SpCas9.

In another example, the modification may be a modification of three or more of the amino acids contained in the REC, HNH and PI domains of the CRISPR enzyme.

In one exemplary embodiment, in the SpCas9 mutant, the modification may be a modification of at least three or more of the A203, H277, G366, F539, I601, K890, T1102 and D1127 amino acids contained in the REC, HNH and PI domains of SpCas9.

In one example, the modification may be a modification of three or more of the amino acids contained in the RuvC, HNH and PI domains of the CRISPR enzyme.

In one exemplary embodiment, in the SpCas9 mutant, the modification may be a modification of at least three or more of the M763, K890, D965, F1038, T1102 and D1127 amino acids contained in the RuvC, HNH and PI domains of SpCas9.

In another example, the modification may be a modification of four or more of the amino acids contained in the REC, RuvC, HNH and PI domains of the CRISPR enzyme.

In one exemplary embodiment, in the SpCas9 mutant, the modification may be a modification of at least four or more of the A203, H277, G366, F539, I601, M763, K890, D965, F1038, T1102 and D1127 amino acids contained in the REC, RuvC, HNH and PI domains of SpCas9.

In addition, in the CRISPR enzyme mutant,

the modification may be a modification of one or two or more of the amino acids participating in the nuclease activity of the CRISPR enzyme.

For example, in the SpCas9 mutant, the modification may be a modification of one or two or more of the group consisting of the amino acids D10, E762, H840, N854, N863 and D986, or one or two or more of the group consisting of the amino acids corresponding to other Cas9 orthologs.

The modification may be a modification for partially inactivating the nuclease activity of the CRISPR enzyme, and such a CRISPR enzyme mutant may be a nickase.

Here, the modification may be a modification for inactivating the nuclease activity of the RuvC domain of the CRISPR enzyme, and such a CRISPR enzyme mutant may not cleave a non-complementary strand of a target gene or nucleic acid, that is, a strand which does not form a complementary bond with gRNA.

In one exemplary embodiment, in the case of SpCas9, when residue 10 of the amino acid sequence of SpCas9 is mutated from aspartic acid to alanine, that is, when mutated to D10A, the nuclease activity of the RuvC domain is inactivated, and thus the SpCas9 may be used as a nickase. The nickase produced thereby may not cleave a non-complementary strand of the target gene or nucleic acid, that is, a strand that does not form a complementary bond with gRNA.

In another exemplary embodiment, in the case of CjCas9, when residue 8 of the amino acid sequence of CjCas9 is mutated from aspartic acid to alanine, that is, when mutated to D8A, the nuclease activity of the RuvC domain is inactivated, and thus the CjCas9 may be used as a nickase. The nickase produced thereby may not cleave a non-complementary strand of the target gene or nucleic acid, that is, a strand that does not form a complementary bond with gRNA.

In addition, here, the modification may be a modification for inactivating the nuclease activity of the HNH domain of the CRISPR enzyme, and such a CRISPR enzyme mutant may not cleave a complementary strand of the target gene or nucleic acid, that is, a strand forming a complementary bond with gRNA.

In one exemplary embodiment, in the case of SpCas9, when residue 840 of the amino acid sequence of SpCas9 is mutated from histidine to alanine, that is, when mutated to H840A, the nuclease activity of the HNH domain is inactivated, and thus the SpCas9 may be used as a nickase. The nickase produced thereby may not cleave a complementary strand of the target gene or nucleic acid, that is, a strand that forms a complementary bond with gRNA.

In another exemplary embodiment, in the case of CjCas9, when residue 559 of the amino acid sequence of CjCas9 is mutated from histidine to alanine, that is, when mutated to H559A, the nuclease activity of the HNH domain is inactivated, and thus the CjCas9 may be used as a nickase. The nickase produced thereby may not cleave a complementary strand of the target gene or nucleic acid, that is, a strand that forms a complementary bond with gRNA.

In addition, the modification may be a modification for completely inactivating the nuclease activity of the CRISPR enzyme, and such a CRISPR enzyme mutant may be an inactive CRISPR enzyme.

Here, the modification may be a modification for inactivating the nuclease activities of the RuvC and HNH domains of the CRISPR enzyme, and such a CRISPR enzyme mutant may does not cleave a double strand of the target gene or nucleic acid.

In one exemplary embodiment, in the case of SpCas9, when the residues 10 and 840 in the amino acid sequence of SpCas9 are mutated from aspartic acid and histidine to alanine, that is, mutated to D10A and H840A, respectively, the nuclease activities of the RuvC domain and the HNH domain are inactivated, the double strand of the target gene or nucleic acid may not be completely cleaved.

In another exemplary embodiment, in the case of CjCas9, when residues 8 and 559 of the amino acid sequence of CjCas9 are mutated from aspartic acid and histidine to alanine, that is, mutated to D8A and H559A, respectively, the nuclease activities by the RuvC and HNH domains are inactivated, and thus the double strand of the target gene or nucleic acid may not be completely cleaved.

In addition, the CRISPR enzyme mutant may further include an optionally functional domain, in addition to the innate characteristics of the CRISPR enzyme, and such a CRISPR enzyme mutant may have an additional characteristic in addition to the innate characteristics.

Here, the functional domain may be a domain having methylase activity, demethylase activity, transcription activation activity, transcription repression activity, transcription release factor activity, histone modification activity, RNA cleavage activity or nucleic acid binding activity, or a tag or reporter gene for isolating and purifying a protein (including a peptide), but the present invention is not limited thereto.

The functional domain, peptide, polypeptide or protein may be a deaminase.

For example, an incomplete or partial CRISPR enzyme may additionally include a cytidine deaminase as a functional domain. In one exemplary embodiment, a cytidine deaminase, for example, apolipoprotein B editing complex 1 (APOBEC1) may be added to SpCas9 nickase, thereby producing a fusion protein. The [SpCas9 nickase]-[APOBEC1] formed thereby may be used in base repair or editing of C into T or U, or G into A.

The tag includes a histidine (His) tag, a V5 tag, a FLAG tag, an influenza hemagglutinin (HA) tag, a Myc tag, a VSV-G tag and a thioredoxin (Trx) tag, and the reporter gene includes glutathione-S-transferase (GST), horseradish peroxidase (HRP), chloramphenicol acetyltransferase (CAT) β-galactosidase, β-glucoronidase, luciferase, autofluorescent proteins including the green fluorescent protein (GFP), HcRed, DsRed, cyan fluorescent protein (CFP), yellow fluorescent protein (YFP) and blue fluorescent protein (BFP), but the present invention is not limited thereto.

In addition, the functional domain may be a nuclear localization sequence or signal (NLS) or a nuclear export sequence or signal (NES).

In one example, the CRISPR enzyme may include one or more NLSs. Here, one or more NLSs may be included at an N-terminus of an CRISPR enzyme or the proximity thereof; a C-terminus of the enzyme or the proximity thereof; or a combination thereof. The NLS may be an NLS sequence derived from the following NLSs, but the present invention is not limited thereto: NLS of a SV40 virus large T-antigen having the amino acid sequence

PKKKRKV; NLS from nucleoplasmin (e.g., nucleoplasmin bipartite NLS having the sequence KRPAATKKAGQAKKKK); c-myc NLS having the amino acid sequence PAAKRVKLD or RQRRNELKRSP; hRNPA1 M9 NLS having the sequence NQSSNFGPMKGGNFGGRSSGPYGGGGQYFAKPRNQGGY; the sequence RMRIZFKNKGKDTAELRRRRVEVSVELRKAKKDEQILKRRNV of the IBB domain from importin-α; the sequences VSRKRPRP and PPKKARED of a myoma T protein; the sequence POPKKKPL of human p53; the sequence SALIKKKKKMAP of mouse c-abl IV; the sequences DRLRR and PKQKKRK of influenza virus NS1; the sequence RKLKKKIKKL of a hepatitis delta virus antigen; the sequence REKKKFLKRR of a mouse Mx1 protein; the sequence KRKGDEVDGVDEVAKKKSKK of a human poly (ADP-ribose) polymerase; or the NLS sequence RKCLQAGMNLEARKTKK, derived from a sequence of a steroid hormone receptor (human) glucocorticoid.

In addition, the CRISPR enzyme mutant may include a split-type CRISPR enzyme prepared by dividing the CRISPR enzyme into two or more parts. The term “split” refers to functional or structural division of a protein or random division of a protein into two or more parts.

Here, the split-type CRISPR enzyme may be a completely, incompletely or partially active enzyme or inactive enzyme.

For example, the SpCas9 may be divided into two parts between the residue 656, tyrosine, and the residue 657, threonine, thereby generating split SpCas9.

In addition, the split-type CRISPR enzyme may selectively include an additional domain, peptide, polypeptide or protein for reconstitution.

Here, the “reconstitution” refers to formation of the split-type CRISPR enzyme to be structurally the same or similar to the wild-type CRISPR enzyme.

The additional domain, peptide, polypeptide or protein for reconstitution may be FRB and FKBP dimerization domains; intein; ERT and VPR domains; or domains which form a heterodimer under specific conditions.

For example, the SpCas9 may be divided into two parts between the residue 713, serine, and the residue 714, glycine, thereby generating split SpCas9. The FRB domain may be connected to one of the two parts, and the FKBP domain may be connected to the other one. In the split SpCas9 produced thereby, the FRB domain and the FKBP domain may be formed in a dimer in an environment in which rapamycine is present, thereby producing a reconstituted CRISPR enzyme.

The CRISPR enzyme or CRISPR enzyme mutant described in the present invention may be a polypeptide, protein or nucleic acid having a sequence encoding the same, and may be codon-optimized for a subject to introduce the CRISPR enzyme or CRISPR enzyme mutant.

The term “codon optimization” refers to a process of modifying a nucleic acid sequence by maintaining a native amino acid sequence while replacing at least one codon of the native sequence with a codon more frequently or the most frequently used in host cells so as to improve expression in the host cells. A variety of species have a specific bias to a specific codon of a specific amino acid, and the codon bias (the difference in codon usage between organisms) is frequently correlated with efficiency of the translation of mRNA, which is considered to be dependent on the characteristic of a translated codon and availability of a specific tRNA molecule. The dominance of tRNA selected in cells generally reflects codons most frequently used in peptide synthesis. Therefore, a gene may be customized by optimal gene expression in a given organism based on codon optimization.

3. Target sequence

The term “target sequence” is a base sequence present in a target gene or nucleic acid, and has complementarity to a guide sequence contained in a guide domain of a guide nucleic acid. The target sequence is a base sequence which may vary according to a target gene or nucleic acid, that is, a subject for gene manipulation or correction, which may be designed in various forms according to the target gene or nucleic acid.

The target sequence may form a complementary bond with the guide sequence contained in the guide domain of the guide nucleic acid, and a length of the target sequence may be the same as that of the guide sequence.

The target sequence may be a 5 to 50-base sequence.

In an embodiment, the target sequence may be a 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25-base sequence.

The target sequence may be a nucleic acid sequence complementary to the guide sequence contained in the guide domain of the guide nucleic acid, which has, for example, at least 70%, 75%, 80%, 85%, 90% or 95% or more complementarity or complete complementarity.

In one example, the target sequence may be or include a 1 to 8-base sequence, which is not complementary to the guide sequence contained in the guide domain of the guide nucleic acid.

In addition, the target sequence may be a base sequence adjacent to a nucleic acid sequence that is able to be recognized by an editor protein.

In one example, the target sequence may be a continuous 5 to 50-base sequence adjacent to the 5’ end and/or 3’ end of the nucleic acid sequence that is able to be recognized by the editor protein.

In one exemplary embodiment, target sequences for a gRNA-CRISPR enzyme complex will be described below.

When the target gene or nucleic acid is targeted by the gRNA-CRISPR enzyme complex,

the target sequence has complementarity to the guide sequence contained in the guide domain of gRNA. The target sequence is a base sequence which varies according to the target gene or nucleic acid, that is, a subject for gene manipulation or correction, which may be designed in various forms according to the target gene or nucleic acid.

In addition, the target sequence may be a base sequence adjacent to a PAM sequence which is able to be recognized by the CRISPR enzyme, that is, Cas9 or Cpf1.

In one example, the target sequence may be a continuous 5 to 50-base sequence adjacent to the 5’ end and/or 3’ end of the PAM sequence which is recognized by the CRISPR enzyme.

In one exemplary embodiment, when the CRISPR enzyme is SpCas9, the target sequence may be a continuous 16 to 25-base sequence adjacent to the 5’ end and/or 3’ end of a 5’-NGG-3’, 5’-NAG-3’ and/or 5’-NGA-3’ (N= A, T, G or C; or A, U, G or C) sequence.

In another exemplary embodiment, when the CRISPR enzyme is StCas9, the target sequence may be a continuous 16 to 25-base sequence adjacent to the 5’ end and/or 3’ end of a 5’-NGGNG-3’ and/or 5’-NNAGAAW-3’ (W = A or T, and N= A, T, G or C; or A, U, G or C) sequence.

In still another exemplary embodiment, when the CRISPR enzyme is NmCas9, the target sequence may be a continuous 16 to 25-base sequence adjacent to the 5’ end and/or 3’ end of a 5’-NNNNGATT-3’ and/or 5’-NNNGCTT-3’ (N= A, T, G or C; or A, U, G or C) sequence.

In one exemplary embodiment, when the CRISPR enzyme is CjCas9, the target sequence may be a continuous 16 to 25-base sequence adjacent to the 5' end and/or 3' end of a 5'-NNNVRYAC-3' (V = G, C or A; R = A or G, Y = C or T, N= A, T, G or C; or A, U, G or C) sequence.

In another exemplary embodiment, when the CRISPR enzyme is SmCas9, the target sequence may be a continuous 16 to 25-base sequence adjacent to the 5' end and/or 3' end of a 5'-NGG-3' and/or 5'-NAAR-3'(R = A or G, N= A, T, G or C; or A, U, G or C) sequence.

In yet another exemplary embodiment, when the CRISPR enzyme is SaCas9, the target sequence may be a continuous 16 to 25-base sequence adjacent to the 5' end and/or 3' end of a 5'-NNGRR-3', 5'-NNGRRT-3' and/or 5'-NNGRRV-3' (R = A or G, V = G, C or A, N= A, T, G or C; or A, U, G or C) sequence.

In one exemplary embodiment, when the CRISPR enzyme is Cpf1, the target sequence may be a continuous 16 to 25-base sequence adjacent to the 5' end and/or 3' end of a 5'-TTN-3' (N= A, T, G or C; or A, U, G or C) sequence.

In one exemplary embodiment of the present invention, the target sequence may be a nucleic acid sequence contained in one or more genes selected from the group consisting of a VEGFA gene, an HIF1A gene, an ANGPT2 gene, an EPAS1 gene, and an ANGPTL4 gene.

The target sequence may be a nucleic acid sequence contained in the VEGFA gene.

The target sequence may be a nucleic acid sequence contained in the HIF1A gene.

The target sequence may be a nucleic acid sequence contained in the ANGPT2 gene.

The target sequence may be a nucleic acid sequence contained in the EPAS1 gene.

The target sequence may be a nucleic acid sequence contained in the ANGPTL4 gene.

Alternatively,

the target sequence may be a partial nucleic acid sequence of one or more genes selected from the group consisting of a VEGFA gene, an HIF1A gene, an ANGPT2 gene, an EPAS1 gene, and an ANGPTL4 gene.

The target sequence may be a partial nucleic acid sequence of the VEGFA gene.

The target sequence may be a partial nucleic acid sequence of the HIF1A gene.

The target sequence may be a partial nucleic acid sequence of the ANGPT2 gene.

The target sequence may be a partial nucleic acid sequence of the EPAS1 gene.

The target sequence may be a partial nucleic acid sequence of the ANGPTL4 gene.

Alternatively, the target sequence may be a nucleic acid sequence of the coding or non-coding region or a mixture thereof of one or more genes selected from the group consisting of a VEGFA gene, an HIF1A gene, an ANGPT2 gene, an EPAS1 gene, and an ANGPTL4 gene.

The target sequence may be a nucleic acid sequence of the coding or non-coding region or a mixture thereof of the VEGFA gene.

The target sequence may be a nucleic acid sequence of the coding or non-coding region or a mixture thereof of the HIF1A gene.

The target sequence may be a nucleic acid sequence of the coding or non-coding region or a mixture thereof of the ANGPT2 gene.

The target sequence may be a nucleic acid sequence of the coding or non-coding region or a mixture thereof of the EPAS1 gene.

The target sequence may be a nucleic acid sequence of the coding or non-coding region or a mixture thereof of the ANGPTL4 gene.

Alternatively, the target sequence may be a nucleic acid sequence of the promoter, enhancer, 3’UTR or polyadenyl (polyA) region or a mixture thereof of one or more genes

selected from the group consisting of a VEGFA gene, an HIF1A gene, an ANGPT2 gene, an EPAS1 gene, and an ANGPTL4 gene.

The target sequence may be a nucleic acid sequence of the promoter, enhancer, 3'UTR or polyadenyl (polyA) region or a mixture thereof of the VEGFA gene.

The target sequence may be a nucleic acid sequence of the promoter, enhancer, 3'UTR or polyadenyl (polyA) region or a mixture thereof of the HIF1A gene.

The target sequence may be a nucleic acid sequence of the promoter, enhancer, 3'UTR or polyadenyl (polyA) region or a mixture thereof of the ANGPT2 gene.

The target sequence may be a nucleic acid sequence of the promoter, enhancer, 3'UTR or polyadenyl (polyA) region or a mixture thereof of the EPAS1 gene.

The target sequence may be a nucleic acid sequence of the promoter, enhancer, 3'UTR or polyadenyl (polyA) region or a mixture thereof of the ANGPTL4 gene.

Alternatively, the target sequence may be a nucleic acid sequence of an exon, an intron or a mixture thereof of one or more genes selected from the group consisting of a VEGFA gene, an HIF1A gene, an ANGPT2 gene, an EPAS1 gene, and an ANGPTL4 gene.

The target sequence may be a nucleic acid sequence of an exon, an intron or a mixture thereof of the VEGFA gene.

The target sequence may be a nucleic acid sequence of an exon, an intron or a mixture thereof of the HIF1A gene.

The target sequence may be a nucleic acid sequence of an exon, an intron or a mixture thereof of the ANGPT2 gene.

The target sequence may be a nucleic acid sequence of an exon, an intron or a mixture thereof of the EPAS1 gene.

The target sequence may be a nucleic acid sequence of an exon, an intron or a mixture thereof of the ANGPTL4 gene.

Alternatively, The target sequence may be a nucleic acid sequence including or adjacent to a mutated region (e.g., a region different from a wild-type gene) of one or more genes selected from the group consisting of a VEGFA gene, an HIF1A gene, an ANGPT2 gene, an EPAS1 gene, and an ANGPTL4 gene.

The target sequence may be a nucleic acid sequence including or adjacent to a mutated region of the VEGFA gene.

The target sequence may be a nucleic acid sequence including or adjacent to a mutated region of the HIF1A gene.

The target sequence may be a nucleic acid sequence including or adjacent to a mutated region of the ANGPT2 gene.

The target sequence may be a nucleic acid sequence including or adjacent to a mutated region of the EPAS1 gene.

The target sequence may be a nucleic acid sequence including or adjacent to a mutated region of the ANGPTL4 gene.

Alternatively, the target sequence may be a continuous 5 to 50-nucleic acid sequence of one or more genes selected from the group consisting of a VEGFA gene, an HIF1A gene, an ANGPT2 gene, an EPAS1 gene, and an ANGPTL4 gene.

The target sequence may be a continuous 5 to 50-nucleic acid sequence of the VEGFA gene.

The target sequence may be a continuous 5 to 50-nucleic acid sequence of the HIF1A gene.

The target sequence may be a continuous 5 to 50-nucleic acid sequence of the ANGPT2 gene.

The target sequence may be a continuous 5 to 50-nucleic acid sequence of the EPAS1 gene.

The target sequence may be a continuous 5 to 50-nucleic acid sequence of the ANGPTL4 gene.

As one exemplary embodiment of the present invention, the above target sequences of the VEGFA gene, HIF1A gene, ANGPT2 gene, EPAS1 gene, and ANGPTL4 gene are summarized in Table 1, Table 2, Table 3, Table 4 and Table 5.

Neovascularization-associated factor-manipulated product

4. Guide nucleic acid-editor protein complex and use thereof

A guide nucleic acid-editor protein complex may modify a target.

The target may be a target nucleic acid, gene, chromosome or protein.

For example, the guide nucleic acid-editor protein complex may be used to ultimately regulate (e.g., inhibit, suppress, reduce, increase or promote) the expression of a protein of interest, remove a protein, regulate (e.g., inhibit, suppress, reduce, increase or promote) protein activity, or express a new protein.

Here, the guide nucleic acid-editor protein complex may act at a DNA, RNA, gene or chromosomal level.

For example, the guide nucleic acid-editor protein complex may regulate (e.g., inhibit, suppress, reduce, increase or promote) the expression of a protein encoded by target DNA, remove a protein, regulate (e.g., inhibit, suppress, reduce, increase or promote) protein activity, or express a modified protein through manipulation or modification of the target DNA.

In another example, the guide nucleic acid-editor protein complex may regulate (e.g., inhibit, suppress, reduce, increase or promote) the expression of a protein encoded by target DNA, remove a protein, regulate (e.g., inhibit, suppress, reduce, increase or promote) protein activity, or express a modified protein through manipulation or modification of target RNA.

In one example, the guide nucleic acid-editor protein complex may regulate (e.g., inhibit, suppress, reduce, increase or promote) the expression of a protein encoded by target DNA, remove a protein, regulate (e.g., inhibit, suppress, reduce, increase or promote) protein activity, or express a modified protein through manipulation or modification of a target gene.

In another example, the guide nucleic acid-editor protein complex may regulate (e.g., inhibit, suppress, reduce, increase or promote) the expression of a protein encoded by target DNA, remove a protein, regulate (e.g., inhibit, suppress, reduce, increase or promote) protein activity, or express a modified protein through manipulation or modification of a target chromosome.

The guide nucleic acid-editor protein complex may act at gene transcription and translation stages.

In one example, the guide nucleic acid-editor protein complex may promote or suppress the transcription of a target gene, thereby regulating (e.g., inhibiting, suppressing, reducing, increasing or promoting) the expression of a protein encoded by the target gene.

In another example, the guide nucleic acid-editor protein complex may promote or suppress the translation of a target gene, thereby regulating (e.g., inhibiting, suppressing, reducing, increasing or promoting) the expression of a protein encoded by the target gene.

The guide nucleic acid-editor protein complex may act at a protein level.

In one example, the guide nucleic acid-editor protein complex may manipulate or modify a target protein, thereby removing the target protein or regulating (e.g., inhibiting, suppressing, reducing, increasing or promoting) protein activity.

In one exemplary embodiment, the present invention provides a guide nucleic acid-editor protein complex used to manipulate a neovascularization-associated factor, for example, a VEGFA gene, an HIF1A gene, an ANGPT2 gene, an EPAS1 gene, and/or an ANGPTL4 gene. Preferably, a gRNA-CRISPR enzyme complex is provided.

Particularly, the present invention may provide gRNA including a guide domain capable of forming a complementary bond with a target sequence from a gene, for example, isolated or non-natural gRNA and DNA encoding the same. The gRNA and the DNA sequence encoding the same may be designed to be able to complementarily bind to a target sequence listed in Table 1, Table 2, Table 3, Table 4 and Table 5.

In addition, a target region of the gRNA is designed to provide a third gene, which has a nucleic acid modification, for example, double or single strand breaks; or a specific function at a target site in a VEGFA gene, an HIF1A gene, an ANGPT2 gene, an EPAS1 gene, and/or an ANGPTL4 gene.

In addition, when two or more gRNAs are used to induce two or more cleaving events in a target gene, for example, a double or single strand break, the two or more cleaving events may occur due to the same or different Cas9 proteins.

The gRNA may target, for example, two or more of the VEGFA gene, the HIF1A gene, the ANGPT2 gene, the EPAS1 gene, and/or the ANGPTL4 gene, or

two or more regions in each of the VEGFA gene, HIF1A gene, ANGPT2 gene, EPAS1 gene, and/or ANGPTL4 gene, and

may independently induce the cleavage of a double strand and/or a single strand of the VEGFA gene, HIF1A gene, ANGPT2 gene, EPAS1 gene, and/or ANGPTL4 gene, or

may induce the insertion of one foreign nucleotide into a cleavage site of the VEGFA gene, the HIF1A gene, the ANGPT2 gene, the EPAS1 gene, and/or the ANGPTL4 gene.

In addition, in another exemplary embodiment of the present invention, a nucleic acid constituting the guide nucleic acid-editor protein complex may include:

(a) a sequence encoding a guide nucleic acid including a guide domain, which is complementary to a target sequence of the VEGFA gene, the HIF1A gene, the ANGPT2 gene, the EPAS1 gene, and/or the ANGPTL4 gene as described herein; and

(b) a sequence encoding an editor protein.

Here, there may be two or more of the (a) according to a target region, and the (b) may employ the same or two or more editor proteins.

In an embodiment, the nucleic acid may be designed to target an enzymatically inactive editor protein or a fusion protein (e.g., a transcription repressor domain fusion) thereof to place it sufficiently adjacent to a knockdown target site in order to reduce, decrease or inhibit expression of the VEGFA gene, the HIF1A gene, the ANGPT2 gene, the EPAS1 gene, and/or the ANGPTL4 gene.

Besides, it should be obvious that the above-described structure, function, and all applications of the guide nucleic acid-editor protein complex will be utilized in manipulation of the VEGFA gene, the HIF1A gene, the ANGPT2 gene, the EPAS1 gene, and/or the ANGPTL4 gene.

Use of guide nucleic acid-editor protein complex

In an embodiment for the use of the guide nucleic acid-editor protein complex of the present invention, the manipulation or modification of target DNA, RNA, genes or chromosomes using the gRNA-CRISPR enzyme complex will be described below.

Gene manipulation

A target gene or nucleic acid may be manipulated or corrected using the above-described gRNA-CRISPR enzyme complex, that is, the CRISPR complex. Here, the manipulation or correction of the target gene or nucleic acid includes all of the stages of i) cleaving or damaging the target gene or nucleic acid and ii) repairing the damaged target gene or nucleic acid.

i) Cleavage or damage of target gene or nucleic acid

i) The cleavage or damage of the target gene or nucleic acid may be cleavage or damage of the target gene or nucleic acid using the CRISPR complex, and particularly, cleavage or damage of a target sequence in the target gene or nucleic acid.

In one example, the cleavage or damage of the target gene or nucleic acid using the CRISPR complex may be complete cleavage or damage to the double strand of a target sequence.

In one exemplary embodiment, when wild-type SpCas9 is used, the double strand of a target sequence forming a complementary bond with gRNA may be completely cleaved.

In another exemplary embodiment, when SpCas9 nickase (D10A) and SpCas9 nickase (H840A) are used, a complementary single strand of a target sequence forming a complementary bond with gRNA may be cleaved by the SpCas9 nickase (D10A), and a non-complementary single strand of the target sequence forming a complementary bond with gRNA may be cleaved by the SpCas9 nickase (H840A), and the cleavages may take place sequentially or simultaneously.

In still another exemplary embodiment, when SpCas9 nickase (D10A) and SpCas9 nickase (H840A), and two gRNAs having different target sequences are used, a complementary single strand of a target sequence forming a complementary bond with the first gRNA may be cleaved by the SpCas9 nickase (D10A), a non-complementary single strand of a target sequence forming a complementary bond with the second gRNS may be cleaved by the SpCas9 nickase (H840A), and the cleavages may take place sequentially or simultaneously.

In another example, the cleavage or damage of a target gene or nucleic acid using the CRISPR complex may be cleavage or damage to only the single strand of a target sequence.

Here, the single strand may be a complementary single strand of a target sequence forming a complementary bond with gRNA, or a non-complementary single strand of the target sequence forming a complementary bond with gRNA.

In one exemplary embodiment, when SpCas9 nickase (D10A) is used, a complementary single strand of a target sequence forming a complementary bond with gRNA may be cleaved by the SpCas9 nickase (D10A), but a non-complementary single strand of the target sequence forming a complementary bond with gRNA may not be cleaved.

In another exemplary embodiment, when SpCas9 nickase (H840A) is used, a complementary single strand of a target sequence forming a complementary bond with gRNA may be cleaved by the SpCas9 nickase (H840A), but a non-complementary single strand of the target sequence forming a complementary bond with gRNA may not be cleaved.

In yet another example, the cleavage or damage of a target gene or nucleic acid using the CRISPR complex may be partial removal of a nucleic acid fragment.

In one exemplary embodiment, when two gRNAs having different target sequences and wild-type SpCas9 are used, a double strand of a target sequence forming a complementary bond with the first gRNA may be cleaved, and a double strand of a target sequence forming a complementary bond with the second gRNA may be cleaved, resulting in the removal of nucleic acid fragments by the first and second gRNAs and SpCas9.

In another exemplary embodiment, when two gRNAs having different target sequences, wild-type SpCas9, SpCas9 nickase (D10A) and SpCas9 nickase (H840A) are used, a double strand of a target sequence forming a complementary bond with the first gRNA may be cleaved by the wild-type SpCas9, a complementary single strand of a target sequence forming a complementary bond with the second gRNA may be cleaved by the SpCas9 nickase (D10A), and a non-complementary single strand nay be cleaved by the SpCas9

nickase (H840A), resulting in the removal of nucleic acid fragments by the first and second gRNAs, the wild-type SpCas9, the SpCas9 nickase (D10A) and the SpCas9 nickase (H840A).

In still another exemplary embodiment, when two gRNAs having different target sequences, SpCas9 nickase (D10A) and SpCas9 nickase (H840A) are used, a complementary single strand of a target sequence forming a complementary bond with the first gRNA may be cleaved by the SpCas9 nickase (D10A), a non-complementary single strand may be cleaved by the SpCas9 nickase (H840A), a complementary double strand of a target sequence forming a complementary bond with the second gRNA may be cleaved by the SpCas9 nickase (D10A), and a non-complementary single strand may be cleaved by the SpCas9 nickase (H840A), resulting in the removal of nucleic acid fragments by the first and second gRNAs, the SpCas9 nickase (D10A) and the SpCas9 nickase (H840A).

In yet another exemplary embodiment, when three gRNAs having different target sequences, wild-type SpCas9, SpCas9 nickase (D10A) and SpCas9 nickase (H840A) are used, a double strand of a target sequence forming a complementary bond with the first gRNA may be cleaved by the wild-type SpCas9, a complementary single strand of a target sequence forming a complementary bond with the second gRNA may be cleaved by the SpCas9 nickase (D10A), and a non-complementary single strand of a target sequence forming a complementary bond with the third gRNA may be cleaved by the SpCas9 nickase (H840A), resulting in the removal of nucleic acid fragments by the first gRNA, the second gRNA, the third gRNA, the wild-type SpCas9, the SpCas9 nickase (D10A) and the SpCas9 nickase (H840A).

In yet another exemplary embodiment, when four gRNAs having different target sequences, SpCas9 nickase (D10A) and SpCas9 nickase (H840A) are used, a complementary single strand of a target sequence forming a complementary bond with the first gRNA may be cleaved by the SpCas9 nickase (D10A), a non-complementary single strand of a target

sequence forming a complementary bond with the second gRNA may be cleaved by the SpCas9 nickase (H840A), a complementary single strand of a target sequence forming a complementary bond with the third gRNA may be cleaved by the SpCas9 nickase (D10A), and a non-complementary single strand of a target sequence forming a complementary bond with fourth gRNA may be cleaved by the SpCas9 nickase (H840A), resulting in the removal of nucleic acid fragments by the first gRNA, the second gRNA, the third gRNA, the fourth gRNA, the SpCas9 nickase (D10A) and the SpCas9 nickase (H840A).

ii) Repair or restoration of damaged target gene or nucleic acid

The target gene or nucleic acid cleaved or damaged by the CRISPR complex may be repaired or restored through NHEJ and homology-directed repairing (HDR).

Non-homologous end joining (NHEJ)

NHEJ is a method of restoration or repairing double strand breaks in DNA by joining both ends of a cleaved double or single strand together, and generally, when two compatible ends formed by breaking of the double strand (for example, cleavage) are frequently in contact with each other to completely join the two ends, the broken double strand is recovered. The NHEJ is a restoration method that is able to be used in the entire cell cycle, and usually occurs when there is no homologous genome to be used as a template in cells, like the G1 phase.

In the repair process of the damaged gene or nucleic acid using NHEJ, some insertions and/or deletions (indels) in the nucleic acid sequence occur in the NHEJ-repaired region, such insertions and/or deletions cause the leading frame to be shifted, resulting in frame-shifted transcriptome mRNA. As a result, innate functions are lost because of nonsense-mediated decay or the failure to synthesize normal proteins. In addition, while the

leading frame is maintained, mutations in which insertion or deletion of a considerable amount of sequence may be caused to destroy the functionality of the proteins. The mutation is locus-dependent because mutations in a significant functional domain is probably less tolerated than mutations in a non-significant region of a protein.

While it is impossible to expect indel mutations produced by NHEJ in a natural state, a specific indel sequence is preferred in a given broken region, and can come from a small region of micro homology. Conventionally, the deletion length ranges from 1 bp to 50 bp, insertions tend to be shorter, and frequently include a short repeat sequence directly surrounding a broken region.

In addition, the NHEJ is a process causing a mutation, and when it is not necessary to produce a specific final sequence, may be used to delete a motif of the small sequence.

A specific knockout of a gene targeted by the CRISPR complex may be performed using such NHEJ. A double strand or two single strands of a target gene or nucleic acid may be cleaved using the CRISPR enzyme such as Cas9 or Cpf1, and the broken double strand or two single strands of the target gene or nucleic acid may have indels through the NHEJ, thereby inducing specific knockout of the target gene or nucleic acid. Here, the site of a target gene or nucleic acid cleaved by the CRISPR enzyme may be a non-coding or coding region, and in addition, the site of the target gene or nucleic acid restored by NHEJ may be a non-coding or coding region.

Homology directed repairing (HDR)

HDR is a correction method without an error, which uses a homologous sequence as a template to repair or restoration a damaged gene or nucleic acid, and generally, to repair or restoration broken DNA, that is, to restore innate information of cells, the broken DNA is repaired using information of a complementary base sequence which is not modified or

information of a sister chromatid. The most common type of HDR is homologous recombination (HR). HDR is a repair or restoration method usually occurring in the S or G2/M phase of actively dividing cells.

To repair or restore damaged DNA using HDR, rather than using a complementary base sequence or sister chromatin of the cells, a DNA template artificially synthesized using information of a complementary base sequence or homologous base sequence, that is, a nucleic acid template including a complementary base sequence or homologous base sequence may be provided to the cells, thereby repairing the broken DNA. Here, when a nucleic acid sequence or nucleic acid fragment is further added to the nucleic acid template to repair the broken DNA, the nucleic acid sequence or nucleic acid fragment further added to the broken DNA may be subjected to knockin. The further added nucleic acid sequence or nucleic acid fragment may be a nucleic acid sequence or nucleic acid fragment for correcting the target gene or nucleic acid modified by a mutation to a normal gene or nucleic acid, or a gene or nucleic acid to be expressed in cells, but the present invention is not limited thereto.

In one example, a double or single strand of a target gene or nucleic acid may be cleaved using the CRISPR complex, a nucleic acid template including a base sequence complementary to a base sequence adjacent to the cleavage site may be provided to cells, and the cleaved base sequence of the target gene or nucleic acid may be repaired or restored through HDR.

Here, the nucleic acid template including the complementary base sequence may have broken DNA, that is, a cleaved double or single strand of a complementary base sequence, and further include a nucleic acid sequence or nucleic acid fragment to be inserted into the broken DNA. An additional nucleic acid sequence or nucleic acid fragment may be inserted into a cleaved site of the broken DNA, that is, the target gene or nucleic acid using the nucleic acid template including a nucleic acid sequence or nucleic acid fragment to be

inserted into the complementary base sequence. Here, the nucleic acid sequence or nucleic acid fragment to be inserted and the additional nucleic acid sequence or nucleic acid fragment may be a nucleic acid sequence or nucleic acid fragment for correcting a target gene or nucleic acid modified by a mutation to a normal gene or nucleic acid or a gene or nucleic acid to be expressed in cells. The complementary base sequence may be a base sequence having complementary bonds with broken DNA, that is, right and left base sequences of the cleaved double or single strand of the target gene or nucleic acid. Alternatively, the complementary base sequence may be a base sequence having complementary bonds with broken DNA, that is, 3' and 5' ends of the cleaved double or single strand of the target gene or nucleic acid. The complementary base sequence may be a 15 to 3000-base sequence, a length or size of the complementary base sequence may be suitably designed according to a size of the nucleic acid template or the target gene. Here, as the nucleic acid template, a double- or single-stranded nucleic acid may be used, or it may be linear or circular, but the present invention is not limited thereto.

In another example, a double- or single-stranded target gene or nucleic acid is cleaved using the CRISPR complex, a nucleic acid template including a homologous base sequence with a base sequence adjacent to a cleavage site is provided to cells, and the cleaved base sequence of the target gene or nucleic acid may be repaired or restored by HDR.

Here, the nucleic acid template including the homologous base sequence may be broken DNA, that is, a cleaved double- or single-stranded homologous base sequence, and further include a nucleic acid sequence or nucleic acid fragment to be inserted into the broken DNA. An additional nucleic acid sequence or nucleic acid fragment may be inserted into broken DNA, that is, a cleaved site of a target gene or nucleic acid using the nucleic acid template including a homologous base sequence and a nucleic acid sequence or nucleic acid fragment to be inserted. Here, the nucleic acid sequence or nucleic acid fragment to be

inserted and the additional nucleic acid sequence or nucleic acid fragment may be a nucleic acid sequence or nucleic acid fragment for correcting a target gene or nucleic acid modified by a mutation to a normal gene or nucleic acid or a gene or nucleic acid to be expressed in cells. The homologous base sequence may be broken DNA, that is, a base sequence having homology with cleaved double-stranded base sequence or right and left single-stranded base sequences of a target gene or nucleic acid. Alternatively, the complementary base sequence may be a base sequence having homology with broken DNA, that is, the 3' and 5' ends of a cleaved double or single strand of a target gene or nucleic acid. The homologous base sequence may be a 15 to 3000-base sequence, and a length or size of the homologous base sequence may be suitably designed according to a size of the nucleic acid template or a target gene or nucleic acid. Here, as the nucleic acid template, a double- or single-stranded nucleic acid may be used and may be linear or circular, but the present invention is not limited thereto.

Other than the NHEJ and HDR, there are methods of repairing or restoring broken DNA.

Single-strand annealing (SSA)

SSA is a method of repairing double strand breaks between two repeat sequences present in a target nucleic acid, and generally uses a repeat sequence of more than 30 bases. The repeat sequence is cleaved (to have sticky ends) to have a single strand with respect to a double strand of the target nucleic acid at each of the broken ends, and after the cleavage, a single-strand overhang containing the repeat sequence is coated with an RPA protein such that it is prevented from inappropriately annealing the repeat sequences to each other. RAD52 binds to each repeat sequence on the overhang, and a sequence capable of annealing a complementary repeat sequence is arranged. After annealing, a single-stranded flap of the

overhang is cleaved, and synthesis of new DNA fills a certain gap to restore a DNA double strand. As a result of this repair, a DNA sequence between two repeats is deleted, and a deletion length may be dependent on various factors including the locations of the two repeats used herein, and a path or degree of the progress of cleavage.

SSA, similar to HDR, utilizes a complementary sequence, that is, a complementary repeat sequence, and in contrast, does not requires a nucleic acid template for modifying or correcting a target nucleic acid sequence.

Single-strand break repair (SSBA)

Single strand breaks in a genome are repaired through a separate mechanism, SSBR, from the above-described repair mechanisms. In the case of single-strand DNA breaks, PARP1 and/or PARP2 recognizes the breaks and recruits a repair mechanism. PARP1 binding and activity with respect to the DNA breaks are temporary, and SSBR is promoted by promoting the stability of an SSBR protein complex in the damaged regions. The most important protein in the SSBR complex is XRCC1, which interacts with a protein promoting 3' and 5' end processing of DNA to stabilize the DNA. End processing is generally involved in repairing the damaged 3' end to a hydroxylated state, and/or the damaged 5' end to a phosphatic moiety, and after the ends are processed, DNA gap filling takes place. There are two methods for the DNA gap filling, that is, short patch repair and long patch repair, and the short patch repair involves insertion of a single base. After DNA gap filling, a DNA ligase promotes end joining.

Mismatch repair (MMR)

MMR works on mismatched DNA bases. Each of an MSH2/6 or MSH2/3 complex has ATPase activity and thus plays an important role in recognizing a mismatch and initiating

a repair, and the MSH2/6 primarily recognizes base-base mismatches and identifies one or two base mismatches, but the MSH2/3 primarily recognizes a larger mismatch.

Base excision repair (BER)

BER is a repair method which is active throughout the entire cell cycle, and used to remove a small non-helix-distorting base damaged region from the genome. In the damaged DNA, damaged bases are removed by cleaving an N-glycoside bond joining a base to the phosphate-deoxyribose backbone, and then the phosphodiester backbone is cleaved, thereby generating breaks in single-strand DNA. The broken single strand ends formed thereby were removed, a gap generated due to the removed single strand is filled with a new complementary base, and then an end of the newly-filled complementary base is ligated with the backbone by a DNA ligase, resulting in repair of the damaged DNA.

Nucleotide excision repair (NER)

NER is an excision mechanism important for removing large helix-distorting damage from DNA, and when the damage is recognized, a short single-strand DNA segment containing the damaged region is removed, resulting in a single strand gap of 22 to 30 bases. The generated gap is filled with a new complementary base, and an end of the newly filled complementary base is ligated with the backbone by a DNA ligase, resulting in the repair of the damaged DNA.

Gene manipulation effects

Manipulation or correction of a target gene or nucleic acid may largely lead to effects of knockout, knockdown, and knockin.

Knockout

The term "knockout" refers to inactivation of a target gene or nucleic acid, and the "inactivation of a target gene or nucleic acid" refers to a state in which transcription and/or translation of a target gene or nucleic acid does not occur. Transcription and translation of a gene causing a disease or a gene having an abnormal function may be inhibited through knockout, resulting in the prevention of protein expression.

For example, when a target gene or nucleic acid is edited or corrected using a gRNA-CRISPR enzyme complex, that is, a CRISPR complex, the target gene or nucleic acid may be cleaved using the CRISPR complex. The damaged target gene or nucleic acid may be repaired through NHEJ using the CRISPR complex. The damaged target gene or nucleic acid may have indels due to NHEJ, and thereby, specific knockout for the target gene or nucleic acid may be induced.

Knockdown

The term "knockdown" refers to a decrease in transcription and/or translation of a target gene or nucleic acid or the expression of a target protein. The onset of a disease may be prevented or a disease may be treated by regulating the overexpression of a gene or protein through the knockdown.

For example, when a target gene or nucleic acid is edited or corrected using a gRNA-CRISPR inactive enzyme-transcription inhibitory activity domain complex, that is, a CRISPR inactive complex including a transcription inhibitory activity domain, the CRISPR inactive complex may specifically bind to the target gene or nucleic acid, transcription of the target gene or nucleic acid may be inhibited by the transcription inhibitory activity domain included in the CRISPR inactive complex, thereby inducing knockdown in which expression of the corresponding gene or nucleic acid is inhibited.

Knockin

The term “knockin” refers to insertion of a specific nucleic acid or gene into a target gene or nucleic acid, and here, the “specific nucleic acid” refers to a gene or nucleic acid of interest to be inserted or expressed. A mutant gene triggering a disease may be utilized in disease treatment by correction to normal or insertion of a normal gene to induce expression of the normal gene through the knockin.

In addition, the knockin may further need a donor.

For example, when a target gene or nucleic acid is edited or corrected using a gRNA-CRISPR enzyme complex, that is, a CRISPR complex, the target gene or nucleic acid may be cleaved using the CRISPR complex. The target gene or nucleic acid damaged using the CRISPR complex may be repaired through HDR. Here, a specific nucleic acid may be inserted into the damaged gene or nucleic acid using a donor.

The term “donor” refers to a nucleic acid sequence that helps HDR-based repair of the damaged gene or nucleic acid, and here, the donor may include a specific nucleic acid.

The donor may be a double- or single-stranded nucleic acid.

The donor may be present in a linear or circular shape.

The donor may include a nucleic acid sequence having homology with a target gene or nucleic acid.

For example, the donor may include a nucleic acid sequence having homology with each of base sequences at a location into which a specific nucleic acid is to be inserted, for example, upstream (left) and downstream (right) of a damaged nucleic acid. Here, the specific nucleic acid to be inserted may be located between a nucleic acid sequence having homology with a base sequence downstream of the damaged nucleic acid and a nucleic acid sequence having homology with a base sequence upstream of the damaged nucleic acid.

Here, the homologous nucleic acid sequence may have at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95% or more homology or complete homology.

The donor may optionally include an additional nucleic acid sequence. Here, the additional nucleic acid sequence may serve to increase donor stability, knockin efficiency or HDR efficiency.

For example, the additional nucleic acid sequence may be an A, T-rich nucleic acid sequence, that is, an A-T rich domain. In addition, the additional nucleic acid sequence may be a scaffold/matrix attachment region (SMAR).

In one exemplary embodiment relating to a gene manipulation effect of the present invention, a manipulated target gene obtained using a gRNA-CRISPR enzyme complex, that is, a manipulated neovascularization-associated factor may have the following constitution.

In one exemplary embodiment, when the neovascularization-associated factor is a gene,

the constitution of the artificially manipulated neovascularization-associated factor by the gRNA-CRISPR enzyme complex may include modification of one or more nucleic acids among

a deletion or insertion of one or more nucleotides;

a substitution with one or more nucleotides different from a wild-type gene; and

an insertion of one or more foreign nucleotides

in a continuous 1 bp to 50 bp, 1 bp to 40 bp or 1 bp to 30 bp, preferably, 3 bp to 25 bp region in the base sequence, which is located in a PAM sequence in a nucleic acid sequence constituting the neovascularization-associated factor or adjacent to a 5’ end and/or 3’ end thereof.

In addition, a chemical modification of one or more nucleotides may be included in the nucleic acid sequence constituting the neovascularization-associated factor.

Here, the "foreign nucleotide" is the concept including all exogeneous, for example, heterologous or artificially-synthesized nucleotides, other than nucleotides innately included in the neovascularization-associated factor. The foreign nucleotide also includes a nucleotide with a size of several hundred, thousand or tens of thousands of bp to express a protein having a specific function, as well as a small ologonucleotide with a size of 50 bp or less. Such a foreign nucleotide may be a donor.

The chemical modification may include methylation, acetylation, phosphorylation, ubiquitination, ADP-ribosylation, myristylation, and glycosylation, for example, substitution of some functional groups contained in a nucleotide with any one of a hydrogen atom, a fluorine atom, an -O-alkyl group, an -O-acyl group, and an amino group, but the present invention is not limited thereto. In addition, to increase transferability of a nucleic acid molecule, the functional groups may also be substituted with any one of -Br, -Cl, -R, -R'OR, -SH, -SR, -N3 and -CN (R= alkyl, aryl, alkylene). In addition, the phosphate backbone of at least one nucleotide may be substituted with any one of an alkylphosphonate form, a phosphoroamidate form and a boranophosphate form. In addition, the chemical modification may be a substitution of at least one type of nucleotide contained in the nucleic acid molecule with any one of a locked nucleic acid (LNA), an unlocked nucleic acid (UNA), a morpholino, and a peptide nucleic acid (PNA), and the chemical modification may be bonding of the nucleic acid molecule with one or more selected from the group consisting of a lipid, a cell-penetrating peptide and a cell-target ligand.

To form a desired neovascularization regulating system, artificial modification using a gRNA-CRISPR enzyme complex may be applied to the nucleic acid constituting the neovascularization-associated factor.

A region including the nucleic acid modification of the neovascularization-associated factor may be a target region or target sequence.

Such a target sequence may be a target for the gRNA-CRISPR enzyme complex, and the target sequence may include or not include a PAM sequence recognized by the CRISPR enzyme. Such a target sequence may provide a critical standard in a gRNA designing stage to those of ordinary skill in the art.

Such nucleic acid modification includes the "cleavage" of a nucleic acid.

The term "cleavage" in a target region refers to breakage of a covalent backbone of polynucleotides. The cleavage includes enzymatic or chemical hydrolysis of a phosphodiester bond, but the present invention is not limited thereto, and also include various other methods. The cleavage is able to be performed on both of a single strand and a double strand, and the cleavage of a double strand may result from distinct single-strand cleavage. The double-strand cleavage may generate blunt ends or staggered ends.

When an inactivated CRISPR enzyme is used, it may induce a factor possessing a specific function to approach a certain region of the target region or neovascularization-associated factor without the cleavage process. Chemical modification of one or more nucleotides in the nucleic acid sequence of the neovascularization-associated factor may be included according to such a specific function.

In one example, various indels may occur due to target and non-target activities through the nucleic acid cleavage formed by the gRNA-CRISPR enzyme complex.

The term "indel" is the generic term for an insertion or deletion mutation occurring in-between some bases in a DNA base sequence. The indel may be introduced into a target sequence during repair by an HDR or NHEJ mechanism when the gRNA-CRISPR enzyme complex cleaves the nucleic acid (DNA or RNA) of the neovascularization-associated factor as described above.

The artificially manipulated neovascularization-associated factor of the present invention refers to modification of the nucleic acid sequence of an original gene by cleavage, indels, or insertion using a donor of such a nucleic acid, and contributes to a desired neovascularization regulating system, for example, exhibition of an effect of promoting or suppressing neovascularization.

For example,

a specific protein may be expressed and its activity may be stimulated by the artificially manipulated neovascularization-associated factor.

A specific protein may be inactivated by the artificially manipulated neovascularization-associated factor.

In one example, a specific target region of each neovascularization-associated factor of the genome, for example, reverse regulatory genes such as a VEGFA gene, an HIF1A gene, an ANGPT2 gene, an EPAS1 gene, and/or an ANGPTL4 gene may be cleaved, resulting in knockdown or knockout of the gene.

In another example, targeted knockdown may be mediated using an enzymatically inactive CRISPR enzyme fused to a transcription repressor domain or chromatin-modified protein to change transcription, for example, to block, negatively regulate or decrease the transcription of a VEGFA gene, an HIF1A gene, an ANGPT2 gene, an EPAS1 gene, and/or an ANGPTL4 gene.

The neovascularization may be regulated by the artificially manipulated neovascularization-associated factor.

A neovascularization-associated disease may be improved or treated by the artificially manipulated neovascularization-associated factor.

In one exemplary embodiment of the present invention, the artificially manipulated neovascularization-associated factor may provide various artificially manipulated neovascularization-associated factors according to the constitutional characteristic of the gRNA-CRISPR enzyme complex (e.g., included in a target region of the neovascularization-associated factor or different in the adjacent major PAM sequence).

Hereinafter, while representative examples of CRISPR enzymes and a neovascularization-regulatory gene have been illustrated, they are merely specific examples, and thus the present invention is not limited thereto.

For example, when the CRISPR enzyme is a SpCas9 protein, the PAM sequence is 5'-NGG-3' (N is A, T, G, or C), and the cleaved base sequence region (target region) may be a continuous 1 bp to 25 bp, for example, 17 bp to 23 bp or 21 bp to 23 bp, region in the base sequence adjacent to the 5' end and/or 3' end of the 5'-NGG-3' sequence in a target gene.

The present invention may provide an artificially manipulated neovascularization-associated factor, for example, an artificially manipulated VEGFA gene, HIF1A gene, ANGPT2 gene, EPAS1 gene, and/or ANGPTL4 gene, which is prepared by

a) deletion of one or more nucleotides of a continuous 1 bp to 25 bp, for example, 17 bp to 23 bp, region in the base sequence adjacent to the 5' end and/or 3' end of the 5'-NGG-3' (N is A, T, C or G) sequence,

b) substitution of one or more nucleotides of a continuous 1 bp to 25 bp, for example, 17 bp to 23 bp, region in the base sequence adjacent to the 5' end and/or 3' end of the 5'-NGG-3' sequence with nucleotides different from those of the wild-type gene,

c) insertion of one or more nucleotides into a continuous 1 bp to 25 bp, for example, 17 bp to 23 bp, region in the base sequence adjacent to the 5' end and/or 3' end of the 5'-NGG-3' sequence, or

d) a combination of two or more selected from a) through c)

in the nucleic acid sequence of the neovascularization-associated factor.

For example, when the CRISPR enzyme is a CjCas9 protein, the PAM sequence is 5'-NNNNRYAC-3' (each N is independently A, T, C or G, R is A or G, and Y is C or T), and the cleaved base sequence region (target region) may be a continuous 1 bp to 25 bp, for example, 17 bp to 23 bp or 21 bp to 23 bp, region in the base sequence adjacent to the 5' end and/or 3' end of the 5'-NNNNRYAC-3' sequence in a target gene.

The present invention may provide an artificially manipulated neovascularization-associated factor, for example, an artificially manipulated VEGFA gene, HIF1A gene, ANGPT2 gene, EPAS1 gene, and/or ANGPTL4 gene, which is prepared by

a') deletion of one or more nucleotides of a continuous 1 bp to 25 bp, for example, 17 bp to 23 bp, region in the base sequence adjacent to the 5' end and/or 3' end of the 5'-NNNNRYAC-3' (each N is independently A, T, C or G, R is A or G, and Y is C or T),

b') substitution of one or more nucleotides of a continuous 1 bp to 25 bp, for example, 17 bp to 23 bp, region in the base sequence adjacent to the 5' end and/or 3' end of the 5'-NNNNRYAC-3' sequence with nucleotides different from those of the wild-type gene,

c') insertion of one or more nucleotides into a continuous 1 bp to 25 bp, for example, 17 bp to 23 bp, region in the base sequence adjacent to the 5' end and/or 3' end of the 5'-NNNNRYAC-3' sequence, or

d') a combination of two or more selected from a') through c') in the nucleic acid sequence of the neovascularization-associated factor.

For example, when the CRISPR enzyme is a StCas9 protein, the PAM sequence is 5'-NNAGAAW-3' (each N is independently A, T, C or G, and W is A or T), and the cleaved base sequence region (target region) may be a continuous 1 bp to 25 bp, for example, 17 bp to

23 bp or 21 bp to 23 bp, region in the base sequence adjacent to the 5’ end and/or 3’ end of the 5’-NNAGAAW-3’ sequence in a target gene.

The present invention may provide an artificially manipulated neovascularization-associated factor, for example, an artificially manipulated VEGFA gene, HIF1A gene, ANGPT2 gene, EPAS1 gene, and/or ANGPTL4 gene, which is prepared by

a’’) deletion of one or more nucleotides of a continuous 1 bp to 25 bp, for example, 17 bp to 23 bp, region in the base sequence adjacent to the 5’ end of the 5’-NNAGAAW-3’ sequence (each N is independently A, T, C or G, and W is A or T),

b’’) substitution of one or more nucleotides of a continuous 1 bp to 25 bp, for example, 17 bp to 23 bp, region in the base sequence adjacent to the 5’ end and/or 3’ end of the 5’-NNAGAAW-3’ sequence with nucleotides different from those of the wild-type gene,

c’’) insertion of one or more nucleotides into a continuous 1 bp to 25 bp, for example, 17 bp to 23 bp, region in the base sequence adjacent to the 5’ end and/or 3’ end of the 5’-NNAGAAW-3’ sequence, or

d’’) a combination of two or more selected from a’’) through c’’) in the nucleic acid sequence of the neovascularization-associated factor.

For example, when the CRISPR enzyme is an NmCas9 protein, the PAM sequence is 5’-NNNNGATT-3’(each N is independently A, T, C or G), and the cleaved base sequence region (target region) may be a continuous 1 bp to 25 bp, for example, 17 bp to 23 bp or 21 bp to 23 bp, region in the base sequence adjacent to the 5’ end and/or 3’ end of the 5’-NNNNGATT-3’ sequence in a target gene.

The present invention may provide an artificially manipulated neovascularization-associated factor, for example, an artificially manipulated VEGFA gene, HIF1A gene, ANGPT2 gene, EPAS1 gene, and/or ANGPTL4 gene, which is prepared by

a’’’) deletion of one or more nucleotides of a continuous 1 bp to 25 bp, for example, 17 bp to 23 bp, region in the base sequence adjacent to the 5’ end and/or the 3’ end of the 5’-NNNNGATT-3’ sequence (each N is independently A, T, C or G),

b’’’) substitution of one or more nucleotides of a continuous 1 bp to 25 bp, for example, 17 bp to 23 bp, region in the base sequence adjacent to the 5’ end and/or 3’ end of the 5’-NNNNGATT-3’ sequence with nucleotides different from those of the wild-type gene,

c’’’) insertion of one or more nucleotides into a continuous 1 bp to 25 bp, for example, 17 bp to 23 bp, region in the base sequence adjacent to the 5’-NNNNGATT-3’ sequence, or

d’’’) a combination of two or more selected from a’’’) through c’’’) in the nucleic acid sequence of the neovascularization-associated factor.

For example, when the CRISPR enzyme is an SaCas9 protein, the PAM sequence is 5’-NNGRR(T)-3’ (each N is independently A, T, C or G, R is A or G, and (T) is a randomly addable sequence), and the cleaved base sequence region (target region) may be a continuous 1 bp to 25 bp, for example, 17 bp to 23 bp or 21 bp to 23 bp, region in the base sequence adjacent to the 5’ end and/or 3’ end of the 5’-NNGRR(T)-3’ sequence in a target gene.

The present invention may provide an artificially manipulated neovascularization-associated factor, for example, an artificially manipulated VEGFA gene, HIF1A gene, ANGPT2 gene, EPAS1 gene, and/or ANGPTL4 gene, which is prepared by

a’’’’) deletion of one or more nucleotides of a continuous 1 bp to 25 bp, for example, 17 bp to 23 bp region, in the base sequence adjacent to the 5’ end and/or the 3’ end of the 5’-NNGRR(T)-3’ sequence (each N is independently A, T, C or G, R is A or G, and (T) is a randomly addable sequence),

b’’’’) substitution of one or more nucleotides of a continuous 1 bp to 25 bp, for example, 17 bp to 23 bp, region in the base sequence adjacent to the 5’ end and/or 3’ end of the 5’-NNGRR(T)-3’ sequence with nucleotides different from those of the wild-type gene,

c’’’’) insertion of one or more nucleotides into a continuous 1 bp to 25 bp, for example, 17 bp to 23 bp, region in the base sequence adjacent to the 5’-NNGRR(T)-3’ sequence, or

d’’’’) a combination of two or more selected from a’’’’) through c’’’’) in the nucleic acid sequence of the neovascularization-associated factor.

For example, when the CRISPR enzyme is a Cpf1 protein, the PAM sequence is 5’-TTN-3’ (N is A, T, C or G), and the cleaved base sequence region (target region) may be a continuous 10 bp to 30 bp, for example, 15 bp to 26 bp, 17 bp to 30 bp or 17 bp to 26 bp, region in the base sequence adjacent to the 5’ end or the 3’ end of the 5’-TTN-3’ sequence.

The Cpf1 protein may be derived from a microorganism such as *Parcubacteria bacterium* (GWC2011_GWC2_44_17), *Lachnospiraceae bacterium* (MC2017), *Butyrivibrio proteoclasiicu*s, *Peregrinibacteria bacterium* (GW2011_GWA_33_10), *Acidaminococcus sp.* (BV3L6), *Porphyromonas macacae*, *Lachnospiraceae bacterium* (ND2006), *Porphyromonas crevioricanis*, *Prevotella disiens*, *Moraxella bovoculi* (237), *Smiihella sp.* (SC_KO8D17), *Leptospira inadai*, *Lachnospiraceae bacterium* (MA2020), *Francisella novicida* (U112), *Candidatus Methanoplasma termitum*, or *Eubacterium eligens*, for example, *Parcubacteria bacterium* (GWC2011_GWC2_44_17), *Peregrinibacteria bacterium* (GW2011_GWA_33_10), *Acidaminococcus sp*. (BV3L6), *Porphyromonas macacae*, *Lachnospiraceae bacterium* (ND2006), *Porphyromonas crevioricanis*, *Prevotella disiens*, *Moraxella bovoculi* (237), *Leptospira inadai*, *Lachnospiraceae bacterium* (MA2020), *Francisella novicida* (U112), *Candidatus Methanoplasma termitum*, or *Eubacterium eligens*, but the present invention is not limited thereto.

The present invention may provide an artificially manipulated neovascularization-associated factor, for example, an artificially manipulated VEGFA gene, HIF1A gene, ANGPT2 gene, EPAS1 gene, and/or ANGPTL4 gene, which is prepared by

a’’’’’) deletion of one or more nucleotides of a continuous 10 bp to 30 bp, for example, 15 bp to 26 bp, region in the base sequence adjacent to the 5’ end and/or the 3’ end of the 5’-TTN-3’ sequence (N is A, T, C or G),

b’’’’’) substitution of one or more nucleotides of a continuous 10 bp to 30 bp, for example, 15 bp to 26 bp, region in the base sequence adjacent to the 5’ end and/or 3’ end of the 5’-TTN-3’ sequence with nucleotides different from those of the wild-type gene,

c’’’’’) insertion of one or more nucleotides of a continuous 10 bp to 30 bp, for example, 15 bp to 26 bp, region in the base sequence adjacent to the 5’ end and/or 3’ end of the 5’-TTN-3’ sequence, or

d’’’’’) a combination of two or more selected from a’’’’’) through c’’’’’) in the nucleic acid sequence of the neovascularization-associated factor.

In another exemplary embodiment, when the neovascularization-associated factor is a protein,

the artificially manipulated protein includes all proteins involved in formation of new or modified blood vessels by a direct or indirect action of the gRNA-CRISPR enzyme complex.

For example, the artificially manipulated protein may be a protein expressed by a neovascularization-associated factor (gene) artificially manipulated by the gRNA-CRISPR enzyme complex or another protein increased or reduced by an influence by such protein activity, but the present invention is not limited thereto.

The artificially manipulated neovascularization-associated factor (protein) may have an amino acid composition and activity corresponding to the composition of the artificially manipulated neovascularization-associated factor (gene).

As an embodiment, an (i) artificially manipulated protein which is changed in expression characteristics may be provided.

For example, protein modification may have one or more characteristics:

a decrease or increase in expression level according to the deletion or insertion of one or more nucleotides in a continuous 1 bp to 50 bp, 1 bp to 40 bp, 1 bp to 30 bp, and preferably 3 bp to 25 bp region in the base sequence of the PAM sequence in the nucleic acid sequence of the neovascularization-associated factor or adjacent to the 5' end and/or the 3' end thereof;

a decrease or increase in expression level according to the substitution with one or more nucleotides different from those of a wild-type gene;

a decrease or increase in expression level, expression of a fusion protein or independent expression of a specific protein according to the insertion of one or more foreign nucleotides; and

a decrease or increase in expression level of a third protein influenced by expression characteristics of the above-described proteins.

An (ii) artificially manipulated protein which is changed in structural characteristics may be provided.

For example, protein modification may have one or more characteristics:

a change in codons, amino acids and three-dimensional structure according to the deletion or insertion of one or more nucleotides in a continuous 1 bp to 50 bp, 1 bp to 40 bp, 1 bp to 30 bp, and preferably 3 bp to 25 bp region in the base sequence of the PAM sequence

in the nucleic acid sequence of the neovascularization-associated factor or adjacent to the 5’ end and/or the 3’ end thereof;

a change in codons, amino acids, and three-dimensional structure thereby according to the substitution with one or more nucleotides different from a wild-type gene;

a change in codons, amino acids, and three-dimensional structure, or a fusion structure with a specific protein or independent structure from which a specific protein is separated according to the insertion of one or more foreign nucleotides; and

a change in codons, amino acids, and three-dimensional structure of a third protein influenced by the above-described protein changed in structural characteristic.

An (iii) artificially manipulated protein changed in functional characteristics may be provided.

For example, protein modification may have one or more characteristics:

the activation or inactivation of a specific function or introduction of a new neovascularization function by protein modification caused by a deletion or insertion of one or more nucleotides in a continuous 1 bp to 50 bp, 1 bp to 40 bp, 1 bp to 30 bp, and preferably 3 bp to 25 bp region in the base sequence of the PAM sequence in the nucleic acid sequence of the neovascularization-associated factor or adjacent to the 5’ end and/or the 3’ end thereof;

the activation or inactivation of a specific function or introduction of a new function by protein modification caused by substitution with one or more nucleotides different from those of a wild-type gene;

the activation or inactivation of a specific function or introduction of a new function by protein modification caused by insertion of one or more foreign nucleotides, particularly, introduction of a third function to an existing function due to fusion or independent expression of a specific protein; and

the change in the function of a third protein influenced by the above-described protein changed in functional characteristics.

In addition, a protein artificially manipulated by the chemical modification of one or more nucleotides in the nucleic acid sequence constituting the neovascularization-associated factor may be included.

For example, one or more of the expression, structural and functional characteristics of a protein caused by methylation, acetylation, phosphorylation, ubiquitination, ADP-ribosylation, myristylation and glycosylation may be changed.

For example, the third structure and function may be achieved by binding of a third protein into the nucleic acid sequence of the gene due to the chemical modification of nucleotides.

5. Other additional components

An additional component may be selectively added to increase the efficiency of a guide nucleic acid-editor protein complex or improve the repair efficiency of a damaged gene or nucleic acid.

The additional component may be selectively used to improve the efficiency of the guide nucleic acid-editor protein complex.

Activator

The additional component may be used as an activator to increase the cleavage efficiency of a target nucleic acid, gene or chromosome of the guide nucleic acid-editor protein complex.

The term “activator” refers to a nucleic acid serving to stabilize the bonding between the guide nucleic acid-editor protein complex and the target nucleic acid, gene or chromosome, or to allow the guide nucleic acid-editor protein complex to more easily approach the target nucleic acid, gene or chromosome.

The activator may be a double-stranded nucleic acid or single-stranded nucleic acid.

The activator may be linear or circular.

The activator may be divided into a “helper” that stabilizes the bonding between the guide nucleic acid-editor protein complex and the target nucleic acid, gene or chromosome, and an “escorter” that serves to allow the guide nucleic acid-editor protein complex to more easily approach the target nucleic acid, gene or chromosome.

The helper may increase the cleavage efficiency of the guide nucleic acid-editor protein complex with respect to the target nucleic acid, gene or chromosome.

For example, the helper includes a nucleic acid sequence having homology with the target nucleic acid, gene or chromosome. Therefore, when the guide nucleic acid-editor protein complex is bonded to the target nucleic acid, gene or chromosome, the homologous nucleic acid sequence included in the helper may form an additional complementary bond with the target nucleic acid, gene or chromosome to stabilize the bonding between the guide nucleic acid-editor protein complex and the target nucleic acid, gene or chromosome.

The escorter may increase the cleavage efficiency of the guide nucleic acid-editor protein complex with respect to the target nucleic acid, gene or chromosome.

For example, the escorter includes a nucleic acid sequence having homology with the target nucleic acid, gene or chromosome. Here, the homologous nucleic acid sequence included in the escorter may partly form a complementary bond with a guide nucleic acid of the guide nucleic acid-editor protein complex. Therefore, the escorter partly forming a complementary bond with the guide nucleic acid-editor protein complex may partly form a

complementary bond with the target nucleic acid, gene or chromosome, and as a result, may allow the guide nucleic acid-editor protein complex to accurately approach the position of the target nucleic acid, gene or chromosome.

The homologous nucleic acid sequence may have at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95% or more homology, or complete homology.

In addition, the additional component may be selectively used to improve the repair efficiency of the damaged gene or nucleic acid.

Assistor

The additional component may be used as an assistor to improve the repair efficiency of the damaged gene or nucleic acid.

The term "assistor" refers to a nucleic acid that serves to participate in a repair process or increase the repair efficiency of the damaged gene or nucleic acid, for example, the gene or nucleic acid cleaved by the guide nucleic acid-editor protein complex.

The assistor may be a double-stranded nucleic acid or single-stranded nucleic acid.

The assistor may be present in a linear or circular shape.

The assistor may be divided into an "NHEJ assistor" that participates in a repair process using NHEJ or improves repair efficiency and an "HDR assistor" that participates in a repair process using HDR or improves repair efficiency according to a repair method.

The NHEJ assistor may participate in a repair process or improve the repair efficiency of the damaged gene or nucleic acid using NHEJ.

For example, the NHEJ assistor may include a nucleic acid sequence having homology with a part of the damaged nucleic acid sequence. Here, the homologous nucleic acid sequence may include a nucleic acid sequence having homology with the nucleic acid sequence at one end (e.g., the 3' end) of the damaged nucleic acid sequence, and include a

nucleic acid sequence having homology with the nucleic acid sequence at the other end (e.g., the 5’ end) of the damaged nucleic acid sequence. In addition, a nucleic acid sequence having homology with each of the base sequences upstream and downstream of the damaged nucleic acid sequence may be included. The nucleic acid sequence having such homology may assist two parts of the damaged nucleic acid sequence to be placed in close proximity, thereby increasing the repair efficiency of the damaged nucleic acid by NHEJ.

The HDR assistor may participate in the repair process or improve repair efficiency of the damaged gene or nucleic acid using HDR.

For example, the HDR assistor may include a nucleic acid sequence having homology with a part of the damaged nucleic acid sequence. Here, the homologous nucleic acid sequence may include a nucleic acid sequence having homology with the nucleic acid sequence at one end (e.g., the 3’ end) of the damaged nucleic acid sequence, and a nucleic acid sequence having homology with the nucleic acid sequence at the other end (e.g., the 5’ end) of the damaged nucleic acid sequence. Alternatively, a nucleic acid sequence having homology with each of the base sequences upstream and downstream of the damaged nucleic acid sequence may be included. The nucleic acid sequence having such homology may serve as a template of the damaged nucleic acid sequence to increase the repair efficiency of the damaged nucleic acid by HDR.

In another example, the HDR assistor may include a nucleic acid sequence having homology with a part of the damaged nucleic acid sequence and a specific nucleic acid, for example, a nucleic acid or gene to be inserted. Here, the homologous nucleic acid sequence may include a nucleic acid sequence having homology with each of the base sequences upstream and downstream of the damaged nucleic acid sequence. The specific nucleic acid may be located between a nucleic acid sequence having homology with a base sequence downstream of the damaged nucleic acid and a nucleic acid sequence having homology with

a base sequence upstream of the damaged nucleic acid. The nucleic acid sequence having such homology and specific nucleic acid may serve as a donor to insert a specific nucleic acid into the damaged nucleic acid, thereby increasing HDR efficiency for knockin.

The homologous nucleic acid sequence may have at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95% or more homology or complete homology.

6. Subject

The term "subject" refers to an organism into which a guide nucleic acid, editor protein or guide nucleic acid-editor protein complex is introduced, an organism in which a guide nucleic acid, editor protein or guide nucleic acid-editor protein complex operates, or a specimen or sample obtained from the organism.

The subject may be an organism including a target nucleic acid, gene, chromosome or protein of the guide nucleic acid-editor protein complex.

The organism may be cells, tissue, a plant, an animal or a human.

The cells may be prokaryotic cells or eukaryotic cells.

The eukaryotic cells may be plant cells, animal cells or human cells, but the present invention is not limited thereto.

The tissue may be animal or human body tissue such as skin, liver, kidney, heart, lung, brain or muscle tissue.

The subject may be a specimen or sample including a target nucleic acid, gene, chromosome or protein of the guide nucleic acid-editor protein complex.

The specimen or sample may be obtained from an organism including a target nucleic acid, gene, chromosome or protein and may be saliva, blood, skin tissue, cancer cells or stem cells.

In the present invention, as a specific example, the subject may include a target gene or nucleic acid of the guide nucleic acid-editor protein complex.

Here, the target gene may be a neovascularization-associated factor, for example, a VEGFA gene, HIF1A gene, ANGPT2 gene, EPAS1 gene, and/or ANGPTL4 gene.

The target gene may be a wild type, or a modified form in the wild-type.

In one exemplary embodiment of the present invention, the subject may include a gene or nucleic acid manipulated by the guide nucleic acid-editor protein complex.

Here, the manipulated gene may be a neovascularization-associated factor, for example, a VEGFA gene, HIF1A gene, ANGPT2 gene, EPAS1 gene, and/or ANGPTL4 gene.

Here, the guide nucleic acid may target a neovascularization-associated factor, for example, a VEGFA gene, HIF1A gene, ANGPT2 gene, EPAS1 gene, and/or ANGPTL4 gene.

The guide nucleic acid may be a nucleic acid sequence complementary to a target sequence of the VEGFA gene, HIF1A gene, ANGPT2 gene, EPAS1 gene, and/or ANGPTL4 gene.

The guide nucleic acid may target one or more genes.

The guide nucleic acid may simultaneously target two or more genes. Here, the two or more genes may be homologous or heterologous genes.

The guide nucleic acid may target one or more target sequences.

The guide nucleic acid may be designed in various forms according to the number or locations of the target sequences.

In one exemplary embodiment of the present invention, the guide nucleic acid may be a nucleic acid sequence complementary to one or more target sequences of the sequences listed in Table 1, Table 2, Table 3, Table 4 and Table 5.

In a certain embodiment, for artificial manipulation of each gene, a guide nucleic acid sequence corresponding to any one of the target sequences of SEQ ID NOs: 1 to 79.

In a certain embodiment, for artificial manipulation of each gene, an editor protein that interacts with a guide nucleic acid sequence corresponding to, for example, forming a complex with any one of the target sequences of SEQ ID NOs: 1 to 1522, for example, SEQ ID NOs: 1 to 79, is provided.

In a certain embodiment, a nucleic acid modification product of each gene in which artificial manipulation occurs at a target sequence region of any one of SEQ ID NOs: 1 to 1522, for example, SEQ ID NOs: 1 to 79, and an expression product thereof are provided.

7. Delivery

The guide nucleic acid, editor protein or guide nucleic acid-editor protein complex may be delivered or introduced into a subject by various delivering methods and various forms.

The guide nucleic acid may be delivered or introduced into a subject in the form of DNA, RNA or a mixed form.

The editor protein may be delivered or introduced into a subject in the form of DNA, RNA, a DNA/RNA mixture, a peptide, a polypeptide, which encodes the editor protein, or a protein.

The guide nucleic acid-editor protein complex may be delivered or introduced into a target in the form of DNA, RNA or a mixture thereof, which encodes each component, that is, a guide nucleic acid or an editor protein.

The guide nucleic acid-editor protein complex may be delivered or introduced into a subject as a complex of a guide nucleic acid having a form of DNA, RNA or a mixture thereof and an editor protein having a form of a peptide, polypeptide or protein.

In addition, an additional component capable of increasing or inhibiting the efficiency of the guide nucleic acid-editor protein complex may be delivered or introduced into a subject by various delivering methods and in various forms.

The additional component may be delivered or introduced into a subject in the form of DNA, RNA, a DNA/RNA mixture, a peptide, a polypeptide or a protein.

i) Delivery in form of DNA, RNA or mixture thereof

The form of DNA, RNA or a mixture thereof, which encodes the guide nucleic acid and/or editor protein may be delivered or introduced into a subject by a method known in the art.

Or, the form of DNA, RNA or a mixture thereof, which encodes the guide nucleic acid and/or editor protein may be delivered or introduced into a subject by a vector, a non-vector or a combination thereof.

The vector may be a viral or non-viral vector (e.g., a plasmid).

The non-vector may be naked DNA, a DNA complex or mRNA.

Vector-based introduction

The nucleic acid sequence encoding the guide nucleic acid and/or editor protein may be delivered or introduced into a subject by a vector.

The vector may include a nucleic acid sequence encoding a guide nucleic acid and/or editor protein.

For example, the vector may simultaneously include nucleic acid sequences, which encode the guide nucleic acid and the editor protein, respectively.

For example, the vector may include the nucleic acid sequence encoding the guide nucleic acid.

As an example, domains included in the guide nucleic acid may be contained all in one vector, or may be divided and then contained in different vectors.

For example, the vector may include the nucleic acid sequence encoding the editor protein.

In one example, in the case of the editor protein, the nucleic acid sequence encoding the editor protein may be contained in one vector, or may be divided and then contained in several vectors.

The vector may include one or more regulatory/control components.

Here, the regulatory/control components may include a promoter, an enhancer, an intron, a polyadenylation signal, a Kozak consensus sequence, an internal ribosome entry site (IRES), a splice acceptor and/or a 2A sequence.

The promoter may be a promoter recognized by RNA polymerase II.

The promoter may be a promoter recognized by RNA polymerase III.

The promoter may be an inducible promoter.

The promoter may be a subject-specific promoter.

The promoter may be a viral or non-viral promoter.

The promoter may use a suitable promoter according to a control region (that is, a nucleic acid sequence encoding a guide nucleic acid or editor protein).

For example, a promoter useful for the guide nucleic acid may be a H1, EF-1a, tRNA or U6 promoter. For example, a promoter useful for the editor protein may be a CMV, EF-1a, EFS, MSCV, PGK or CAG promoter.

The vector may be a viral vector or recombinant viral vector.

The virus may be a DNA virus or an RNA virus.

Here, the DNA virus may be a double-stranded DNA (dsDNA) virus or single-stranded DNA (ssDNA) virus.

Here, the RNA virus may be a single-stranded RNA (ssRNA) virus.

The virus may be a retrovirus, a lentivirus, an adenovirus, adeno-associated virus (AAV), vaccinia virus, a poxvirus or a herpes simplex virus, but the present invention is not limited thereto.

Generally, the virus may infect a host (e.g., cells), thereby introducing a nucleic acid encoding the genetic information of the virus into the host or inserting a nucleic acid encoding the genetic information into the host genome. The guide nucleic acid and/or editor protein may be introduced into a subject using a virus having such a characteristic. The guide nucleic acid and/or editor protein introduced using the virus may be temporarily expressed in the subject (e.g., cells). Alternatively, the guide nucleic acid and/or editor protein introduced using the virus may be continuously expressed in a subject (e.g., cells) for a long time (e.g., 1, 2 or 3 weeks, 1, 2, 3, 6 or 9 months, 1 or 2 years, or permanently).

The packaging capability of the virus may vary from at least 2 kb to 50 kb according to the type of virus. Depending on such a packaging capability, a viral vector including a guide nucleic acid or an editor protein or a viral vector including both of a guide nucleic acid and an editor protein may be designed. Alternatively, a viral vector including a guide nucleic acid, an editor protein and additional components may be designed.

In one example, a nucleic acid sequence encoding a guide nucleic acid and/or editor protein may be delivered or introduced by a recombinant lentivirus.

In another example, a nucleic acid sequence encoding a guide nucleic acid and/or editor protein may be delivered or introduced by a recombinant adenovirus.

In still another example, a nucleic acid sequence encoding a guide nucleic acid and/or editor protein may be delivered or introduced by recombinant AAV.

In yet another example, a nucleic acid sequence encoding a guide nucleic acid and/or editor protein may be delivered or introduced by a hybrid virus, for example, one or more hybrids of the virus listed herein.

Non-vector-based introduction

A nucleic acid sequence encoding a guide nucleic acid and/or editor protein may be delivered or introduced into a subject using a non-vector.

The non-vector may include a nucleic acid sequence encoding a guide nucleic acid and/or editor protein.

The non-vector may be naked DNA, a DNA complex, mRNA, or a mixture thereof.

The non-vector may be delivered or introduced into a subject by electroporation, particle bombardment, sonoporation, magnetofection, transient cell compression or squeezing (e.g., described in the literature [Lee, et al, (2012) Nano Lett., 12, 6322-6327]), lipid-mediated transfection, a dendrimer, nanoparticles, calcium phosphate, silica, a silicate (Ormosil), or a combination thereof.

As an example, the delivery through electroporation may be performed by mixing cells and a nucleic acid sequence encoding a guide nucleic acid and/or editor protein in a cartridge, chamber or cuvette, and applying electrical stimuli with a predetermined duration and amplitude to the cells.

In another example, the non-vector may be delivered using nanoparticles. The nanoparticles may be inorganic nanoparticles (e.g., magnetic nanoparticles, silica, etc.) or organic nanoparticles (e.g., a polyethylene glycol (PEG)-coated lipid, etc.). The outer surface of the nanoparticles may be conjugated with a positively-charged polymer which is attachable (e.g., polyethyleneimine, polylysine, polyserine, etc.).

In a certain embodiment, the non-vector may be delivered using a lipid shell.

In a certain embodiment, the non-vector may be delivered using an exosome. The exosome is an endogenous nano-vesicle for transferring a protein and RNA, which can deliver RNA to the brain and another target organ.

In a certain embodiment, the non-vector may be delivered using a liposome. The liposome is a spherical vesicle structure which is composed of single or multiple lamellar lipid bilayers surrounding internal aqueous compartments and an external, lipophilic phospholipid bilayer which is relatively non-transparent. While the liposome may be made from several different types of lipids; phospholipids are most generally used to produce the liposome as a drug carrier.

Other additives may be included.

ii) Delivery in form of peptide, polypeptide or protein

An editor protein in the form of a peptide, polypeptide or protein may be delivered or introduced into a subject by a method known in the art

The peptide, polypeptide or protein form may be delivered or introduced into a subject by electroporation, microinjection, transient cell compression or squeezing (e.g., described in the literature [Lee, et al, (2012) Nano Lett., 12, 6322-6327]), lipid-mediated transfection, nanoparticles, a liposome, peptide-mediated delivery or a combination thereof.

The peptide, polypeptide or protein may be delivered with a nucleic acid sequence encoding a guide nucleic acid.

In one example, the transfer through electroporation may be performed by mixing cells into which the editor protein will be introduced with or without a guide nucleic acid in a cartridge, chamber or cuvette, and applying electrical stimuli with a predetermined duration and amplitude to the cells.

iii) Delivery in form of nucleic acid-protein mixture

The guide nucleic acid and the editor protein may be delivered or introduced into a subject in the form of a guide nucleic acid-editor protein complex.

For example, the guide nucleic acid may be DNA, RNA or a mixture thereof. The editor protein may be a peptide, polypeptide or protein.

In one example, the guide nucleic acid and the editor protein may be delivered or introduced into a subject in the form of a guide nucleic acid-editor protein complex containing an RNA-type guide nucleic acid and a protein-type editor protein, that is, a ribonucleoprotein (RNP).

In the present invention, as an embodiment of a method for delivering the guide nucleic acid and/or editor protein into a subject, the delivery of gRNA, a CRISPR enzyme or a gRNA-CRISPR enzyme complex will be described below.

In an embodiment of the present invention, a nucleic acid sequence encoding the gRNA and/or CRISPR enzyme will be delivered or introduced into a subject using a vector.

The vector may include the nucleic acid sequence encoding the gRNA and/or CRISPR enzyme.

For example, the vector may simultaneously include the nucleic acid sequences encoding the gRNA and the CRISPR enzyme.

For example, the vector may include the nucleic acid sequence encoding the gRNA.

In one example, domains contained in the gRNA may be contained in one vector, or may be divided and then contained in different vectors.

For example, the vector may include the nucleic acid sequence encoding the CRISPR enzyme.

In one example, in the case of the CRISPR enzyme, the nucleic acid sequence encoding the CRISPR enzyme may be contained in one vector, or may be divided and then contained in several vectors.

The vector may include one or more regulatory/control components.

Here, the regulatory/control components may include a promoter, an enhancer, an intron, a polyadenylation signal, a Kozak consensus sequence, an internal ribosome entry site (IRES), a splice acceptor and/or a 2A sequence.

The promoter may be a promoter recognized by RNA polymerase II.

The promoter may be a promoter recognized by RNA polymerase III.

The promoter may be an inducible promoter.

The promoter may be a subject-specific promoter.

The promoter may be a viral or non-viral promoter.

The promoter may use a suitable promoter according to a control region (that is, a nucleic acid sequence encoding the gRNA and/or CRISPR enzyme).

For example, a promoter useful for the gRNA may be a H1, EF-1a, tRNA or U6 promoter. For example, a promoter useful for the CRISPR enzyme may be a CMV, EF-1a, EFS, MSCV, PGK or CAG promoter.

The vector may be a viral vector or recombinant viral vector.

The virus may be a DNA virus or an RNA virus.

Here, the DNA virus may be a double-stranded DNA (dsDNA) virus or single-stranded DNA (ssDNA) virus.

Here, the RNA virus may be a single-stranded RNA (ssRNA) virus.

The virus may be a retrovirus, a lentivirus, an adenovirus, adeno-associated virus (AAV), vaccinia virus, a poxvirus or a herpes simplex virus, but the present invention is not limited thereto.

Generally, the virus may infect a host (e.g., cells), thereby introducing a nucleic acid encoding the genetic information of the virus into the host or inserting a nucleic acid encoding the genetic information into the host genome. The gRNA and/or CRISPR enzyme may be introduced into a subject using a virus having such a characteristic. The gRNA and/or CRISPR enzyme introduced using the virus may be temporarily expressed in the subject (e.g., cells). Alternatively, the gRNA and/or CRISPR enzyme introduced using the virus may be continuously expressed in a subject (e.g., cells) for a long time (e.g., 1, 2 or 3 weeks, 1, 2, 3, 6 or 9 months, 1 or 2 years, or permanently).

The packaging capability of the virus may vary from at least 2 kb to 50 kb according to the type of virus. Depending on such a packaging capability, a viral vector only including gRNA or a CRISPR enzyme or a viral vector including both of gRNA and a CRISPR enzyme may be designed. Alternatively, a viral vector including gRNA, a CRISPR enzyme and additional components may be designed.

In one example, a nucleic acid sequence encoding gRNA and/or a CRISPR enzyme may be delivered or introduced by a recombinant lentivirus.

In another example, a nucleic acid sequence encoding gRNA and/or a CRISPR enzyme may be delivered or introduced by a recombinant adenovirus.

In still another example, a nucleic acid sequence encoding gRNA and/or a CRISPR enzyme may be delivered or introduced by recombinant AAV.

In yet another example, a nucleic acid sequence encoding gRNA and/or a CRISPR enzyme may be delivered or introduced by one or more hybrids of hybrid viruses, for example, the viruses described herein.

In one exemplary embodiment of the present invention, the gRNA-CRISPR enzyme complex may be delivered or introduced into a subject.

For example, the gRNA may be present in the form of DNA, RNA or a mixture thereof. The CRISPR enzyme may be present in the form of a peptide, polypeptide or protein.

In one example, the gRNA and CRISPR enzyme may be delivered or introduced into a subject in the form of a gRNA-CRISPR enzyme complex including RNA-type gRNA and a protein-type CRISPR, that is, a ribonucleoprotein (RNP).

The gRNA-CRISPR enzyme complex may be delivered or introduced into a subject by electroporation, microinjection, transient cell compression or squeezing (e.g., described in the literature [Lee, et al, (2012) Nano Lett., 12, 6322-6327]), lipid-mediated transfection, nanoparticles, a liposome, peptide-mediated delivery or a combination thereof.

8. Transformant

The term "transformant" refers to an organism into which a guide nucleic acid, editor protein or guide nucleic acid-editor protein complex is introduced, an organism in which a guide nucleic acid, editor protein or guide nucleic acid-editor protein complex is expressed, or a specimen or sample obtained from the organism.

The transformant may be an organism into which a guide nucleic acid, editor protein or guide nucleic acid-editor protein complex is introduced in the form of DNA, RNA or a mixture thereof.

For example, the transformant may be an organism into which a vector including a nucleic acid sequence encoding a guide nucleic acid and/or editor protein is introduced. Here, the vector may be a non-viral vector, viral vector or recombinant viral vector.

In another example, the transformant may be an organism into which a nucleic acid sequence encoding a guide nucleic acid and/or editor protein is introduced in a non-vector

form. Here, the non-vector may be naked DNA, a DNA complex, mRNA or a mixture thereof.

The transformant may be an organism into which a guide nucleic acid, editor protein or guide nucleic acid-editor protein complex is introduced in the form of a peptide, polypeptide or protein.

The transformant may be an organism into which a guide nucleic acid, editor protein or guide nucleic acid-editor protein complex is introduced in the form of DNA, RNA, a peptide, a polypeptide, a protein or a mixture thereof.

For example, the transformant may be an organism into which a guide nucleic acid-editor protein complex including an RNA-type guide nucleic acid and a protein-type editor protein is introduced.

The transformant may be an organism including a target nucleic acid, gene, chromosome or protein of the guide nucleic acid-editor protein complex.

The organism may be cells, tissue, a plant, an animal or a human.

The cells may be prokaryotic cells or eukaryotic cells.

The eukaryotic cells may be plant cells, animal cells, or human cells, but the present invention is not limited thereto.

The tissue may be an animal or human body tissue such as skin, liver, kidney, heart, lung, brain, or muscle tissue.

The transformant may be an organism in(to)to which a guide nucleic acid, editor protein or guide nucleic acid-editor protein complex is introduced or expressed, or a specimen or sample obtained from the organism.

The specimen or sample may be saliva, blood, skin tissue, cancer cells or stem cells.

[Use]

One exemplary embodiment of the present invention relates to a use of treating a neovascularization-associated disease using a method of administering a composition for artificially manipulating a neovascularization-associated factor or an artificially manipulated neovascularization-associated factor to a subject.

Targets for the treatment may be mammals including primates such as a human or a monkey, rodents such as a mouse or a rat, etc.

Diseases to be treated

In an embodiment, diseases to be treated may be neovascularization-associated diseases.

The term "neovascularization-associated diseases" refer to all states including excessive and/or abnormal neovascularization. The neovascularization-associated diseases refer to disorders characterized by vascularization which is not changed or regulated, except tumorigenesis or neoplastic transformation, that is, cancer. The neovascularization-associated diseases include an ocular neovascularization disease.

Neovascular diseases include neovascularization-dependent cancer, for example, solid tumors, hematomas such as leukemia and tumor metastasis; benign tumors such as hemangiomas, acoustic neuromas, neurofibroma, trachomas and pyogenic granulomas; rheumatoid arthritis; psoriasis; ocular neovascularization diseases such as diabetic retinopathy, retinopathy of prematurity, macular degenerations including dry age-related macular degeneration and wet age-related macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis; Osler-Webber Syndrome; myocardial neovascularization blindness; plaque neovascularization; telangiectasia; hemophiliac joint; angiofibromas; and wound granulation, but the present invention is not limited thereto.

In a certain embodiment, the neovascularization-associated diseases may be one or more diseases selected from the group consisting of rheumatoid arthritis, psoriasis, Osler-Webber Syndrome, myocardial neovascularization blindness, plaque neovascularization, telangiectasia, hemophiliac joint, angiofibromas, and wound granulation.

In an embodiment, the neovascularization-associated disease may be a disease including excessive and/or abnormal neovascularization.

In an embodiment, the neovascularization-associated disease may be neovascularization-dependent cancer.

Here, the neovascularization-dependent cancer includes solid tumors, hematologic tumors such as leukemia and tumor metastasis.

The neovascularization-dependent cancer may be, for example, solid tumors, hematologic tumors such as leukemia and tumor metastasis; benign tumors such as hemangiomas, acoustic neuromas, neurofibromas, trachomas and pyogenic granulomas.

In a certain embodiment, the neovascularization-associated disease may be a benign tumor.

The benign tumor may include hemangiomas, acoustic neuromas, trachomas and pyogenic granulomas.

In a certain embodiment, the neovascularization-associated disease may be an ocular neovascularization disease.

The term “ocular neovascularization disease” refers to all ocular diseases including excessive and/or abnormal neovascularization. The ocular neovascularization disease includes a disorder characterized by vascularization that is not changed or regulated in the eyes.

As an example, the ocular neovascularization diseases may include:

ischemic retinopathy, optic papillary neovascularization, iris neovascularization, retinal neovascularization, choroidal neovascularization, corneal neovascularization, vitreous neovascularization, glaucoma, panus, pterygiums, macular edemas, diabetic retinopathy, proliferative diabetic retinopathy, diabetic macular edemas, vascular retinopathy, retinal degeneration, uveitis, inflammatory diseases of the retina, and proliferative vitreoretinopathy.

In one exemplary embodiment, the ocular neovascularization disease may be diabetic retinopathy or macular degeneration.

- Diabetic retinopathy

Diabetic retinopathy is a diabetic complication occurring in approximately 40 to 45% of the patients diagnosed with any one of Type 1 diabetes or Type 2 diabetes.

Diabetic retinopathy usually affects both eyes, and generally progresses in four stages. The first stage, that is, mild nonproliferative retinopathy is characterized by ocular microaneurysms. Small scaled swelling occurs in a retinal capillary tube and a small vessel. In the second stage, that is, moderate nonproliferative retinopathy, a blood vessel provided to the retina starts to be blocked. In the third stage, that is, severe nonproliferative retinopathy, the occlusion leads to a decrease in blood supply to the retina, and the retina sends a neovascularization signal to the eye in order to provide blood supply to the retina. In the fourth stage, that is, proliferative retinopathy, which is the most advanced stage, angiogenesis occurs, but the new blood vessel is abnormal, weak, and grows on the surface of a vitreous gel contained in the retina and the eyes.

The diabetic retinopathy includes insulin-dependent diabetes, insulin-independent diabetes, retinal detachment, diabetic retinopathy, and vitreous hemorrhage.

- Macular degeneration

The macular degeneration refers to a disease in which visual impairment is caused by macular degeneration, and is also called age-related macular degeneration (AMD).

The AMD includes early, intermediate and advanced AMD, and also includes all of dry AMD, for example, geographic atrophy, and wet AMD which is also known as neovascular or exudative AMD.

Dry macular degeneration is a prevalent type accounting for approximately 90% of the AMDs when a lesion such as a druse (the state in which waste is accumulated in the macula) or retinal pigment epithelial atrophy is generated in the retina. Wet macular degeneration is characterized by production of choroidal neovascularization under the retina.

Dry macular degeneration includes macular degeneration caused by a missense mutation in an immunoregulatory complement factor H (CFH) gene.

In another exemplary embodiment, a use of a system for regulating an additional, third *in vivo* mechanism, accompanied with various functions of specific factors artificially modified in function (e.g., a gene known as a neovascularization-associated factor, etc.) may be provided.

For example, the specific factors artificially modified in function may be one or more genes of a VEGFA gene, an HIF1A gene, an ANGPT2 gene, an EPAS1 gene and an ANGPTL4 gene.

The third mechanism may be an *in vivo* mechanism in which the genes are involved, other than neovascularization.

Pharmaceutical composition

One exemplary embodiment of the present invention relates to a composition to be used in treatment of a disease using an artificially manipulated neovascularization-associated factor.

The composition may include an artificially manipulated neovascularization-associated factor or a manipulation composition capable of artificially manipulating the neovascularization-associated factor. The composition may be referred to as a therapeutic composition or pharmaceutical composition.

In an exemplary embodiment, the composition may include an artificially manipulated neovascularization-associated factor, that is, a gene and/or protein.

In an exemplary embodiment, the composition may include a manipulation composition capable of artificially manipulating a neovascularization-associated factor.

The manipulation composition may include a guide nucleic acid-editor protein complex.

The manipulation composition may include a guide nucleic acid and/or editor protein.

The manipulation composition may include a nucleic acid encoding the guide nucleic acid and/or editor protein.

The manipulation composition may include a virus comprising a nucleic acid encoding the guide nucleic acid and/or editor protein.

In another exemplary embodiment, the composition may further include an additional element.

The additional element may include a suitable carrier for delivery into the body of a subject.

In one exemplary embodiment, the composition may include an expression product of a neovascularization-associated factor manipulated in a sufficient amount to suppress an angiovascular disorder.

The “sufficient amount to suppress an angiovascular disorder” refers to an effective amount necessary to treat or prevent an angiovascular disorder or a symptom thereof.

In one exemplary embodiment, the following therapeutic compositions will be provided:

a composition for treating an angiovascular disorder, which includes a guide nucleic acid capable of forming a complementary bond with each of one or more target sequences in nucleic acid sequences of one or more genes selected from the group consisting of a VEGFA gene, an HIF1A gene, an ANGPT2 gene, an EPAS1 gene and an ANGPTL4 gene, or a nucleic acid sequence encoding the same, and

an editor protein or a nucleic acid sequence encoding the same;

a composition for treating an angiovascular disorder, which includes a guide nucleic acid capable of forming a complementary bond with each of the target sequences of SEQ ID NOs: 1 to 1522, for example, SEQ ID NOs: 1 to 79, of nucleic acid sequences of one or more genes selected from the group consisting of a VEGFA gene, an HIF1A gene, an ANGPT2 gene, an EPAS1 gene and an ANGPTL4 gene, or a nucleic acid sequence encoding the same, and

an editor protein or a nucleic acid sequence encoding the same; and

a composition for treating an angiovascular disorder, which includes a complex formed of a guide nucleic acid capable of forming a complementary bond with each of the target sequences of SEQ ID NOs: 1 to 1522, for example, SEQ ID NOs: 1 to 79, of nucleic acid sequences of one or more genes selected from the group consisting of a VEGFA gene, an HIF1A gene, an ANGPT2 gene, an EPAS1 gene and an ANGPTL4 gene or a nucleic acid sequence encoding the same; and an editor protein.

Here, the guide nucleic acid or nucleic acid sequence encoding the same; and a nucleic acid sequence encoding the editor protein may be present in the form of one or more vectors. The guide nucleic acid or nucleic acid sequence encoding the same; and a nucleic

acid sequence encoding the editor protein may be present in the form of homologous or heterologous vectors.

Treatment method

In another exemplary embodiment of the present invention, a method for treating a disease in a patient, which includes producing the above-described composition and administering an effective amount of the composition to a patient requiring the same, is provided.

Gene manipulating treatment

A treatment method for regulating neovascularization by manipulating a gene of a living organism may be used. Such a treatment method may be achieved by directly injecting a composition for manipulating a gene to manipulate the gene of a living organism into the organism.

The composition for gene manipulation may include a guide nucleic acid-editor protein complex.

The composition for gene manipulation may be injected into a specific location of the body.

Here, the specific location of the body may be tissue in which neovascularization excessively and/or abnormally occurs, or a location close thereto. For example, the specific location of the body may be, for example, the eyeball.

Subjects for administration of the composition may be mammals including primates such as a human or a monkey, rodents such as a mouse or a rat, etc.

The composition may be administered by any convenient method such as injection, transfusion, implantation or transplantation. The composition may be administered

subcutaneously, intradermally, intraocularly, intravitreally, intratumorally, intranodally, intramedullarily, intramuscularly, intravenously, intralymphatically, or intraperitoneally.

A dose (pharmaceutically effective amount to obtain a predetermined, desired effect) of the composition may be selected from all integers in the value ranges of 104-109 cells, for example, 105 to 106 cells/kg (body weight), per kg of the subject of administration, but the present invention is not limited thereto. The composition may be suitably prescribed in consideration of the age, health condition and body weight of the subject of administration, the types of treatments simultaneously received, if they were, frequency of the co-treatments, and characteristics of a desired effect.

In one aspect, the present invention provides a method for modifying a target polynucleotide in prokaryotic cells, which may be achieved *in vivo*, *ex vivo*, or *in vitro*.

In some embodiments, the method may include sampling cells or a cell population from a human or non-human animal, and modifying the cell or cells. Culturing may occur at any step *ex vivo*. The cell or cells may also be reintroduced into a non-human animal or plant. The reintroduced cells are most preferably stem cells.

In still another exemplary embodiment, the present invention may provide a method for artificially manipulating cells, which includes: introducing (a) a guide nucleic acid capable of forming a complementary bond with the target sequences of SEQ ID NOs: 1 to 1522, for example, SEQ ID NOs: 1 to 79, of nucleic acid sequences of one or more genes selected from the group consisting of a VEGFA gene, an HIF1A gene, an ANGPT2 gene, an EPAS1 gene and an ANGPTL4 gene or a nucleic acid sequence encoding the same; and (b) an editor protein including one or more proteins selected from the group consisting of a *Streptococcus pyogenes*-derived Cas9 protein, a *Campylobacter jejuni*-derived Cas9 protein,

a *Streptococcus thermophilus*-derived Cas9 protein, a *Streptocuccus aureus*-derived Cas9 protein, a *Neisseria meningitidis*-derived Cas9 protein, and a Cpf1 protein, or a nucleic acid sequence encoding the same to cells.

The guide nucleic acid and the editor protein may be present in one or more vectors in the form of a nucleic acid sequence, or in a complex of a combination of the guide nucleic acid and the editor protein.

The introduction step may be carried out *in vivo* or *ex vivo*.

A technique of the above-described "7. Delivery" section may be referenced before the introduction step.

For example, the introduction stage may be achieved by one or more methods selected from electroporation, liposomes, plasmids, viral vectors, nanoparticles, and a protein translocation domain (PTD) fusion protein method.

For example, the viral vector may be one or more selected from the group consisting of a retrovirus, a lentivirus, an adenovirus, adeno-associated virus (AAV), vaccinia virus, a poxvirus and a herpes simplex virus.

When a neovascularization-associated factor is artificially manipulated using the method and composition of some embodiments of the present invention, it is possible to regulate, for example, inhibit, suppress, stimulate and/or increase neovascularization, and therefore an effect of suppressing or improving excessive and/or abnormal neovascularization may be obtained.

Additional uses

In a certain embodiment, the present invention may provide a kit for preparing a composition for treating AMD or diabetic retinopathy.

The kit may be prepared by a conventional preparation method known in the art.

The kit may further include a detectable label. The term “detectable label” refers to an atom or molecule for specifically detecting a molecule containing a label among the same type of molecules without a label. The detectable label may be attached to an antibody specifically binding to a protein or a fragment thereof, an interaction protein, a ligand, nanoparticles, or an aptamer. The detectable label may include a radionuclide, a fluorophore, and an enzyme.

In a certain embodiment, the present invention may provide a method for screening a material capable of regulating the expression level of one or more genes of an artificially manipulated VEGFA gene, HIF1A gene, ANGPT2 gene, EPAS1 gene and ANGPTL4 gene.

In a certain embodiment, the present invention may provide a method for providing information on the sequence of a target site which is able to be artificially manipulated in a subject by analyzing the sequences of one or more genes selected from the group consisting of a VEGFA gene, an HIF1A gene, an ANGPT2 gene, an EPAS1 gene and an ANGPTL4 gene.

In addition, a method for constructing a library using the information provided by such a method.

Here, a known database may be used.

In specific embodiments, an animal or cells which can be used for research using the method of the present invention may be provided.

An animal or cells which includes chromosome editing in one or more nucleic acid sequences associated with a disease may be prepared using the above-described method. Such a nucleic acid sequence may be a reference sequence which may encode a disease-associated protein sequence or may be associated with a disease.

In one exemplary embodiment, an effect of mutation and occurrence and/or progression of a disease may be studied in an animal or cells using measurements conventionally used in disease research with the animal or cells prepared by the method of the present invention. Alternatively, a pharmaceutical effect of an active compound in a disease using such an animal or cells may be studied.

In another exemplary embodiment, the effect of the strategy of a possible gene therapy may be evaluated using the animal or cells prepared by the method of the present invention. That is, the development and/or progression of a corresponding disease may be suppressed or reduced by modifying a chromosome sequence encoding a disease-associated protein. Particularly, this method includes forming a modified protein by editing a chromosome sequence encoding a disease-associated protein, resulting in the achievement of a modified response of the animal or cells. Therefore, in some embodiments, a genetically-modified animal may be compared with an animal vulnerable to the development of a corresponding disease, thereby evaluating the effect of a gene therapy process.

Such uses may include a disease model, a pharmacological model, a developmental model, a cell function model, and a humanized model. For example, a neovascularization-associated disease model, a pharmacological model, a developmental model, a cell function model, and a humanized model may be included.

An artificially manipulated neovascularization-associated factor and a neovascularization system artificially modified in function thereby can be effectively used to treat a neovascularization-associated disease, for example, a neovascularization-associated ocular disease (eye disease). The efficiency of a neovascularization system may be improved by regulating various in vivo mechanisms in which various neovascularization-associated factors are involved.

Hereinafter, the present invention will be described in further detail with reference to examples.

The examples are merely provided to describe the present invention in further detail, and it might be obvious to those of ordinary skill in the art that the scope of the present invention is not limited to the following examples.

Experimental methods

1. Design of sgRNA

CRISPR/Cas9 target regions of human VEGFA gene (NCBI Accession No. NM_001025366.2), HIF1A gene (NCBI Accession No. NM_001243084.1), ANGPT2 gene (NCBI Accession No. NM_001118887.1), EPAS1 gene (NCBI Accession No. NM_001430.4) and ANGPTL4 gene (NCBI Accession No. NM_001039667.2) were selected using CRISPR RGEN Tools (Institute for Basic Science, Korea). The target regions of the genes may be different according to the type of CRISPR enzyme. Target sequences of the genes for CjCas9 are summarized in Tables 1 to 5 and Tables 6 to 9 listed above, and target sequences of the genes for SpCas9 are summarized in Tables 10 to 14.

[Table 6]

Target sequences of VEGFA gene

Gene	No.	Target sequence
VEGFA	1	AAATCCCGGTATAAGTCCTGGA (SEQ ID NO: 80)
VEGFA	2	GCACCAACGTACACGCTCCAGG (SEQ ID NO: 81)
VEGFA	3	CATTAGACAGCAGCGGGCACCA (SEQ ID NO: 82)
VEGFA	4	GGCATTAGACAGCAGCGGGCAC (SEQ ID NO: 83)
VEGFA	5	GGCTCCAGGGCATTAGACAGCA (SEQ ID NO: 84)
VEGFA	6	GCTCAGAGCGGAGAAAGCATTT (SEQ ID NO: 85)
VEGFA	7	GGAACATTTACACGTCTGCGGA (SEQ ID NO: 86)
VEGFA	8	GCAGACGTGTAAATGTTCCTGC (SEQ ID NO: 87)
VEGFA	9	GAGTCTGTGTTTTTGCAGGAAC (SEQ ID NO: 88)
VEGFA	10	GGCGAGGCAGCTTGAGTTAAAC (SEQ ID NO: 89)

[Table 7]

Target sequences of HIF1A gene

Gene	No.	Target sequence
HIF1A	1	ACTAAAGGACAAGTCACCACAG (SEQ ID NO: 90)
HIF1A	2	TATCCACCTCTTTTGGCAAGCA (SEQ ID NO: 91)
HIF1A	3	TGAAACTCAAGCAACTGTCATA (SEQ ID NO: 92)
HIF1A	4	CTCACAACGTAATTCACACATA (SEQ ID NO: 93)
HIF1A	5	TACTTACCTCACAACGTAATTC (SEQ ID NO: 94)
HIF1A	6	AACTTACTTACCTCACAACGTA (SEQ ID NO: 95)
HIF1A	7	TCTTGTTTTGACAGTGGTATTA (SEQ ID NO: 96)
HIF1A	8	GGGAGAAAATCAAGTCGTGCTG (SEQ ID NO: 97)
HIF1A	9	TATCTGAAGATTCAACCGGTTT (SEQ ID NO: 98)
HIF1A	10	GCTATTCACCAAAGTTGAATCA (SEQ ID NO: 99)
HIF1A	11	AACTTTGCTGGCCCCAGCCGCT (SEQ ID NO: 100)
HIF1A	12	AAACTGATGACCAGCAACTTGA (SEQ ID NO: 101)
HIF1A	13	GGGGAGCATTACATCATTATAT (SEQ ID NO: 102)
HIF1A	14	AGCCACTTCGAAGTAGTGCTGA (SEQ ID NO: 103)
HIF1A	15	CAACTTCTTGATTGAGTGCAGG (SEQ ID NO: 104)
HIF1A	16	TTACCATGCCCCAGATTCAGGA (SEQ ID NO: 105)
HIF1A	17	TCAGACACCTAGTCCTTCCGAT (SEQ ID NO: 106)
HIF1A	18	ATTGGTAGAAAAACTTTTTGCT (SEQ ID NO: 107)
HIF1A	19	AACTCATGTATTTGCTGTTTTA (SEQ ID NO: 108)
HIF1A	20	AAGCCCTGAAAGCGCAAGTCCT (SEQ ID NO: 109)
HIF1A	21	CAGTTACAGTATTCCAGCAGAC (SEQ ID NO: 110)
HIF1A	22	AGGTTCTTGTATTTGAGTCTGC (SEQ ID NO: 111)
HIF1A	23	ATGCAATCAATATTTTAATGTC (SEQ ID NO: 112)
HIF1A	24	TGATTGCATCTCCATCTCCTAC (SEQ ID NO: 113)
HIF1A	25	TAGTGCCACATCATCACCATAT (SEQ ID NO: 114)
HIF1A	26	GAGTATCTCTATATGGTGATGA (SEQ ID NO: 115)
HIF1A	27	ATACCTTTGACTCAAAGCGACA (SEQ ID NO: 116)
HIF1A	28	TTCCTGAGGAAGAACTAAATCC (SEQ ID NO: 117)
HIF1A	29	TCTGTTCACTAGATTTGCATCC (SEQ ID NO: 118)
HIF1A	30	GAATGGAGCAAAAGACAATTAT (SEQ ID NO: 119)
HIF1A	31	GTTATGATTGTGAAGTTAATGC (SEQ ID NO: 120)

[Table 8]

Target sequences of EPAS1 gene

Gene	No.	Target sequence
EPAS1	1	AACACCTCCGTCTCCTTGCTCC (SEQ ID NO: 121)
EPAS1	2	TGGAGGCCTTGTCCAGATGGGA (SEQ ID NO: 122)
EPAS1	3	TGCGACTGGCAATCAGCTTCCT (SEQ ID NO: 123)
EPAS1	4	CGACTGGCAATCAGCTTCCTGC (SEQ ID NO: 124)
EPAS1	5	GAAGCTGACCAGCAGATGGACA (SEQ ID NO: 125)
EPAS1	6	GCAATGAAACCCTCCAAGGCTT (SEQ ID NO: 126)
EPAS1	7	AAAACATCAGCAAGTTCATGGG (SEQ ID NO: 127)
EPAS1	8	GCAAGTTCATGGGACTTACACA (SEQ ID NO: 128)
EPAS1	9	GGTCGCAGGGATGAGTGAAGTC (SEQ ID NO: 129)
EPAS1	10	GCGGGACTTCTTCATGAGGATG (SEQ ID NO: 130)
EPAS1	11	GAAGTGCACGGTCACCAACAGA (SEQ ID NO: 131)
EPAS1	12	ACAGTACGGCCTCTGTTGGTGA (SEQ ID NO: 132)
EPAS1	13	TCCAGGTGGCTGACTTGAGGTT (SEQ ID NO: 133)
EPAS1	14	TCCACGCCTGTCTCAGGTCTTG (SEQ ID NO: 134)
EPAS1	15	CAGGACAGCAGGGGCTCCTTGT (SEQ ID NO: 135)
EPAS1	16	CTCATCATCATGTGTGAACCAA (SEQ ID NO: 136)
EPAS1	17	ATGTGGGATGGGTGCTGGATTG (SEQ ID NO: 137)
EPAS1	18	GCCACTTACTACCTGACCCTTG (SEQ ID NO: 138)
EPAS1	19	TGGCCACTTACTACCTGACCCT (SEQ ID NO: 139)
EPAS1	20	ACCAAGGGTCAGGTAGTAAGTG (SEQ ID NO: 140)
EPAS1	21	TAGCCCCCATGCTTTGCGAGCA (SEQ ID NO: 141)
EPAS1	22	GATGACCGTCCCCTGGGTCTCC (SEQ ID NO: 142)
EPAS1	23	CTCAGGACGTAGTTGACACACA (SEQ ID NO: 143)
EPAS1	24	CATGCTTACCTCAGGACGTAGT (SEQ ID NO: 144)
EPAS1	25	CACATGCTTACCTCAGGACGTA (SEQ ID NO: 145)
EPAS1	26	CAGGGATTCAGTCTGGTCCATG (SEQ ID NO: 146)
EPAS1	27	GGTGAATAGGAAGTTACTCTTC (SEQ ID NO: 147)
EPAS1	28	ATGGGCCACGGAGTTGAGGAGC (SEQ ID NO: 148)
EPAS1	29	CTAGCCCAATAGCCCTGAAGAC (SEQ ID NO: 149)
EPAS1	30	AGTGATTGAGAAGCTCTTCGCC (SEQ ID NO: 150)
EPAS1	31	GGACACAGAGGCCAAGGACCAA (SEQ ID NO: 151)
EPAS1	32	CCTGATCTCCACAGCCATCTAC (SEQ ID NO: 152)
EPAS1	33	CGGATTTCAATGAGCTGGACTT (SEQ ID NO: 153)
EPAS1	34	TCAATGAGCTGGACTTGGAGAC (SEQ ID NO: 154)

EPAS1	35	GCGGAGAACCCACAGTCCACCC (SEQ ID NO: 155)
EPAS1	36	CCAGTGGCTGGAAGATGTTTGT (SEQ ID NO: 156)
EPAS1	37	TTCCAGCCACTGGCCCCTGTAG (SEQ ID NO: 157)
EPAS1	38	CTGGAGAGCAAGAAGACAGAGC (SEQ ID NO: 158)
EPAS1	39	GAGAGAGGGGTGCTGGCCTGGC (SEQ ID NO: 159)
EPAS1	40	CCTGCCACCGTGCTGTGGCCAG (SEQ ID NO: 160)
EPAS1	41	TCTCTCTTCCATGGGGGGCAGA (SEQ ID NO: 161)
EPAS1	42	CACAAAGTGGGCCGTCGGGGAT (SEQ ID NO: 162)
EPAS1	43	GGAGAGGGCTACCATGGCCGGA (SEQ ID NO: 163)
EPAS1	44	CTCAGGTCCTGGAAGGCTTGCT (SEQ ID NO: 164)
EPAS1	45	CTCCCAGGGGGACCCACCTGGT (SEQ ID NO: 165)
EPAS1	46	TGCCGGACAAGCCACTGAGCGC (SEQ ID NO: 166)
EPAS1	47	TTCCCCCCACAGTGCTACGCCA (SEQ ID NO: 167)
EPAS1	48	CTGTAGTCCTGGTACTGGGTGG (SEQ ID NO: 168)
EPAS1	49	TGGGCTGACGACAGGCTGTAGT (SEQ ID NO: 169)
EPAS1	50	TCCTTGCAGGAGCGTGGAGCTT (SEQ ID NO: 170)

[Table 9]

Target sequences of ANGPT2 gene

Gene	No.	Target sequence
ANGPT2	1	GACTGTGCTGAAGTATTCAAAT (SEQ ID NO: 171)
ANGPT2	2	CTGTGCTGAAGTATTCAAATCA (SEQ ID NO: 172)
ANGPT2	3	TGGTGTGTCCTGATTTGAATAC (SEQ ID NO: 173)
ANGPT2	4	GCCATTCGTGGTGTGTCCTGAT (SEQ ID NO: 174)
ANGPT2	5	ATCAGGACACACCACGAATGGC (SEQ ID NO: 175)
ANGPT2	6	CTAATCAGCAACGCTATGTGCT (SEQ ID NO: 176)
ANGPT2	7	AATCAGCAACGCTATGTGCTTA (SEQ ID NO: 177)
ANGPT2	8	TTCCCAGTCTTTAAGGTGTATT (SEQ ID NO: 178)
ANGPT2	9	TCTTCACTTGAGAGATAGAAAT (SEQ ID NO: 179)
ANGPT2	10	CATCAGCCAACCAGGAAATGAT (SEQ ID NO: 180)
ANGPT2	11	CTGTTAGCATTTGTGAACATTT (SEQ ID NO: 181)
ANGPT2	12	TGTGGTCCTTCCAACTTGAACG (SEQ ID NO: 182)
ANGPT2	13	CGGAATGTACTATCCACAGAGG (SEQ ID NO: 183)
ANGPT2	14	TTATTTGTGTTCTGCCTCTGTG (SEQ ID NO: 184)
ANGPT2	15	ACAAATAAGTTCAACGGCATTA (SEQ ID NO: 185)
ANGPT2	16	AGCGAATAGCCTGAGCCTTTCC (SEQ ID NO: 186)

[Table 10]

Target sequences of VEGFA gene for SpCas9

Gene	No.	Target sequence
VEGFA-Sp1	1	CAGCTGACCAGTCGCGCTGA (SEQ ID NO: 187)
VEGFA-Sp2	2	GTGGTAGCTGGGGCTGGGGG (SEQ ID NO: 188)
VEGFA-Sp3	3	GAGGTGGTAGCTGGGGCTGG (SEQ ID NO: 189)
VEGFA-Sp4	4	GGAGGTGGTAGCTGGGGCTG (SEQ ID NO: 190)
VEGFA-Sp5	5	AGGAGGTGGTAGCTGGGGCT (SEQ ID NO: 191)
VEGFA-Sp6	6	GAGGAGGTGGTAGCTGGGGC (SEQ ID NO: 192)
VEGFA-Sp7	7	CGGGGAGGAGGTGGTAGCTG (SEQ ID NO: 193)
VEGFA-Sp8	8	CCCAGCTACCACCTCCTCCC (SEQ ID NO: 194)
VEGFA-Sp9	9	CCGGGGAGGAGGTGGTAGCT (SEQ ID NO: 195)
VEGFA-Sp10	10	GCCGGGGAGGAGGTGGTAGC (SEQ ID NO: 196)
VEGFA-Sp11	11	GCTACCACCTCCTCCCCGGC (SEQ ID NO: 197)
VEGFA-Sp12	12	ACCACCTCCTCCCCGGCCGG (SEQ ID NO: 198)
VEGFA-Sp13	13	GCCGCCGGCCGGGGAGGAGG (SEQ ID NO: 199)
VEGFA-Sp14	14	ACCTCCTCCCCGGCCGGCGG (SEQ ID NO: 200)
VEGFA-Sp15	15	TCCGCCGCCGGCCGGGGAGG (SEQ ID NO: 201)
VEGFA-Sp16	16	CTGTCCGCCGCCGGCCGGGG (SEQ ID NO: 202)
VEGFA-Sp17	17	CCCCGGCCGGCGGCGGACAG (SEQ ID NO: 203)
VEGFA-Sp18	18	CCACTGTCCGCCGCCGGCCG (SEQ ID NO: 204)
VEGFA-Sp19	19	TCCACTGTCCGCCGCCGGCC (SEQ ID NO: 205)
VEGFA-Sp20	20	GTCCACTGTCCGCCGCCGGC (SEQ ID NO: 206)
VEGFA-Sp21	21	CCGGCGGCGGACAGTGGACG (SEQ ID NO: 207)
VEGFA-Sp22	22	CCGCGTCCACTGTCCGCCGC (SEQ ID NO: 208)
VEGFA-Sp23	23	GCGGCGGACAGTGGACGCGG (SEQ ID NO: 209)
VEGFA-Sp24	24	GTGGACGCGGCGGCGAGCCG (SEQ ID NO: 210)
VEGFA-Sp25	25	TGGACGCGGCGGCGAGCCGC (SEQ ID NO: 211)
VEGFA-Sp26	26	CGCGGCGGCGAGCCGCGGGC (SEQ ID NO: 212)
VEGFA-Sp27	27	GCGGCGGCGAGCCGCGGGCA (SEQ ID NO: 213)
VEGFA-Sp28	28	CGGCGGCGAGCCGCGGGCAG (SEQ ID NO: 214)
VEGFA-Sp29	29	GGCGAGCCGCGGGCAGGGGC (SEQ ID NO: 215)
VEGFA-Sp30	30	CGGGCTCCGGCCCCTGCCCG (SEQ ID NO: 216)
VEGFA-Sp31	31	CAGGGGCCGGAGCCCGCGCC (SEQ ID NO: 217)
VEGFA-Sp32	32	GGGCCGGAGCCCGCGCCCGG (SEQ ID NO: 218)
VEGFA-Sp33	33	CCGGAGCCCGCGCCCGGAGG (SEQ ID NO: 219)
VEGFA-Sp34	34	CCGCCTCCGGGCGCGGGCTC (SEQ ID NO: 220)
VEGFA-Sp35	35	CGGAGCCCGCGCCCGGAGGC (SEQ ID NO: 221)

VEGFA-Sp36	36	GGAGCCCGCGCCCGGAGGCG (SEQ ID NO: 222)
VEGFA-Sp37	37	GCCCGCGCCCGGAGGCGGGG (SEQ ID NO: 223)
VEGFA-Sp38	38	TCCACCCCGCCTCCGGGCGC (SEQ ID NO: 224)
VEGFA-Sp39	39	CTCCACCCCGCCTCCGGGCG (SEQ ID NO: 225)
VEGFA-Sp40	40	CGCGCCCGGAGGCGGGGTGG (SEQ ID NO: 226)
VEGFA-Sp41	41	GCGCCCGGAGGCGGGGTGGA (SEQ ID NO: 227)
VEGFA-Sp42	42	CGCCCGGAGGCGGGGTGGAG (SEQ ID NO: 228)
VEGFA-Sp43	43	GCCCGGAGGCGGGGTGGAGG (SEQ ID NO: 229)
VEGFA-Sp44	44	ACCCCCTCCACCCCGCCTCC (SEQ ID NO: 230)
VEGFA-Sp45	45	GACCCCCTCCACCCCGCCTC (SEQ ID NO: 231)
VEGFA-Sp46	46	GGAGGCGGGGTGGAGGGGGT (SEQ ID NO: 232)
VEGFA-Sp47	47	GAGGCGGGGTGGAGGGGGTC (SEQ ID NO: 233)
VEGFA-Sp48	48	AGGCGGGGTGGAGGGGGTCG (SEQ ID NO: 234)
VEGFA-Sp49	49	GTGGAGGGGGTCGGGGCTCG (SEQ ID NO: 235)
VEGFA-Sp50	50	GAAACTTTTCGTCCAACTTC (SEQ ID NO: 236)
VEGFA-Sp51	51	AAACTTTTCGTCCAACTTCT (SEQ ID NO: 237)
VEGFA-Sp52	52	AGCGAGAACAGCCCAGAAGT (SEQ ID NO: 238)
VEGFA-Sp53	53	CTTCTGGGCTGTTCTCGCTT (SEQ ID NO: 239)
VEGFA-Sp54	54	CTGGGCTGTTCTCGCTTCGG (SEQ ID NO: 240)
VEGFA-Sp55	55	TTCTCGCTTCGGAGGAGCCG (SEQ ID NO: 241)
VEGFA-Sp56	56	CGGAGGAGCCGTGGTCCGCG (SEQ ID NO: 242)
VEGFA-Sp57	57	GGAGGAGCCGTGGTCCGCGC (SEQ ID NO: 243)
VEGFA-Sp58	58	GAGGAGCCGTGGTCCGCGCG (SEQ ID NO: 244)
VEGFA-Sp59	59	AGGAGCCGTGGTCCGCGCGG (SEQ ID NO: 245)
VEGFA-Sp60	60	GGCTTCCCCCGCGCGGACCA (SEQ ID NO: 246)
VEGFA-Sp61	61	TCGGCTCGGCTTCCCCCGCG (SEQ ID NO: 247)
VEGFA-Sp62	62	GCGGGGGAAGCCGAGCCGAG (SEQ ID NO: 248)
VEGFA-Sp63	63	TCTCGCGGCTCCGCTCGGCT (SEQ ID NO: 249)
VEGFA-Sp64	64	GCACTTCTCGCGGCTCCGCT (SEQ ID NO: 250)
VEGFA-Sp65	65	AGCCGCGAGAAGTGCTAGCT (SEQ ID NO: 251)
VEGFA-Sp66	66	GCCGCGAGAAGTGCTAGCTC (SEQ ID NO: 252)
VEGFA-Sp67	67	GCCCGAGCTAGCACTTCTCG (SEQ ID NO: 253)
VEGFA-Sp68	68	CGAGAAGTGCTAGCTCGGGC (SEQ ID NO: 254)
VEGFA-Sp69	69	GAGAAGTGCTAGCTCGGGCC (SEQ ID NO: 255)
VEGFA-Sp70	70	AAGTGCTAGCTCGGGCCGGG (SEQ ID NO: 256)
VEGFA-Sp71	71	GGGCCGGGAGGAGCCGCAGC (SEQ ID NO: 257)

VEGFA-Sp72	72	CCGGGAGGAGCCGCAGCCGG (SEQ ID NO: 258)
VEGFA-Sp73	73	CCTCCGGCTGCGGCTCCTCC (SEQ ID NO: 259)
VEGFA-Sp74	74	GGAGGAGCCGCAGCCGGAGG (SEQ ID NO: 260)
VEGFA-Sp75	75	GAGGAGCCGCAGCCGGAGGA (SEQ ID NO: 261)
VEGFA-Sp76	76	AGGAGCCGCAGCCGGAGGAG (SEQ ID NO: 262)
VEGFA-Sp77	77	GGAGCCGCAGCCGGAGGAGG (SEQ ID NO: 263)
VEGFA-Sp78	78	GCCGCAGCCGGAGGAGGGGG (SEQ ID NO: 264)
VEGFA-Sp79	79	TCCTCCCCCTCCTCCGGCTG (SEQ ID NO: 265)
VEGFA-Sp80	80	GCAGCCGGAGGAGGGGGAGG (SEQ ID NO: 266)
VEGFA-Sp81	81	TCTTCCTCCTCCCCCTCCTC (SEQ ID NO: 267)
VEGFA-Sp82	82	GAAGAGAAGGAAGAGGAGAG (SEQ ID NO: 268)
VEGFA-Sp83	83	AAGAGAAGGAAGAGGAGAGG (SEQ ID NO: 269)
VEGFA-Sp84	84	AAGAGGAGAGGGGGCCGCAG (SEQ ID NO: 270)
VEGFA-Sp85	85	AGGGGGCCGCAGTGGCGACT (SEQ ID NO: 271)
VEGFA-Sp86	86	CGAGCGCCGAGTCGCCACTG (SEQ ID NO: 272)
VEGFA-Sp87	87	CGCAGTGGCGACTCGGCGCT (SEQ ID NO: 273)
VEGFA-Sp88	88	GCGACTCGGCGCTCGGAAGC (SEQ ID NO: 274)
VEGFA-Sp89	89	CGACTCGGCGCTCGGAAGCC (SEQ ID NO: 275)
VEGFA-Sp90	90	GCGCTCGGAAGCCGGGCTCA (SEQ ID NO: 276)
VEGFA-Sp91	91	TCGGAAGCCGGGCTCATGGA (SEQ ID NO: 277)
VEGFA-Sp92	92	CGGAAGCCGGGCTCATGGAC (SEQ ID NO: 278)
VEGFA-Sp93	93	GCCGGGCTCATGGACGGGTG (SEQ ID NO: 279)
VEGFA-Sp94	94	GCCTCACCCGTCCATGAGCC (SEQ ID NO: 280)
VEGFA-Sp95	95	GGGCTCATGGACGGGTGAGG (SEQ ID NO: 281)
VEGFA-Sp96	96	CTCATGGACGGGTGAGGCGG (SEQ ID NO: 282)
VEGFA-Sp97	97	TCCAGCCGCGCGCGCTCCCC (SEQ ID NO: 283)
VEGFA-Sp98	98	GCCTGGGGAGCGCGCGCGGC (SEQ ID NO: 284)
VEGFA-Sp99	99	CAGGGCCTGGGGAGCGCGCG (SEQ ID NO: 285)
VEGFA-Sp100	100	CGCGCGCGCTCCCCAGGCCC (SEQ ID NO: 286)
VEGFA-Sp101	101	GCGCTCCCCAGGCCCTGGCC (SEQ ID NO: 287)
VEGFA-Sp102	102	CGCTCCCCAGGCCCTGGCCC (SEQ ID NO: 288)
VEGFA-Sp103	103	GAGGCCCGGGCCAGGGCCTG (SEQ ID NO: 289)
VEGFA-Sp104	104	CGAGGCCCGGGCCAGGGCCT (SEQ ID NO: 290)
VEGFA-Sp105	105	CCAGGCCCTGGCCCGGGCCT (SEQ ID NO: 291)
VEGFA-Sp106	106	CCGAGGCCCGGGCCAGGGCC (SEQ ID NO: 292)
VEGFA-Sp107	107	CAGGCCCTGGCCCGGGCCTC (SEQ ID NO: 293)

VEGFA-Sp108	108	CCCTGGCCCGGGCCTCGGGC (SEQ ID NO: 294)
VEGFA-Sp109	109	CCGGCCCGAGGCCCGGGCCA (SEQ ID NO: 295)
VEGFA-Sp110	110	CCTGGCCCGGGCCTCGGGCC (SEQ ID NO: 296)
VEGFA-Sp111	111	CCCGGCCCGAGGCCCGGGCC (SEQ ID NO: 297)
VEGFA-Sp112	112	CTGGCCCGGGCCTCGGGCCG (SEQ ID NO: 298)
VEGFA-Sp113	113	GCCCGGGCCTCGGGCCGGGG (SEQ ID NO: 299)
VEGFA-Sp114	114	TCCTCCCCGGCCCGAGGCCC (SEQ ID NO: 300)
VEGFA-Sp115	115	TTCCTCCCCGGCCCGAGGCC (SEQ ID NO: 301)
VEGFA-Sp116	116	TACTCTTCCTCCCCGGCCCG (SEQ ID NO: 302)
VEGFA-Sp117	117	GGCGAGCTACTCTTCCTCCC (SEQ ID NO: 303)
VEGFA-Sp118	118	GGAGGAAGAGTAGCTCGCCG (SEQ ID NO: 304)
VEGFA-Sp119	119	AGTAGCTCGCCGAGGCGCCG (SEQ ID NO: 305)
VEGFA-Sp120	120	CGCCGAGGCGCCGAGGAGAG (SEQ ID NO: 306)
VEGFA-Sp121	121	GCCGAGGCGCCGAGGAGAGC (SEQ ID NO: 307)
VEGFA-Sp122	122	GCCCGCTCTCCTCGGCGCCT (SEQ ID NO: 308)
VEGFA-Sp123	123	GTGGGGCGGCCCGCTCTCCT (SEQ ID NO: 309)
VEGFA-Sp124	124	GGCCGCCCCACAGCCCGAGC (SEQ ID NO: 310)
VEGFA-Sp125	125	CTCCGGCTCGGGCTGTGGGG (SEQ ID NO: 311)
VEGFA-Sp126	126	CCCCACAGCCCGAGCCGGAG (SEQ ID NO: 312)
VEGFA-Sp127	127	CCTCTCCGGCTCGGGCTGTG (SEQ ID NO: 313)
VEGFA-Sp128	128	CCCACAGCCCGAGCCGGAGA (SEQ ID NO: 314)
VEGFA-Sp129	129	CCCTCTCCGGCTCGGGCTGT (SEQ ID NO: 315)
VEGFA-Sp130	130	TCCCTCTCCGGCTCGGGCTG (SEQ ID NO: 316)
VEGFA-Sp131	131	TCGCGCTCCCTCTCCGGCTC (SEQ ID NO: 317)
VEGFA-Sp132	132	CTCGCGCTCCCTCTCCGGCT (SEQ ID NO: 318)
VEGFA-Sp133	133	CGCGGCTCGCGCTCCCTCTC (SEQ ID NO: 319)
VEGFA-Sp134	134	AGAGGGAGCGCGAGCCGCGC (SEQ ID NO: 320)
VEGFA-Sp135	135	CGAGCCGCGCCGGCCCCGGT (SEQ ID NO: 321)
VEGFA-Sp136	136	GAGCCGCGCCGGCCCCGGTC (SEQ ID NO: 322)
VEGFA-Sp137	137	AGGCCCGACCGGGGCCGGCG (SEQ ID NO: 323)
VEGFA-Sp138	138	TTCGGAGGCCCGACCGGGGC (SEQ ID NO: 324)
VEGFA-Sp139	139	TGGTTTCGGAGGCCCGACCG (SEQ ID NO: 325)
VEGFA-Sp140	140	ATGGTTTCGGAGGCCCGACC (SEQ ID NO: 326)
VEGFA-Sp141	141	CATGGTTTCGGAGGCCCGAC (SEQ ID NO: 327)
VEGFA-Sp142	142	CAGAAAGTTCATGGTTTCGG (SEQ ID NO: 328)
VEGFA-Sp143	143	CAGCAGAAAGTTCATGGTTT (SEQ ID NO: 329)

VEGFA-Sp144	144	CCATGAACTTTCTGCTGTCT (SEQ ID NO: 330)
VEGFA-Sp145	145	CCAAGACAGCAGAAAGTTCA (SEQ ID NO: 331)
VEGFA-Sp146	146	CATGAACTTTCTGCTGTCTT (SEQ ID NO: 332)
VEGFA-Sp147	147	TTCTGCTGTCTTGGGTGCAT (SEQ ID NO: 333)
VEGFA-Sp148	148	GGAGGTAGAGCAGCAAGGCA (SEQ ID NO: 334)
VEGFA-Sp149	149	ATGGTGGAGGTAGAGCAGCA (SEQ ID NO: 335)
VEGFA-Sp150	150	GCTCTACCTCCACCATGCCA (SEQ ID NO: 336)
VEGFA-Sp151	151	CGCTTACCTTGGCATGGTGG (SEQ ID NO: 337)
VEGFA-Sp152	152	GACCGCTTACCTTGGCATGG (SEQ ID NO: 338)
VEGFA-Sp153	153	CACGACCGCTTACCTTGGCA (SEQ ID NO: 339)
VEGFA-Sp154	154	TTTCTGTCCTCAGTGGTCCC (SEQ ID NO: 340)
VEGFA-Sp155	155	GGTGCAGCCTGGGACCACTG (SEQ ID NO: 341)
VEGFA-Sp156	156	GTGGTCCCAGGCTGCACCCA (SEQ ID NO: 342)
VEGFA-Sp157	157	TTCTGCCATGGGTGCAGCCT (SEQ ID NO: 343)
VEGFA-Sp158	158	CTTCTGCCATGGGTGCAGCC (SEQ ID NO: 344)
VEGFA-Sp159	159	CAGGCTGCACCCATGGCAGA (SEQ ID NO: 345)
VEGFA-Sp160	160	GCTGCACCCATGGCAGAAGG (SEQ ID NO: 346)
VEGFA-Sp161	161	GCACCCATGGCAGAAGGAGG (SEQ ID NO: 347)
VEGFA-Sp162	162	CACCCATGGCAGAAGGAGGA (SEQ ID NO: 348)
VEGFA-Sp163	163	TGCCCTCCTCCTTCTGCCAT (SEQ ID NO: 349)
VEGFA-Sp164	164	CTGCCCTCCTCCTTCTGCCA (SEQ ID NO: 350)
VEGFA-Sp165	165	GGAGGGCAGAATCATCACGA (SEQ ID NO: 351)
VEGFA-Sp166	166	TCATGCAGTGGTGAAGTTCA (SEQ ID NO: 352)
VEGFA-Sp167	167	CTGCCATCCAATCGAGACCC (SEQ ID NO: 353)
VEGFA-Sp168	168	CCATCCAATCGAGACCCTGG (SEQ ID NO: 354)
VEGFA-Sp169	169	CCACCAGGGTCTCGATTGGA (SEQ ID NO: 355)
VEGFA-Sp170	170	ATGTCCACCAGGGTCTCGAT (SEQ ID NO: 356)
VEGFA-Sp171	171	GACCCTGGTGGACATCTTCC (SEQ ID NO: 357)
VEGFA-Sp172	172	CTCCTGGAAGATGTCCACCA (SEQ ID NO: 358)
VEGFA-Sp173	173	ACTCCTGGAAGATGTCCACC (SEQ ID NO: 359)
VEGFA-Sp174	174	CGATCTCATCAGGGTACTCC (SEQ ID NO: 360)
VEGFA-Sp175	175	AGATGTACTCGATCTCATCA (SEQ ID NO: 361)
VEGFA-Sp176	176	AAGATGTACTCGATCTCATC (SEQ ID NO: 362)
VEGFA-Sp177	177	CGCATCAGGGGCACACAGGA (SEQ ID NO: 363)
VEGFA-Sp178	178	GCATCGCATCAGGGGCACAC (SEQ ID NO: 364)
VEGFA-Sp179	179	TGTGTGCCCCTGATGCGATG (SEQ ID NO: 365)

VEGFA-Sp180	180	GTGTGCCCCTGATGCGATGC (SEQ ID NO: 366)
VEGFA-Sp181	181	TGTGCCCCTGATGCGATGCG (SEQ ID NO: 367)
VEGFA-Sp182	182	GTGCCCCTGATGCGATGCGG (SEQ ID NO: 368)
VEGFA-Sp183	183	CAGCCCCCGCATCGCATCAG (SEQ ID NO: 369)
VEGFA-Sp184	184	GCAGCCCCCGCATCGCATCA (SEQ ID NO: 370)
VEGFA-Sp185	185	AGCAGCCCCCGCATCGCATC (SEQ ID NO: 371)
VEGFA-Sp186	186	CGGGGGCTGCTGCAATGACG (SEQ ID NO: 372)
VEGFA-Sp187	187	GGGGGCTGCTGCAATGACGA (SEQ ID NO: 373)
VEGFA-Sp188	188	CTGCTGCAATGACGAGGGCC (SEQ ID NO: 374)
VEGFA-Sp189	189	CCTGGAGTGTGTGCCCACTG (SEQ ID NO: 375)
VEGFA-Sp190	190	CCTCAGTGGGCACACACTCC (SEQ ID NO: 376)
VEGFA-Sp191	191	GTGATGTTGGACTCCTCAGT (SEQ ID NO: 377)
VEGFA-Sp192	192	GGTGATGTTGGACTCCTCAG (SEQ ID NO: 378)
VEGFA-Sp193	193	GGAGTCCAACATCACCATGC (SEQ ID NO: 379)
VEGFA-Sp194	194	GTCCAACATCACCATGCAGG (SEQ ID NO: 380)
VEGFA-Sp195	195	TCCAACATCACCATGCAGGT (SEQ ID NO: 381)
VEGFA-Sp196	196	GCCCACCTGCATGGTGATGT (SEQ ID NO: 382)
VEGFA-Sp197	197	CCCAAAGATGCCCACCTGCA (SEQ ID NO: 383)
VEGFA-Sp198	198	TCCTTCCTTTCCAGATTATG (SEQ ID NO: 384)
VEGFA-Sp199	199	GAGGTTTGATCCGCATAATC (SEQ ID NO: 385)
VEGFA-Sp200	200	ATGCGGATCAAACCTCACCA (SEQ ID NO: 386)
VEGFA-Sp201	201	CCTCACCAAGGCCAGCACAT (SEQ ID NO: 387)
VEGFA-Sp202	202	CCTATGTGCTGGCCTTGGTG (SEQ ID NO: 388)
VEGFA-Sp203	203	TCTCTCCTATGTGCTGGCCT (SEQ ID NO: 389)
VEGFA-Sp204	204	AGCTCATCTCTCCTATGTGC (SEQ ID NO: 390)
VEGFA-Sp205	205	ATTCACATTTGTTGTGCTGT (SEQ ID NO: 391)
VEGFA-Sp206	206	AGCACAACAAATGTGAATGC (SEQ ID NO: 392)
VEGFA-Sp207	207	AACAAATGTGAATGCAGGTG (SEQ ID NO: 393)
VEGFA-Sp208	208	TGTCTTGCTCTATCTTTCTT (SEQ ID NO: 394)
VEGFA-Sp209	209	TTTTCCAGAAAATCAGTTCG (SEQ ID NO: 395)
VEGFA-Sp210	210	CTTTCCTCGAACTGATTTTC (SEQ ID NO: 396)
VEGFA-Sp211	211	CAGAAAATCAGTTCGAGGAA (SEQ ID NO: 397)
VEGFA-Sp212	212	AGAAAATCAGTTCGAGGAAA (SEQ ID NO: 398)
VEGFA-Sp213	213	ATCAGTTCGAGGAAAGGGAA (SEQ ID NO: 399)
VEGFA-Sp214	214	TCAGTTCGAGGAAAGGGAAA (SEQ ID NO: 400)
VEGFA-Sp215	215	CAGTTCGAGGAAAGGGAAAG (SEQ ID NO: 401)

VEGFA-Sp216	216	AACGAAAGCGCAAGAAATCC (SEQ ID NO: 402)
VEGFA-Sp217	217	AGAAATCCCGGTATAAGTCC (SEQ ID NO: 403)
VEGFA-Sp218	218	CACGCTCCAGGACTTATACC (SEQ ID NO: 404)
VEGFA-Sp219	219	ACACGCTCCAGGACTTATAC (SEQ ID NO: 405)
VEGFA-Sp220	220	AAGTCCTGGAGCGTGTACGT (SEQ ID NO: 406)
VEGFA-Sp221	221	GGCACCAACGTACACGCTCC (SEQ ID NO: 407)
VEGFA-Sp222	222	CCCGCTGCTGTCTAATGCCC (SEQ ID NO: 408)
VEGFA-Sp223	223	CCAGGGCATTAGACAGCAGC (SEQ ID NO: 409)
VEGFA-Sp224	224	TCCAGGGCATTAGACAGCAG (SEQ ID NO: 410)
VEGFA-Sp225	225	CTAATGCCCTGGAGCCTCCC (SEQ ID NO: 411)
VEGFA-Sp226	226	TGGGGGCCAGGGAGGCTCCA (SEQ ID NO: 412)
VEGFA-Sp227	227	CTGGGGGCCAGGGAGGCTCC (SEQ ID NO: 413)
VEGFA-Sp228	228	GGTTGTACTGGGGGCCAGGG (SEQ ID NO: 414)
VEGFA-Sp229	229	GGAGGTTGTACTGGGGGCCA (SEQ ID NO: 415)
VEGFA-Sp230	230	CGGAGGTTGTACTGGGGGCC (SEQ ID NO: 416)
VEGFA-Sp231	231	GCAGGCGGAGGTTGTACTGG (SEQ ID NO: 417)
VEGFA-Sp232	232	TTGCCTTTTTGCAGTCCCTG (SEQ ID NO: 418)
VEGFA-Sp233	233	TGCCTTTTTGCAGTCCCTGT (SEQ ID NO: 419)
VEGFA-Sp234	234	CGCTCTGAGCAAGGCCCACA (SEQ ID NO: 420)
VEGFA-Sp235	235	CCTGTGGGCCTTGCTCAGAG (SEQ ID NO: 421)
VEGFA-Sp236	236	CCGCTCTGAGCAAGGCCCAC (SEQ ID NO: 422)
VEGFA-Sp237	237	TGCTTTCTCCGCTCTGAGCA (SEQ ID NO: 423)
VEGFA-Sp238	238	CAGGAACATTTACACGTCTG (SEQ ID NO: 424)
VEGFA-Sp239	239	ACGCGAGTCTGTGTTTTTGC (SEQ ID NO: 425)
VEGFA-Sp240	240	AAACACAGACTCGCGTTGCA (SEQ ID NO: 426)
VEGFA-Sp241	241	CAGACTCGCGTTGCAAGGCG (SEQ ID NO: 427)
VEGFA-Sp242	242	AGTTAAACGAACGTACTTGC (SEQ ID NO: 428)
VEGFA-Sp243	243	AAACGAACGTACTTGCAGGT (SEQ ID NO: 429)
VEGFA-Sp244	244	CCCTCAGATGTGACAAGCCG (SEQ ID NO: 430)
VEGFA-Sp245	245	GCCTCGGCTTGTCACATCTG (SEQ ID NO: 431)
VEGFA-Sp246	246	TCAGATGTGACAAGCCGAGG (SEQ ID NO: 432)
VEGFA-Sp247	247	ACAAGCCGAGGCGGTGAGCC (SEQ ID NO: 433)
VEGFA-Sp248	248	GCCGAGGCGGTGAGCCGGGC (SEQ ID NO: 434)
VEGFA-Sp249	249	TCCTGCCCGGCTCACCGCCT (SEQ ID NO: 435)

[Table 11]

Target sequences of HIF1A gene for SpCas9

Gene	No.	Target sequence
HIF1A-Sp1	1	TTTAAATGAGCTCCCAATGT (SEQ ID NO: 436)
HIF1A-Sp2	2	GAGCTCCCAATGTCGGAGTT (SEQ ID NO: 437)
HIF1A-Sp3	3	GTTTTCCAAACTCCGACATT (SEQ ID NO: 438)
HIF1A-Sp4	4	TGTTTTCCAAACTCCGACAT (SEQ ID NO: 439)
HIF1A-Sp5	5	AAATTTGTCTTTTTAAAAGA (SEQ ID NO: 440)
HIF1A-Sp6	6	GTCTTTTTAAAAGAAGGTCT (SEQ ID NO: 441)
HIF1A-Sp7	7	AAACTCAAAACCTGAAGAAT (SEQ ID NO: 442)
HIF1A-Sp8	8	CTGATTTCTTCCAATTCTTC (SEQ ID NO: 443)
HIF1A-Sp9	9	GAAGAAATCAGAATAGAAAA (SEQ ID NO: 444)
HIF1A-Sp10	10	AAGAAATCAGAATAGAAAAT (SEQ ID NO: 445)
HIF1A-Sp11	11	ATCAGAATAGAAAATGGGTA (SEQ ID NO: 446)
HIF1A-Sp12	12	CTCGAGATGCAGCCAGATCT (SEQ ID NO: 447)
HIF1A-Sp13	13	TTCTTTACTTCGCCGAGATC (SEQ ID NO: 448)
HIF1A-Sp14	14	GAACTCACATTATGTGGAAG (SEQ ID NO: 449)
HIF1A-Sp15	15	AGATGCGAACTCACATTATG (SEQ ID NO: 450)
HIF1A-Sp16	16	TGTGAGTTCGCATCTTGATA (SEQ ID NO: 451)
HIF1A-Sp17	17	TTGATAAGGCCTCTGTGATG (SEQ ID NO: 452)
HIF1A-Sp18	18	GATGGTAAGCCTCATCACAG (SEQ ID NO: 453)
HIF1A-Sp19	19	CCATCAGCTATTTGCGTGTG (SEQ ID NO: 454)
HIF1A-Sp20	20	CCTCACACGCAAATAGCTGA (SEQ ID NO: 455)
HIF1A-Sp21	21	TTTGCGTGTGAGGAAACTTC (SEQ ID NO: 456)
HIF1A-Sp22	22	GTGAGGAAACTTCTGGATGC (SEQ ID NO: 457)
HIF1A-Sp23	23	TGTGCCCTTTTTAGGTGATT (SEQ ID NO: 458)
HIF1A-Sp24	24	TTGCTTTTATTTGAAAGCCT (SEQ ID NO: 459)
HIF1A-Sp25	25	TTTTATTTGAAAGCCTTGGA (SEQ ID NO: 460)
HIF1A-Sp26	26	AGCCTTGGATGGTTTTGTTA (SEQ ID NO: 461)
HIF1A-Sp27	27	AACCATAACAAAACCATCCA (SEQ ID NO: 462)
HIF1A-Sp28	28	GTTATGGTTCTCACAGATGA (SEQ ID NO: 463)
HIF1A-Sp29	29	TGATAATGTGAACAAATACA (SEQ ID NO: 464)
HIF1A-Sp30	30	GATAATGTGAACAAATACAT (SEQ ID NO: 465)
HIF1A-Sp31	31	CAAATACATGGGATTAACTC (SEQ ID NO: 466)
HIF1A-Sp32	32	TGTTTACAGTTTGAACTAAC (SEQ ID NO: 467)
HIF1A-Sp33	33	TACTCATCCATGTGACCATG (SEQ ID NO: 468)
HIF1A-Sp34	34	CTCATTTCCTCATGGTCACA (SEQ ID NO: 469)
HIF1A-Sp35	35	GCATTTCTCTCATTTCCTCA (SEQ ID NO: 470)

HIF1A-Sp36	36	GAAATGCTTACACACAGAAA (SEQ ID NO: 471)
HIF1A-Sp37	37	TTCATTAGGCCTTGTGAAAA (SEQ ID NO: 472)
HIF1A-Sp38	38	TCATTAGGCCTTGTGAAAAA (SEQ ID NO: 473)
HIF1A-Sp39	39	GTTCTTTACCCTTTTTCACA (SEQ ID NO: 474)
HIF1A-Sp40	40	AAGTGTACCCTAACTAGCCG (SEQ ID NO: 475)
HIF1A-Sp41	41	AGTTCTTCCTCGGCTAGTTA (SEQ ID NO: 476)
HIF1A-Sp42	42	TAGTTCTTCCTCGGCTAGTT (SEQ ID NO: 477)
HIF1A-Sp43	43	TTATGTTCATAGTTCTTCCT (SEQ ID NO: 478)
HIF1A-Sp44	44	TGAACATAAAGTCTGCAACA (SEQ ID NO: 479)
HIF1A-Sp45	45	CATAAAGTCTGCAACATGGA (SEQ ID NO: 480)
HIF1A-Sp46	46	ACACAGGTATTGCACTGCAC (SEQ ID NO: 481)
HIF1A-Sp47	47	TGGTATCATATACGTGAATG (SEQ ID NO: 482)
HIF1A-Sp48	48	ACACTGAGGTTGGTTACTGT (SEQ ID NO: 483)
HIF1A-Sp49	49	AACAGTAACCAACCTCAGTG (SEQ ID NO: 484)
HIF1A-Sp50	50	ACAGTAACCAACCTCAGTGT (SEQ ID NO: 485)
HIF1A-Sp51	51	TCTTATACCCACACTGAGGT (SEQ ID NO: 486)
HIF1A-Sp52	52	GGTTTCTTATACCCACACTG (SEQ ID NO: 487)
HIF1A-Sp53	53	GAAACCACCTATGACCTGCT (SEQ ID NO: 488)
HIF1A-Sp54	54	AGCACCAAGCAGGTCATAGG (SEQ ID NO: 489)
HIF1A-Sp55	55	ATCAGCACCAAGCAGGTCAT (SEQ ID NO: 490)
HIF1A-Sp56	56	TTCACAAATCAGCACCAAGC (SEQ ID NO: 491)
HIF1A-Sp57	57	ATATTTGATGGGTGAGGAAT (SEQ ID NO: 492)
HIF1A-Sp58	58	AATATTTGATGGGTGAGGAA (SEQ ID NO: 493)
HIF1A-Sp59	59	ATTTCAATATTTGATGGGTG (SEQ ID NO: 494)
HIF1A-Sp60	60	AAGGAATTTCAATATTTGAT (SEQ ID NO: 495)
HIF1A-Sp61	61	AAAGGAATTTCAATATTTGA (SEQ ID NO: 496)
HIF1A-Sp62	62	AGGAAAGTCTTGCTATCTAA (SEQ ID NO: 497)
HIF1A-Sp63	63	TTTCCTCAGTCGACACAGCC (SEQ ID NO: 498)
HIF1A-Sp64	64	TATCCAGGCTGTGTCGACTG (SEQ ID NO: 499)
HIF1A-Sp65	65	AATAAGAAAATTTCATATCC (SEQ ID NO: 500)
HIF1A-Sp66	66	AATTTTCTTATTGTGATGAA (SEQ ID NO: 501)
HIF1A-Sp67	67	TAACAGAATTACCGAATTGA (SEQ ID NO: 502)
HIF1A-Sp68	68	AACAGAATTACCGAATTGAT (SEQ ID NO: 503)
HIF1A-Sp69	69	TGGCTCATATCCCATCAATT (SEQ ID NO: 504)
HIF1A-Sp70	70	TATGAGCCAGAAGAACTTTT (SEQ ID NO: 505)
HIF1A-Sp71	71	GAGCGGCCTAAAAGTTCTTC (SEQ ID NO: 506)

HIF1A-Sp72	72	GATAATATTCATAAATTGAG (SEQ ID NO: 507)
HIF1A-Sp73	73	TTATGAATATTATCATGCTT (SEQ ID NO: 508)
HIF1A-Sp74	74	CTTACTATCATGATGAGTTT (SEQ ID NO: 509)
HIF1A-Sp75	75	TCCCCCCTAGTGTTTACTAA (SEQ ID NO: 510)
HIF1A-Sp76	76	TTGTCCTTTAGTAAACACTA (SEQ ID NO: 511)
HIF1A-Sp77	77	CTTGTCCTTTAGTAAACACT (SEQ ID NO: 512)
HIF1A-Sp78	78	ACTAAAGGACAAGTCACCAC (SEQ ID NO: 513)
HIF1A-Sp79	79	AAGTCACCACAGGACAGTAC (SEQ ID NO: 514)
HIF1A-Sp80	80	AAGCATCCTGTACTGTCCTG (SEQ ID NO: 515)
HIF1A-Sp81	81	TACAGGATGCTTGCCAAAAG (SEQ ID NO: 516)
HIF1A-Sp82	82	AGGATGCTTGCCAAAAGAGG (SEQ ID NO: 517)
HIF1A-Sp83	83	CCAAAAGAGGTGGATATGTC (SEQ ID NO: 518)
HIF1A-Sp84	84	CCAGACATATCCACCTCTTT (SEQ ID NO: 519)
HIF1A-Sp85	85	CAAAAGAGGTGGATATGTCT (SEQ ID NO: 520)
HIF1A-Sp86	86	GCACTGTGGTTGAGAATTCT (SEQ ID NO: 521)
HIF1A-Sp87	87	TTCACACATACAATGCACTG (SEQ ID NO: 522)
HIF1A-Sp88	88	TATGTGTGAATTACGTTGTG (SEQ ID NO: 523)
HIF1A-Sp89	89	GACACATTCTGTTTGTTGAA (SEQ ID NO: 524)
HIF1A-Sp90	90	GGACACATTCTGTTTGTTGA (SEQ ID NO: 525)
HIF1A-Sp91	91	AACAGAATGTGTCCTTAAAC (SEQ ID NO: 526)
HIF1A-Sp92	92	CTGAAGATTCAACCGGTTTA (SEQ ID NO: 527)
HIF1A-Sp93	93	TTCATATCTGAAGATTCAAC (SEQ ID NO: 528)
HIF1A-Sp94	94	TGTATCTTCTGATTCAACTT (SEQ ID NO: 529)
HIF1A-Sp95	95	CCTCTTTGACAAACTTAAGA (SEQ ID NO: 530)
HIF1A-Sp96	96	CCTTCTTAAGTTTGTCAAAG (SEQ ID NO: 531)
HIF1A-Sp97	97	ACCTGATGCTTTAACTTTGC (SEQ ID NO: 532)
HIF1A-Sp98	98	GCCAGCAAAGTTAAAGCATC (SEQ ID NO: 533)
HIF1A-Sp99	99	ACTTTGCTGGCCCCAGCCGC (SEQ ID NO: 534)
HIF1A-Sp100	100	GATTGTGTCTCCAGCGGCTG (SEQ ID NO: 535)
HIF1A-Sp101	101	TGATTGTGTCTCCAGCGGCT (SEQ ID NO: 536)
HIF1A-Sp102	102	ATGATTGTGTCTCCAGCGGC (SEQ ID NO: 537)
HIF1A-Sp103	103	AGATATGATTGTGTCTCCAG (SEQ ID NO: 538)
HIF1A-Sp104	104	ACAATCATATCTTTAGATTT (SEQ ID NO: 539)
HIF1A-Sp105	105	TCTTTAGATTTTGGCAGCAA (SEQ ID NO: 540)
HIF1A-Sp106	106	GTCATCAGTTTCTGTGTCTG (SEQ ID NO: 541)
HIF1A-Sp107	107	AACTGATGACCAGCAACTTG (SEQ ID NO: 542)

HIF1A-Sp108	108	ATGGTACTTCCTCAAGTTGC (SEQ ID NO: 543)
HIF1A-Sp109	109	AGCATTACATCATTATATAA (SEQ ID NO: 544)
HIF1A-Sp110	110	GTAATTTTTCGTTGGGTGAG (SEQ ID NO: 545)
HIF1A-Sp111	111	TGTAATTTTTCGTTGGGTGA (SEQ ID NO: 546)
HIF1A-Sp112	112	CTGTAATTTTTCGTTGGGTG (SEQ ID NO: 547)
HIF1A-Sp113	113	ATATTCTGTAATTTTTCGTT (SEQ ID NO: 548)
HIF1A-Sp114	114	TATATTCTGTAATTTTTCGT (SEQ ID NO: 549)
HIF1A-Sp115	115	AAAATTACAGAATATAAATT (SEQ ID NO: 550)
HIF1A-Sp116	116	GGCGTTTCAGCGGTGGGTAA (SEQ ID NO: 551)
HIF1A-Sp117	117	GGCTTTGGCGTTTCAGCGGT (SEQ ID NO: 552)
HIF1A-Sp118	118	TGGCTTTGGCGTTTCAGCGG (SEQ ID NO: 553)
HIF1A-Sp119	119	AAGTGGCTTTGGCGTTTCAG (SEQ ID NO: 554)
HIF1A-Sp120	120	GCACTACTTCGAAGTGGCTT (SEQ ID NO: 555)
HIF1A-Sp121	121	GGGTCAGCACTACTTCGAAG (SEQ ID NO: 556)
HIF1A-Sp122	122	CAACTTCTTGATTGAGTGCA (SEQ ID NO: 557)
HIF1A-Sp123	123	GCAACTTCTTGATTGAGTGC (SEQ ID NO: 558)
HIF1A-Sp124	124	AGAACCAAATCCAGAGTCAC (SEQ ID NO: 559)
HIF1A-Sp125	125	AGTTCCAGTGACTCTGGATT (SEQ ID NO: 560)
HIF1A-Sp126	126	AAAGAAAGTTCCAGTGACTC (SEQ ID NO: 561)
HIF1A-Sp127	127	TTTTACCATGCCCCAGATTC (SEQ ID NO: 562)
HIF1A-Sp128	128	CTGATCCTGAATCTGGGGCA (SEQ ID NO: 563)
HIF1A-Sp129	129	GGTGTCTGATCCTGAATCTG (SEQ ID NO: 564)
HIF1A-Sp130	130	AGGTGTCTGATCCTGAATCT (SEQ ID NO: 565)
HIF1A-Sp131	131	TAGGTGTCTGATCCTGAATC (SEQ ID NO: 566)
HIF1A-Sp132	132	CAGACACCTAGTCCTTCCGA (SEQ ID NO: 567)
HIF1A-Sp133	133	GTGCTTCCATCGGAAGGACT (SEQ ID NO: 568)
HIF1A-Sp134	134	TGTCTAGTGCTTCCATCGGA (SEQ ID NO: 569)
HIF1A-Sp135	135	ACTTTGTCTAGTGCTTCCAT (SEQ ID NO: 570)
HIF1A-Sp136	136	CACTAGACAAAGTTCACCTG (SEQ ID NO: 571)
HIF1A-Sp137	137	AGACAAAGTTCACCTGAGGT (SEQ ID NO: 572)
HIF1A-Sp138	138	TATATCATGACACCTACCTC (SEQ ID NO: 573)
HIF1A-Sp139	139	CAATATTCACTGGGACTATT (SEQ ID NO: 574)
HIF1A-Sp140	140	ACATAAAAACAATATTCACT (SEQ ID NO: 575)
HIF1A-Sp141	141	CACATAAAAACAATATTCAC (SEQ ID NO: 576)
HIF1A-Sp142	142	CAGTGAATATTGTTTTTATG (SEQ ID NO: 577)
HIF1A-Sp143	143	TTTTTATGTGGATAGTGATA (SEQ ID NO: 578)

HIF1A-Sp144	144	TATGGTCAATGAATTCAAGT (SEQ ID NO: 579)
HIF1A-Sp145	145	CAATGAATTCAAGTTGGAAT (SEQ ID NO: 580)
HIF1A-Sp146	146	AAAGAACCCATTTTCTACTC (SEQ ID NO: 581)
HIF1A-Sp147	147	CATATACCTGAGTAGAAAAT (SEQ ID NO: 582)
HIF1A-Sp148	148	TCATATACCTGAGTAGAAAA (SEQ ID NO: 583)
HIF1A-Sp149	149	AAAGGACACAGATTTAGACT (SEQ ID NO: 584)
HIF1A-Sp150	150	GTTAGCTCCCTATATCCCAA (SEQ ID NO: 585)
HIF1A-Sp151	151	TCATCATCCATTGGGATATA (SEQ ID NO: 586)
HIF1A-Sp152	152	GTCATCATCCATTGGGATAT (SEQ ID NO: 587)
HIF1A-Sp153	153	ACTGGAAGTCATCATCCATT (SEQ ID NO: 588)
HIF1A-Sp154	154	AACTGGAAGTCATCATCCAT (SEQ ID NO: 589)
HIF1A-Sp155	155	ACTGATCGAAGGAACGTAAC (SEQ ID NO: 590)
HIF1A-Sp156	156	TAATGGTGACAACTGATCGA (SEQ ID NO: 591)
HIF1A-Sp157	157	CTTGCGGAACTGCTTTCTAA (SEQ ID NO: 592)
HIF1A-Sp158	158	ACTTGCGCTTTCAGGGCTTG (SEQ ID NO: 593)
HIF1A-Sp159	159	TTTGAGGACTTGCGCTTTCA (SEQ ID NO: 594)
HIF1A-Sp160	160	CTTTGAGGACTTGCGCTTTC (SEQ ID NO: 595)
HIF1A-Sp161	161	AATACTGTAACTGTGCTTTG (SEQ ID NO: 596)
HIF1A-Sp162	162	GTTCTTGTATTTGAGTCTGC (SEQ ID NO: 597)
HIF1A-Sp163	163	GTAGTGGTGGCATTAGCAGT (SEQ ID NO: 598)
HIF1A-Sp164	164	AGTGGTGGCAGTGGTAGTGG (SEQ ID NO: 599)
HIF1A-Sp165	165	ATCAGTGGTGGCAGTGGTAG (SEQ ID NO: 600)
HIF1A-Sp166	166	TAATTCATCAGTGGTGGCAG (SEQ ID NO: 601)
HIF1A-Sp167	167	TGTTTTTAATTCATCAGTGG (SEQ ID NO: 602)
HIF1A-Sp168	168	CACTGTTTTTAATTCATCAG (SEQ ID NO: 603)
HIF1A-Sp169	169	AACAGTGACAAAAGACCGTA (SEQ ID NO: 604)
HIF1A-Sp170	170	ATATTTTAATGTCTTCCATA (SEQ ID NO: 605)
HIF1A-Sp171	171	TTATGTATGTGGGTAGGAGA (SEQ ID NO: 606)
HIF1A-Sp172	172	GTTTCTTTATGTATGTGGGT (SEQ ID NO: 607)
HIF1A-Sp173	173	AGTAGTTTCTTTATGTATGT (SEQ ID NO: 608)
HIF1A-Sp174	174	TAGTAGTTTCTTTATGTATG (SEQ ID NO: 609)
HIF1A-Sp175	175	ATCTCTATATGGTGATGATG (SEQ ID NO: 610)
HIF1A-Sp176	176	CGACTTTGAGTATCTCTATA (SEQ ID NO: 611)
HIF1A-Sp177	177	CATATAGAGATACTCAAAGT (SEQ ID NO: 612)
HIF1A-Sp178	178	ACAGCCTCACCAAACAGAGC (SEQ ID NO: 613)
HIF1A-Sp179	179	TTTTCCTGCTCTGTTTGGTG (SEQ ID NO: 614)

HIF1A-Sp180	180	TCACCAAACAGAGCAGGAAA (SEQ ID NO: 615)
HIF1A-Sp181	181	ACTCCTTTTCCTGCTCTGTT (SEQ ID NO: 616)
HIF1A-Sp182	182	GATAACACGTTAGGGCTTCT (SEQ ID NO: 617)
HIF1A-Sp183	183	AAGCGACAGATAACACGTTA (SEQ ID NO: 618)
HIF1A-Sp184	184	AAAGCGACAGATAACACGTT (SEQ ID NO: 619)
HIF1A-Sp185	185	TATCTGTCGCTTTGAGTCAA (SEQ ID NO: 620)
HIF1A-Sp186	186	TTTCAGAACTACAGTTCCTG (SEQ ID NO: 621)
HIF1A-Sp187	187	TTTGGATTTAGTTCTTCCTC (SEQ ID NO: 622)
HIF1A-Sp188	188	TTCTGCAAAGCTAGTATCTT (SEQ ID NO: 623)
HIF1A-Sp189	189	TGCTCAGAGAAAGCGAAAAA (SEQ ID NO: 624)
HIF1A-Sp190	190	AAGCGAAAAATGGAACATGA (SEQ ID NO: 625)
HIF1A-Sp191	191	GTAGTAGCTGCATGATCGTC (SEQ ID NO: 626)
HIF1A-Sp192	192	CAGCTACTACATCACTTTCT (SEQ ID NO: 627)
HIF1A-Sp193	193	CTTTCTTGGAAACGTGTAAA (SEQ ID NO: 628)
HIF1A-Sp194	194	ACAATTATTTTAATACCCTC (SEQ ID NO: 629)
HIF1A-Sp195	195	AAAAGAATAAACTAACCAGA (SEQ ID NO: 630)
HIF1A-Sp196	196	AAAAAGAATAAACTAACCAG (SEQ ID NO: 631)
HIF1A-Sp197	197	AGATTTAGCATGTAGACTGC (SEQ ID NO: 632)
HIF1A-Sp198	198	GATTTAGCATGTAGACTGCT (SEQ ID NO: 633)
HIF1A-Sp199	199	ATTTAGCATGTAGACTGCTG (SEQ ID NO: 634)
HIF1A-Sp200	200	TAGACTGCTGGGGCAATCAA (SEQ ID NO: 635)
HIF1A-Sp201	201	GGGCAATCAATGGATGAAAG (SEQ ID NO: 636)
HIF1A-Sp202	202	CAATCATAACTGGTCAGCTG (SEQ ID NO: 637)
HIF1A-Sp203	203	ATTAACTTCACAATCATAAC (SEQ ID NO: 638)
HIF1A-Sp204	204	GAAGTTAATGCTCCTATACA (SEQ ID NO: 639)
HIF1A-Sp205	205	AGGTTTCTGCTGCCTTGTAT (SEQ ID NO: 640)
HIF1A-Sp206	206	AGGCAGCAGAAACCTACTGC (SEQ ID NO: 641)
HIF1A-Sp207	207	GGCAGCAGAAACCTACTGCA (SEQ ID NO: 642)
HIF1A-Sp208	208	GTAATTCTTCACCCTGCAGT (SEQ ID NO: 643)
HIF1A-Sp209	209	TGAAGAATTACTCAGAGCTT (SEQ ID NO: 644)

[Table 12]

Target sequences of EPAS1 gene for SpCas9

Gene	No.	Target sequence
EPAS1-Sp1	1	AGCGACAATGACAGCTGACA (SEQ ID NO: 645)
EPAS1-Sp2	2	CAGCTGACAAGGAGAAGAAA (SEQ ID NO: 646)
EPAS1-Sp3	3	TTCTCCACTTAGGAGTAGCT (SEQ ID NO: 647)
EPAS1-Sp4	4	CACTTAGGAGTAGCTCGGAG (SEQ ID NO: 648)
EPAS1-Sp5	5	TTAGGAGTAGCTCGGAGAGG (SEQ ID NO: 649)
EPAS1-Sp6	6	GAGTAGCTCGGAGAGGAGGA (SEQ ID NO: 650)
EPAS1-Sp7	7	AGAGGAGGAAGGAGAAGTCC (SEQ ID NO: 651)
EPAS1-Sp8	8	GAGGAGGAAGGAGAAGTCCC (SEQ ID NO: 652)
EPAS1-Sp9	9	AGAAGTCCCGGGATGCTGCG (SEQ ID NO: 653)
EPAS1-Sp10	10	CCCGGGATGCTGCGCGGTGC (SEQ ID NO: 654)
EPAS1-Sp11	11	CCGGCACCGCGCAGCATCCC (SEQ ID NO: 655)
EPAS1-Sp12	12	GCCGGCACCGCGCAGCATCC (SEQ ID NO: 656)
EPAS1-Sp13	13	GGGATGCTGCGCGGTGCCGG (SEQ ID NO: 657)
EPAS1-Sp14	14	TGCGCGGTGCCGGCGGAGCA (SEQ ID NO: 658)
EPAS1-Sp15	15	GTGCCGGCGGAGCAAGGAGA (SEQ ID NO: 659)
EPAS1-Sp16	16	CCGGCGGAGCAAGGAGACGG (SEQ ID NO: 660)
EPAS1-Sp17	17	CCTCCGTCTCCTTGCTCCGC (SEQ ID NO: 661)
EPAS1-Sp18	18	GACGGAGGTGTTCTATGAGC (SEQ ID NO: 662)
EPAS1-Sp19	19	GTGGGGCAGAGGCAGCTCAT (SEQ ID NO: 663)
EPAS1-Sp20	20	TGTGGGGCAGAGGCAGCTCA (SEQ ID NO: 664)
EPAS1-Sp21	21	GAGCTCACACTGTGGGGCAG (SEQ ID NO: 665)
EPAS1-Sp22	22	AGATGGGAGCTCACACTGTG (SEQ ID NO: 666)
EPAS1-Sp23	23	CAGATGGGAGCTCACACTGT (SEQ ID NO: 667)
EPAS1-Sp24	24	CCACAGTGTGAGCTCCCATC (SEQ ID NO: 668)
EPAS1-Sp25	25	CCAGATGGGAGCTCACACTG (SEQ ID NO: 669)
EPAS1-Sp26	26	TGTGAGCTCCCATCTGGACA (SEQ ID NO: 670)
EPAS1-Sp27	27	GATGGAGGCCTTGTCCAGAT (SEQ ID NO: 671)
EPAS1-Sp28	28	TGATGGAGGCCTTGTCCAGA (SEQ ID NO: 672)
EPAS1-Sp29	29	CAAGGCCTCCATCATGCGAC (SEQ ID NO: 673)
EPAS1-Sp30	30	GATTGCCAGTCGCATGATGG (SEQ ID NO: 674)
EPAS1-Sp31	31	GCTGATTGCCAGTCGCATGA (SEQ ID NO: 675)
EPAS1-Sp32	32	AGAGGAGCTTGTGTGTTCGC (SEQ ID NO: 676)
EPAS1-Sp33	33	ACACACAAGCTCCTCTCCTC (SEQ ID NO: 677)
EPAS1-Sp34	34	CAAGCTCCTCTCCTCAGGTA (SEQ ID NO: 678)
EPAS1-Sp35	35	TGCTGGCCTTACCTGAGGAG (SEQ ID NO: 679)

EPAS1-Sp36	36	GAGCCTGCTGGCCTTACCTG (SEQ ID NO: 680)
EPAS1-Sp37	37	GACTCGTTTTCAGAGCAAAC (SEQ ID NO: 681)
EPAS1-Sp38	38	CTGCTGGTCAGCTTCGGCTT (SEQ ID NO: 682)
EPAS1-Sp39	39	AGCCGAAGCTGACCAGCAGA (SEQ ID NO: 683)
EPAS1-Sp40	40	GTCCATCTGCTGGTCAGCTT (SEQ ID NO: 684)
EPAS1-Sp41	41	GGTACAAGTTGTCCATCTGC (SEQ ID NO: 685)
EPAS1-Sp42	42	CAACTTGTACCTGAAAGCCT (SEQ ID NO: 686)
EPAS1-Sp43	43	CTTGTACCTGAAAGCCTTGG (SEQ ID NO: 687)
EPAS1-Sp44	44	TTGTACCTGAAAGCCTTGGA (SEQ ID NO: 688)
EPAS1-Sp45	45	TGAAACCCTCCAAGGCTTTC (SEQ ID NO: 689)
EPAS1-Sp46	46	CACGGCAATGAAACCCTCCA (SEQ ID NO: 690)
EPAS1-Sp47	47	CTTGGAGGGTTTCATTGCCG (SEQ ID NO: 691)
EPAS1-Sp48	48	ATTGCCGTGGTGACCCAAGA (SEQ ID NO: 692)
EPAS1-Sp49	49	GTCGCCATCTTGGGTCACCA (SEQ ID NO: 693)
EPAS1-Sp50	50	AAAGATCATGTCGCCATCTT (SEQ ID NO: 694)
EPAS1-Sp51	51	GAAAGATCATGTCGCCATCT (SEQ ID NO: 695)
EPAS1-Sp52	52	AGAAAACATCAGCAAGTTCA (SEQ ID NO: 696)
EPAS1-Sp53	53	GAAAACATCAGCAAGTTCAT (SEQ ID NO: 697)
EPAS1-Sp54	54	CAAGTTCATGGGACTTACAC (SEQ ID NO: 698)
EPAS1-Sp55	55	TTGAAACAGGTGGAGCTAAC (SEQ ID NO: 699)
EPAS1-Sp56	56	CACTCATCCCTGCGACCATG (SEQ ID NO: 700)
EPAS1-Sp57	57	CGAATCTCCTCATGGTCGCA (SEQ ID NO: 701)
EPAS1-Sp58	58	ACGAATCTCCTCATGGTCGC (SEQ ID NO: 702)
EPAS1-Sp59	59	GGTTCTCACGAATCTCCTCA (SEQ ID NO: 703)
EPAS1-Sp60	60	GAGAACCTGAGTCTCAAAAA (SEQ ID NO: 704)
EPAS1-Sp61	61	GGATACCATTTTTGAGACTC (SEQ ID NO: 705)
EPAS1-Sp62	62	ATCCTTCCACATCCAGGCTC (SEQ ID NO: 706)
EPAS1-Sp63	63	CCACATCCAGGCTCTGGTTT (SEQ ID NO: 707)
EPAS1-Sp64	64	CACATCCAGGCTCTGGTTTT (SEQ ID NO: 708)
EPAS1-Sp65	65	TTTTTCCCAAAACCAGAGCC (SEQ ID NO: 709)
EPAS1-Sp66	66	GCAAAGACATGTCCACAGAG (SEQ ID NO: 710)
EPAS1-Sp67	67	CAAAGACATGTCCACAGAGC (SEQ ID NO: 711)
EPAS1-Sp68	68	CATGAAGAAGTCCCGCTCTG (SEQ ID NO: 712)
EPAS1-Sp69	69	CAGAGCGGGACTTCTTCATG (SEQ ID NO: 713)
EPAS1-Sp70	70	CTTCATGAGGATGAAGTGCA (SEQ ID NO: 714)
EPAS1-Sp71	71	AAGTGCACGGTCACCAACAG (SEQ ID NO: 715)

EPAS1-Sp72	72	GTTGACAGTACGGCCTCTGT (SEQ ID NO: 716)
EPAS1-Sp73	73	CTGACTTGAGGTTGACAGTA (SEQ ID NO: 717)
EPAS1-Sp74	74	TCAACCTCAAGTCAGCCACC (SEQ ID NO: 718)
EPAS1-Sp75	75	CCTCAAGTCAGCCACCTGGA (SEQ ID NO: 719)
EPAS1-Sp76	76	CCTTCCAGGTGGCTGACTTG (SEQ ID NO: 720)
EPAS1-Sp77	77	AAGTCAGCCACCTGGAAGGT (SEQ ID NO: 721)
EPAS1-Sp78	78	AGTCAGCCACCTGGAAGGTA (SEQ ID NO: 722)
EPAS1-Sp79	79	ATGTTGCCCTACCTTCCAGG (SEQ ID NO: 723)
EPAS1-Sp80	80	CTGATGTTGCCCTACCTTCC (SEQ ID NO: 724)
EPAS1-Sp81	81	GTCTCAGGTCTTGCACTGCA (SEQ ID NO: 725)
EPAS1-Sp82	82	TCTCAGGTCTTGCACTGCAC (SEQ ID NO: 726)
EPAS1-Sp83	83	GGTCTTGCACTGCACGGGCC (SEQ ID NO: 727)
EPAS1-Sp84	84	AGTTGTTGTAGACTTTCACC (SEQ ID NO: 728)
EPAS1-Sp85	85	CACACAGACTATTGTGAGGA (SEQ ID NO: 729)
EPAS1-Sp86	86	CCTCCTCACAATAGTCTGTG (SEQ ID NO: 730)
EPAS1-Sp87	87	CCACACAGACTATTGTGAGG (SEQ ID NO: 731)
EPAS1-Sp88	88	TAGCCACACAGACTATTGTG (SEQ ID NO: 732)
EPAS1-Sp89	89	CAATAGTCTGTGTGGCTACA (SEQ ID NO: 733)
EPAS1-Sp90	90	ATGATGAGGCAGGACAGCAG (SEQ ID NO: 734)
EPAS1-Sp91	91	GATGATGAGGCAGGACAGCA (SEQ ID NO: 735)
EPAS1-Sp92	92	TGATGATGAGGCAGGACAGC (SEQ ID NO: 736)
EPAS1-Sp93	93	TTCACACATGATGATGAGGC (SEQ ID NO: 737)
EPAS1-Sp94	94	TTGGTTCACACATGATGATG (SEQ ID NO: 738)
EPAS1-Sp95	95	ATGTGGGATGGGTGCTGGAT (SEQ ID NO: 739)
EPAS1-Sp96	96	AATCCAGCACCCATCCCACA (SEQ ID NO: 740)
EPAS1-Sp97	97	TGTCCATGTGGGATGGGTGC (SEQ ID NO: 741)
EPAS1-Sp98	98	GGGGGATGTCCATGTGGGAT (SEQ ID NO: 742)
EPAS1-Sp99	99	AGGGGGATGTCCATGTGGGA (SEQ ID NO: 743)
EPAS1-Sp100	100	ATCCCACATGGACATCCCCC (SEQ ID NO: 744)
EPAS1-Sp101	101	ATCCAGGGGGATGTCCATGT (SEQ ID NO: 745)
EPAS1-Sp102	102	TATCCAGGGGGATGTCCATG (SEQ ID NO: 746)
EPAS1-Sp103	103	GGAAGGTCTTGCTATCCAGG (SEQ ID NO: 747)
EPAS1-Sp104	104	AGGAAGGTCTTGCTATCCAG (SEQ ID NO: 748)
EPAS1-Sp105	105	CAGGAAGGTCTTGCTATCCA (SEQ ID NO: 749)
EPAS1-Sp106	106	TCAGGAAGGTCTTGCTATCC (SEQ ID NO: 750)
EPAS1-Sp107	107	CATGCTGTGGCGGCTCAGGA (SEQ ID NO: 751)

EPAS1-Sp108	108	CTTCCTGAGCCGCCACAGCA (SEQ ID NO: 752)
EPAS1-Sp109	109	TGTCCATGCTGTGGCGGCTC (SEQ ID NO: 753)
EPAS1-Sp110	110	ACTTCATGTCCATGCTGTGG (SEQ ID NO: 754)
EPAS1-Sp111	111	TGAACTTCATGTCCATGCTG (SEQ ID NO: 755)
EPAS1-Sp112	112	AGTTCACCTACTGTGATGAC (SEQ ID NO: 756)
EPAS1-Sp113	113	CACCTACTGTGATGACAGGT (SEQ ID NO: 757)
EPAS1-Sp114	114	ACCTACTGTGATGACAGGTA (SEQ ID NO: 758)
EPAS1-Sp115	115	CCCCTACCTGTCATCACAGT (SEQ ID NO: 759)
EPAS1-Sp116	116	CTCAGAATCACAGAACTGAT (SEQ ID NO: 760)
EPAS1-Sp117	117	ACTGATTGGTTACCACCCTG (SEQ ID NO: 761)
EPAS1-Sp118	118	TACCACCCTGAGGAGCTGCT (SEQ ID NO: 762)
EPAS1-Sp119	119	GGCCAAGCAGCTCCTCAGGG (SEQ ID NO: 763)
EPAS1-Sp120	120	AGCGGCCAAGCAGCTCCTCA (SEQ ID NO: 764)
EPAS1-Sp121	121	GAGCGGCCAAGCAGCTCCTC (SEQ ID NO: 765)
EPAS1-Sp122	122	GGTAGAATTCATAGGCTGAG (SEQ ID NO: 766)
EPAS1-Sp123	123	TAGCGCATGGTAGAATTCAT (SEQ ID NO: 767)
EPAS1-Sp124	124	TGTTCTCGGAGTCTAGCGCA (SEQ ID NO: 768)
EPAS1-Sp125	125	GTGACTCTTGGTCATGTTCT (SEQ ID NO: 769)
EPAS1-Sp126	126	CTCACAGTTCTGGTGACTCT (SEQ ID NO: 770)
EPAS1-Sp127	127	ACTCCTGGAACTCACAGTTC (SEQ ID NO: 771)
EPAS1-Sp128	128	TCCTCCCCTAGTGTGCACCA (SEQ ID NO: 772)
EPAS1-Sp129	129	CCTCCCCTAGTGTGCACCAA (SEQ ID NO: 773)
EPAS1-Sp130	130	CTGACCCTTGGTGCACACTA (SEQ ID NO: 774)
EPAS1-Sp131	131	CCTAGTGTGCACCAAGGGTC (SEQ ID NO: 775)
EPAS1-Sp132	132	CCTGACCCTTGGTGCACACT (SEQ ID NO: 776)
EPAS1-Sp133	133	ACCAAGGGTCAGGTAGTAAG (SEQ ID NO: 777)
EPAS1-Sp134	134	GCCACTTACTACCTGACCCT (SEQ ID NO: 778)
EPAS1-Sp135	135	AGGTAGTAAGTGGCCAGTAC (SEQ ID NO: 779)
EPAS1-Sp136	136	GCTTTGCGAGCATCCGGTAC (SEQ ID NO: 780)
EPAS1-Sp137	137	TACCGGATGCTCGCAAAGCA (SEQ ID NO: 781)
EPAS1-Sp138	138	ACCGGATGCTCGCAAAGCAT (SEQ ID NO: 782)
EPAS1-Sp139	139	CCGGATGCTCGCAAAGCATG (SEQ ID NO: 783)
EPAS1-Sp140	140	CCCCATGCTTTGCGAGCATC (SEQ ID NO: 784)
EPAS1-Sp141	141	CGGATGCTCGCAAAGCATGG (SEQ ID NO: 785)
EPAS1-Sp142	142	CAAAGCATGGGGGCTACGTG (SEQ ID NO: 786)
EPAS1-Sp143	143	GCATGGGGGCTACGTGTGGC (SEQ ID NO: 787)

EPAS1-Sp144	144	CTACGTGTGGCTGGAGACCC (SEQ ID NO: 788)
EPAS1-Sp145	145	TACGTGTGGCTGGAGACCCA (SEQ ID NO: 789)
EPAS1-Sp146	146	ACGTGTGGCTGGAGACCCAG (SEQ ID NO: 790)
EPAS1-Sp147	147	GTGGCTGGAGACCCAGGGGA (SEQ ID NO: 791)
EPAS1-Sp148	148	GTTGTAGATGACCGTCCCCT (SEQ ID NO: 792)
EPAS1-Sp149	149	GGTTGTAGATGACCGTCCCC (SEQ ID NO: 793)
EPAS1-Sp150	150	ACTGGGGCTGCAGGTTGCGA (SEQ ID NO: 794)
EPAS1-Sp151	151	CACTGGGGCTGCAGGTTGCG (SEQ ID NO: 795)
EPAS1-Sp152	152	ACATGATGCACTGGGGCTGC (SEQ ID NO: 796)
EPAS1-Sp153	153	TTGACACACATGATGCACTG (SEQ ID NO: 797)
EPAS1-Sp154	154	GTTGACACACATGATGCACT (SEQ ID NO: 798)
EPAS1-Sp155	155	AGTTGACACACATGATGCAC (SEQ ID NO: 799)
EPAS1-Sp156	156	TGTGTGTCAACTACGTCCTG (SEQ ID NO: 800)
EPAS1-Sp157	157	AGCCCTCACATGCTTACCTC (SEQ ID NO: 801)
EPAS1-Sp158	158	TGAGATTGAGAAGAATGACG (SEQ ID NO: 802)
EPAS1-Sp159	159	GAATGACGTGGTGTTCTCCA (SEQ ID NO: 803)
EPAS1-Sp160	160	CAGGGATTCAGTCTGGTCCA (SEQ ID NO: 804)
EPAS1-Sp161	161	GCTTGAACAGGGATTCAGTC (SEQ ID NO: 805)
EPAS1-Sp162	162	CATCAGGTGGGGCTTGAACA (SEQ ID NO: 806)
EPAS1-Sp163	163	CCTGTTCAAGCCCCACCTGA (SEQ ID NO: 807)
EPAS1-Sp164	164	CCATCAGGTGGGGCTTGAAC (SEQ ID NO: 808)
EPAS1-Sp165	165	CTGTTCATGGCCATCAGGTG (SEQ ID NO: 809)
EPAS1-Sp166	166	GCTGTTCATGGCCATCAGGT (SEQ ID NO: 810)
EPAS1-Sp167	167	TGCTGTTCATGGCCATCAGG (SEQ ID NO: 811)
EPAS1-Sp168	168	AGATGCTGTTCATGGCCATC (SEQ ID NO: 812)
EPAS1-Sp169	169	GCTATCAAAGATGCTGTTCA (SEQ ID NO: 813)
EPAS1-Sp170	170	AACAGCATCTTTGATAGCAG (SEQ ID NO: 814)
EPAS1-Sp171	171	CATCTTTGATAGCAGTGGCA (SEQ ID NO: 815)
EPAS1-Sp172	172	ATCTTTGATAGCAGTGGCAA (SEQ ID NO: 816)
EPAS1-Sp173	173	TCTTTGATAGCAGTGGCAAG (SEQ ID NO: 817)
EPAS1-Sp174	174	CTTTGATAGCAGTGGCAAGG (SEQ ID NO: 818)
EPAS1-Sp175	175	CTTCCTATTCACCAAGCTAA (SEQ ID NO: 819)
EPAS1-Sp176	176	CCTATTCACCAAGCTAAAGG (SEQ ID NO: 820)
EPAS1-Sp177	177	CCTCCTTTAGCTTGGTGAAT (SEQ ID NO: 821)
EPAS1-Sp178	178	CTCGGGCTCCTCCTTTAGCT (SEQ ID NO: 822)
EPAS1-Sp179	179	CAAGCTAAAGGAGGAGCCCG (SEQ ID NO: 823)

EPAS1-Sp180	180	AAAGGAGGAGCCCGAGGAGC (SEQ ID NO: 824)
EPAS1-Sp181	181	GCCCGAGGAGCTGGCCCAGC (SEQ ID NO: 825)
EPAS1-Sp182	182	GCCAGCTGGGCCAGCTCCTC (SEQ ID NO: 826)
EPAS1-Sp183	183	AGCCAGCTGGGCCAGCTCCT (SEQ ID NO: 827)
EPAS1-Sp184	184	GCCCAGCTGGCTCCCACCCC (SEQ ID NO: 828)
EPAS1-Sp185	185	TCCTGGGGTGGGAGCCAGCT (SEQ ID NO: 829)
EPAS1-Sp186	186	CTCCTGGGGTGGGAGCCAGC (SEQ ID NO: 830)
EPAS1-Sp187	187	ATGATGGCGTCTCCTGGGGT (SEQ ID NO: 831)
EPAS1-Sp188	188	GATGATGGCGTCTCCTGGGG (SEQ ID NO: 832)
EPAS1-Sp189	189	AGAGATGATGGCGTCTCCTG (SEQ ID NO: 833)
EPAS1-Sp190	190	GAGAGATGATGGCGTCTCCT (SEQ ID NO: 834)
EPAS1-Sp191	191	AGAGAGATGATGGCGTCTCC (SEQ ID NO: 835)
EPAS1-Sp192	192	AGGAGACGCCATCATCTCTC (SEQ ID NO: 836)
EPAS1-Sp193	193	GCCATCATCTCTCTGGATTT (SEQ ID NO: 837)
EPAS1-Sp194	194	ACCGAAATCCAGAGAGATGA (SEQ ID NO: 838)
EPAS1-Sp195	195	ATCATCTCTCTGGATTTCGG (SEQ ID NO: 839)
EPAS1-Sp196	196	TCATCTCTCTGGATTTCGGT (SEQ ID NO: 840)
EPAS1-Sp197	197	CTCGAAGTTCTGATTCCCTG (SEQ ID NO: 841)
EPAS1-Sp198	198	CACAGGGAATCAGAACTTCG (SEQ ID NO: 842)
EPAS1-Sp199	199	TTCGAGGAGTCCTCAGCCTA (SEQ ID NO: 843)
EPAS1-Sp200	200	GGAGTCCTCAGCCTATGGCA (SEQ ID NO: 844)
EPAS1-Sp201	201	GATGGCCTTGCCATAGGCTG (SEQ ID NO: 845)
EPAS1-Sp202	202	GGGCAGGATGGCCTTGCCAT (SEQ ID NO: 846)
EPAS1-Sp203	203	TGGCTGGCTCGGGGGCAGGA (SEQ ID NO: 847)
EPAS1-Sp204	204	TCCTGCCCCCGAGCCAGCCA (SEQ ID NO: 848)
EPAS1-Sp205	205	CCTGCCCCCGAGCCAGCCAT (SEQ ID NO: 849)
EPAS1-Sp206	206	CCCATGGCTGGCTCGGGGGC (SEQ ID NO: 850)
EPAS1-Sp207	207	GTGGCCCATGGCTGGCTCGG (SEQ ID NO: 851)
EPAS1-Sp208	208	CGTGGCCCATGGCTGGCTCG (SEQ ID NO: 852)
EPAS1-Sp209	209	CCCGAGCCAGCCATGGGCCA (SEQ ID NO: 853)
EPAS1-Sp210	210	CCGTGGCCCATGGCTGGCTC (SEQ ID NO: 854)
EPAS1-Sp211	211	TCCGTGGCCCATGGCTGGCT (SEQ ID NO: 855)
EPAS1-Sp212	212	TCAACTCCGTGGCCCATGGC (SEQ ID NO: 856)
EPAS1-Sp213	213	AGCCATGGGCCACGGAGTTG (SEQ ID NO: 857)
EPAS1-Sp214	214	CTCCTCAACTCCGTGGCCCA (SEQ ID NO: 858)
EPAS1-Sp215	215	GCTGTGGCTCCTCAACTCCG (SEQ ID NO: 859)

EPAS1-Sp216	216	GAGCCACAGCACCCAGAGCG (SEQ ID NO: 860)
EPAS1-Sp217	217	CAGCCTCGCTCTGGGTGCTG (SEQ ID NO: 861)
EPAS1-Sp218	218	CACAGCACCCAGAGCGAGGC (SEQ ID NO: 862)
EPAS1-Sp219	219	ACAGCACCCAGAGCGAGGCT (SEQ ID NO: 863)
EPAS1-Sp220	220	CAGGCTCCCAGCCTCGCTCT (SEQ ID NO: 864)
EPAS1-Sp221	221	GCAGGCTCCCAGCCTCGCTC (SEQ ID NO: 865)
EPAS1-Sp222	222	GGGGCACGGTGAAGGCAGGC (SEQ ID NO: 866)
EPAS1-Sp223	223	GCCTGCCTTCACCGTGCCCC (SEQ ID NO: 867)
EPAS1-Sp224	224	GCCTGGGGCACGGTGAAGGC (SEQ ID NO: 868)
EPAS1-Sp225	225	AGCTGCCTGGGGCACGGTGA (SEQ ID NO: 869)
EPAS1-Sp226	226	CGGGGCAGCTGCCTGGGGCA (SEQ ID NO: 870)
EPAS1-Sp227	227	CGTGCCCCAGGCAGCTGCCC (SEQ ID NO: 871)
EPAS1-Sp228	228	GTGCCCCAGGCAGCTGCCCC (SEQ ID NO: 872)
EPAS1-Sp229	229	CTGCCCGGGGCAGCTGCCTG (SEQ ID NO: 873)
EPAS1-Sp230	230	GCTGCCCGGGGCAGCTGCCT (SEQ ID NO: 874)
EPAS1-Sp231	231	TGCTGCCCGGGGCAGCTGCC (SEQ ID NO: 875)
EPAS1-Sp232	232	ACTGGGGGTGGTGCTGCCCG (SEQ ID NO: 876)
EPAS1-Sp233	233	CACTGGGGGTGGTGCTGCCC (SEQ ID NO: 877)
EPAS1-Sp234	234	GCACTGGGGGTGGTGCTGCC (SEQ ID NO: 878)
EPAS1-Sp235	235	GCTGCTGGTGGCACTGGGGG (SEQ ID NO: 879)
EPAS1-Sp236	236	GCTGCTGCTGGTGGCACTGG (SEQ ID NO: 880)
EPAS1-Sp237	237	TGCTGCTGCTGGTGGCACTG (SEQ ID NO: 881)
EPAS1-Sp238	238	CTGCTGCTGCTGGTGGCACT (SEQ ID NO: 882)
EPAS1-Sp239	239	GCTGCTGCTGCTGGTGGCAC (SEQ ID NO: 883)
EPAS1-Sp240	240	GCAGCTGCTGCTGCTGCTGG (SEQ ID NO: 884)
EPAS1-Sp241	241	CAGCAGCAGCAGCTGCTCCA (SEQ ID NO: 885)
EPAS1-Sp242	242	TCTTCAGGGCTATTGGGCTA (SEQ ID NO: 886)
EPAS1-Sp243	243	GTCTTCAGGGCTATTGGGCT (SEQ ID NO: 887)
EPAS1-Sp244	244	TAATAGTCTTCAGGGCTATT (SEQ ID NO: 888)
EPAS1-Sp245	245	GTAATAGTCTTCAGGGCTAT (SEQ ID NO: 889)
EPAS1-Sp246	246	AAGATGTGTAATAGTCTTCA (SEQ ID NO: 890)
EPAS1-Sp247	247	AAAGATGTGTAATAGTCTTC (SEQ ID NO: 891)
EPAS1-Sp248	248	TGAAGACTATTACACATCTT (SEQ ID NO: 892)
EPAS1-Sp249	249	TCTCAATCACTTCAATCTTC (SEQ ID NO: 893)
EPAS1-Sp250	250	GATTGAGAAGCTCTTCGCCA (SEQ ID NO: 894)
EPAS1-Sp251	251	GCTCTTCGCCATGGACACAG (SEQ ID NO: 895)

EPAS1-Sp252	252	CGCCATGGACACAGAGGCCA (SEQ ID NO: 896)
EPAS1-Sp253	253	GTCCTTGGCCTCTGTGTCCA (SEQ ID NO: 897)
EPAS1-Sp254	254	CTGGGTACTGCATTGGTCCT (SEQ ID NO: 898)
EPAS1-Sp255	255	CAAGGACCAATGCAGTACCC (SEQ ID NO: 899)
EPAS1-Sp256	256	CATCTACCTGGGTACTGCAT (SEQ ID NO: 900)
EPAS1-Sp257	257	AGCTCATTGAAATCCGTCTG (SEQ ID NO: 901)
EPAS1-Sp258	258	TCAGACGGATTTCAATGAGC (SEQ ID NO: 902)
EPAS1-Sp259	259	GGATTTCAATGAGCTGGACT (SEQ ID NO: 903)
EPAS1-Sp260	260	TGAGCTGGACTTGGAGACAC (SEQ ID NO: 904)
EPAS1-Sp261	261	ACTGGCACCCTATATCCCCA (SEQ ID NO: 905)
EPAS1-Sp262	262	GCACCCTATATCCCCATGGA (SEQ ID NO: 906)
EPAS1-Sp263	263	CACCCTATATCCCCATGGAC (SEQ ID NO: 907)
EPAS1-Sp264	264	ACCCTATATCCCCATGGACG (SEQ ID NO: 908)
EPAS1-Sp265	265	TCCCCGTCCATGGGGATATA (SEQ ID NO: 909)
EPAS1-Sp266	266	TTCCCCGTCCATGGGGATAT (SEQ ID NO: 910)
EPAS1-Sp267	267	GGAAGTCTTCCCCGTCCATG (SEQ ID NO: 911)
EPAS1-Sp268	268	TGGAAGTCTTCCCCGTCCAT (SEQ ID NO: 912)
EPAS1-Sp269	269	CTGGAAGTCTTCCCCGTCCA (SEQ ID NO: 913)
EPAS1-Sp270	270	CGGGGCAGATGGGGCTTAGC (SEQ ID NO: 914)
EPAS1-Sp271	271	GCTAAGCCCCATCTGCCCCG (SEQ ID NO: 915)
EPAS1-Sp272	272	GCCCCATCTGCCCCGAGGAG (SEQ ID NO: 916)
EPAS1-Sp273	273	GCCGCTCCTCGGGGCAGATG (SEQ ID NO: 917)
EPAS1-Sp274	274	AGCCGCTCCTCGGGGCAGAT (SEQ ID NO: 918)
EPAS1-Sp275	275	GAGCCGCTCCTCGGGGCAGA (SEQ ID NO: 919)
EPAS1-Sp276	276	CTGCCCCGAGGAGCGGCTCT (SEQ ID NO: 920)
EPAS1-Sp277	277	CCCCGAGGAGCGGCTCTTGG (SEQ ID NO: 921)
EPAS1-Sp278	278	CCGCCAAGAGCCGCTCCTCG (SEQ ID NO: 922)
EPAS1-Sp279	279	TCCGCCAAGAGCCGCTCCTC (SEQ ID NO: 923)
EPAS1-Sp280	280	CTCCGCCAAGAGCCGCTCCT (SEQ ID NO: 924)
EPAS1-Sp281	281	AGTGCTGGGGGGTGGACTGT (SEQ ID NO: 925)
EPAS1-Sp282	282	CAGTGCTGGGGGGTGGACTG (SEQ ID NO: 926)
EPAS1-Sp283	283	ACTGAAGCAGTGCTGGGGGG (SEQ ID NO: 927)
EPAS1-Sp284	284	GGCACTGAAGCAGTGCTGGG (SEQ ID NO: 928)
EPAS1-Sp285	285	TGGCACTGAAGCAGTGCTGG (SEQ ID NO: 929)
EPAS1-Sp286	286	ATGGCACTGAAGCAGTGCTG (SEQ ID NO: 930)
EPAS1-Sp287	287	CATGGCACTGAAGCAGTGCT (SEQ ID NO: 931)

EPAS1-Sp288	288	TCATGGCACTGAAGCAGTGC (SEQ ID NO: 932)
EPAS1-Sp289	289	TGGCTGGAAGATGTTTGTCA (SEQ ID NO: 933)
EPAS1-Sp290	290	GACAAACATCTTCCAGCCAC (SEQ ID NO: 934)
EPAS1-Sp291	291	GGGCTACAGGGGCCAGTGGC (SEQ ID NO: 935)
EPAS1-Sp292	292	TGCGGGGCTACAGGGGCCAG (SEQ ID NO: 936)
EPAS1-Sp293	293	GGGACTGTGCGGGGCTACAG (SEQ ID NO: 937)
EPAS1-Sp294	294	AGGGACTGTGCGGGGCTACA (SEQ ID NO: 938)
EPAS1-Sp295	295	AAGGGACTGTGCGGGGCTAC (SEQ ID NO: 939)
EPAS1-Sp296	296	CAGGAGGAAGGGACTGTGCG (SEQ ID NO: 940)
EPAS1-Sp297	297	CCCGCACAGTCCCTTCCTCC (SEQ ID NO: 941)
EPAS1-Sp298	298	CCAGGAGGAAGGGACTGTGC (SEQ ID NO: 942)
EPAS1-Sp299	299	TCCAGGAGGAAGGGACTGTG (SEQ ID NO: 943)
EPAS1-Sp300	300	TGAAACTTGTCCAGGAGGAA (SEQ ID NO: 944)
EPAS1-Sp301	301	CTGAAACTTGTCCAGGAGGA (SEQ ID NO: 945)
EPAS1-Sp302	302	GCTGCTGAAACTTGTCCAGG (SEQ ID NO: 946)
EPAS1-Sp303	303	GCTGCTGCTGAAACTTGTCC (SEQ ID NO: 947)
EPAS1-Sp304	304	GGACAAGTTTCAGCAGCAGC (SEQ ID NO: 948)
EPAS1-Sp305	305	AGAAGACAGAGCCCGAGCAC (SEQ ID NO: 949)
EPAS1-Sp306	306	GAGGACATGGGCCGGTGCTC (SEQ ID NO: 950)
EPAS1-Sp307	307	GGAGGACATGGGCCGGTGCT (SEQ ID NO: 951)
EPAS1-Sp308	308	AGAAGATGGAGGACATGGGC (SEQ ID NO: 952)
EPAS1-Sp309	309	TCAAAGAAGATGGAGGACAT (SEQ ID NO: 953)
EPAS1-Sp310	310	ATCAAAGAAGATGGAGGACA (SEQ ID NO: 954)
EPAS1-Sp311	311	TCCTCCATCTTCTTTGATGC (SEQ ID NO: 955)
EPAS1-Sp312	312	TCCGGCATCAAAGAAGATGG (SEQ ID NO: 956)
EPAS1-Sp313	313	GCTTCCGGCATCAAAGAAGA (SEQ ID NO: 957)
EPAS1-Sp314	314	TGGCAGGGATGCTTTGCTTC (SEQ ID NO: 958)
EPAS1-Sp315	315	GCATCCCTGCCACCGTGCTG (SEQ ID NO: 959)
EPAS1-Sp316	316	CTGGCCACAGCACGGTGGCA (SEQ ID NO: 960)
EPAS1-Sp317	317	CCTGCCACCGTGCTGTGGCC (SEQ ID NO: 961)
EPAS1-Sp318	318	CCTGGCCACAGCACGGTGGC (SEQ ID NO: 962)
EPAS1-Sp319	319	CTGGCCTGGCCACAGCACGG (SEQ ID NO: 963)
EPAS1-Sp320	320	GTGCTGGCCTGGCCACAGCA (SEQ ID NO: 964)
EPAS1-Sp321	321	AAGAGAGAGGGGTGCTGGCC (SEQ ID NO: 965)
EPAS1-Sp322	322	CATGGAAGAGAGAGGGGTGC (SEQ ID NO: 966)
EPAS1-Sp323	323	CAGCACCCCTCTCTCTTCCA (SEQ ID NO: 967)

EPAS1-Sp324	324	AGCACCCCTCTCTCTTCCAT (SEQ ID NO: 968)
EPAS1-Sp325	325	GCACCCCTCTCTCTTCCATG (SEQ ID NO: 969)
EPAS1-Sp326	326	CACCCCTCTCTCTTCCATGG (SEQ ID NO: 970)
EPAS1-Sp327	327	ACCCCTCTCTCTTCCATGGG (SEQ ID NO: 971)
EPAS1-Sp328	328	GCCCCCCATGGAAGAGAGAG (SEQ ID NO: 972)
EPAS1-Sp329	329	TGCCCCCCATGGAAGAGAGA (SEQ ID NO: 973)
EPAS1-Sp330	330	CTGCCCCCCATGGAAGAGAG (SEQ ID NO: 974)
EPAS1-Sp331	331	GGTATTGGATCTGCCCCCCA (SEQ ID NO: 975)
EPAS1-Sp332	332	GGGGCAGATCCAATACCCAG (SEQ ID NO: 976)
EPAS1-Sp333	333	ATCTGGGGGCCACTGGGTAT (SEQ ID NO: 977)
EPAS1-Sp334	334	TGGTGGATCTGGGGGCCACT (SEQ ID NO: 978)
EPAS1-Sp335	335	ATGGTGGATCTGGGGGCCAC (SEQ ID NO: 979)
EPAS1-Sp336	336	AAATGTAATGGTGGATCTGG (SEQ ID NO: 980)
EPAS1-Sp337	337	AAAATGTAATGGTGGATCTG (SEQ ID NO: 981)
EPAS1-Sp338	338	CAAAATGTAATGGTGGATCT (SEQ ID NO: 982)
EPAS1-Sp339	339	CCAGATCCACCATTACATTT (SEQ ID NO: 983)
EPAS1-Sp340	340	CCAAAATGTAATGGTGGATC (SEQ ID NO: 984)
EPAS1-Sp341	341	CAGATCCACCATTACATTTT (SEQ ID NO: 985)
EPAS1-Sp342	342	GTGGGCCCAAAATGTAATGG (SEQ ID NO: 986)
EPAS1-Sp343	343	TTTGTGGGCCCAAAATGTAA (SEQ ID NO: 987)
EPAS1-Sp344	344	TACATTTTGGGCCCACAAAG (SEQ ID NO: 988)
EPAS1-Sp345	345	ACATTTTGGGCCCACAAAGT (SEQ ID NO: 989)
EPAS1-Sp346	346	GGGCCCACAAAGTGGGCCGT (SEQ ID NO: 990)
EPAS1-Sp347	347	GGCCCACAAAGTGGGCCGTC (SEQ ID NO: 991)
EPAS1-Sp348	348	GCCCACAAAGTGGGCCGTCG (SEQ ID NO: 992)
EPAS1-Sp349	349	TCCCCGACGGCCCACTTTGT (SEQ ID NO: 993)
EPAS1-Sp350	350	ATCCCCGACGGCCCACTTTG (SEQ ID NO: 994)
EPAS1-Sp351	351	CTCTGTGCGCTGATCCCCGA (SEQ ID NO: 995)
EPAS1-Sp352	352	GGATCAGCGCACAGAGTTCT (SEQ ID NO: 996)
EPAS1-Sp353	353	GATCAGCGCACAGAGTTCTT (SEQ ID NO: 997)
EPAS1-Sp354	354	GTTCTTGGGAGCAGCGCCGT (SEQ ID NO: 998)
EPAS1-Sp355	355	TTCTTGGGAGCAGCGCCGTT (SEQ ID NO: 999)
EPAS1-Sp356	356	TCTTGGGAGCAGCGCCGTTG (SEQ ID NO: 1000)
EPAS1-Sp357	357	GGAGAGACAGGGGGCCCCAA (SEQ ID NO: 1001)
EPAS1-Sp358	358	ACATGGGGTGGAGAGACAGG (SEQ ID NO: 1002)
EPAS1-Sp359	359	GACATGGGGTGGAGAGACAG (SEQ ID NO: 1003)

EPAS1-Sp360	360	AGACATGGGGTGGAGAGACA (SEQ ID NO: 1004)
EPAS1-Sp361	361	GAGACATGGGGTGGAGAGAC (SEQ ID NO: 1005)
EPAS1-Sp362	362	TTGAAGGTGGAGACATGGGG (SEQ ID NO: 1006)
EPAS1-Sp363	363	GTCTTGAAGGTGGAGACATG (SEQ ID NO: 1007)
EPAS1-Sp364	364	TGTCTTGAAGGTGGAGACAT (SEQ ID NO: 1008)
EPAS1-Sp365	365	TTGTCTTGAAGGTGGAGACA (SEQ ID NO: 1009)
EPAS1-Sp366	366	ATGTCTCCACCTTCAAGACA (SEQ ID NO: 1010)
EPAS1-Sp367	367	TGCCACTTACCTTGTCTTGA (SEQ ID NO: 1011)
EPAS1-Sp368	368	CGGGCTTGGCAGGTCTGCAA (SEQ ID NO: 1012)
EPAS1-Sp369	369	GGGCTTGGCAGGTCTGCAAA (SEQ ID NO: 1013)
EPAS1-Sp370	370	GGCAGGTCTGCAAAGGGTTT (SEQ ID NO: 1014)
EPAS1-Sp371	371	GCAGGTCTGCAAAGGGTTTT (SEQ ID NO: 1015)
EPAS1-Sp372	372	CAGGTCTGCAAAGGGTTTTG (SEQ ID NO: 1016)
EPAS1-Sp373	373	GCAAAGGGTTTTGGGGCTCG (SEQ ID NO: 1017)
EPAS1-Sp374	374	AGGCCCAGACGTGCTGAGTC (SEQ ID NO: 1018)
EPAS1-Sp375	375	TGGCCGGACTCAGCACGTCT (SEQ ID NO: 1019)
EPAS1-Sp376	376	ATGGCCGGACTCAGCACGTC (SEQ ID NO: 1020)
EPAS1-Sp377	377	AGACGTGCTGAGTCCGGCCA (SEQ ID NO: 1021)
EPAS1-Sp378	378	TTGGAGAGGGCTACCATGGC (SEQ ID NO: 1022)
EPAS1-Sp379	379	CTTGTTGGAGAGGGCTACCA (SEQ ID NO: 1023)
EPAS1-Sp380	380	CAGCTTCAGCTTGTTGGAGA (SEQ ID NO: 1024)
EPAS1-Sp381	381	TCAGCTTCAGCTTGTTGGAG (SEQ ID NO: 1025)
EPAS1-Sp382	382	TCGCTTCAGCTTCAGCTTGT (SEQ ID NO: 1026)
EPAS1-Sp383	383	GCTGAAGCTGAAGCGACAGC (SEQ ID NO: 1027)
EPAS1-Sp384	384	GTATGAAGAGCAAGCCTTCC (SEQ ID NO: 1028)
EPAS1-Sp385	385	CAAGCCTTCCAGGACCTGAG (SEQ ID NO: 1029)
EPAS1-Sp386	386	AAGCCTTCCAGGACCTGAGC (SEQ ID NO: 1030)
EPAS1-Sp387	387	AGCCTTCCAGGACCTGAGCG (SEQ ID NO: 1031)
EPAS1-Sp388	388	CACCCCGCTCAGGTCCTGGA (SEQ ID NO: 1032)
EPAS1-Sp389	389	GACTCACCCCGCTCAGGTCC (SEQ ID NO: 1033)
EPAS1-Sp390	390	GGGGATGACTCACCCCGCTC (SEQ ID NO: 1034)
EPAS1-Sp391	391	TACTCCCAGGGGGACCCACC (SEQ ID NO: 1035)
EPAS1-Sp392	392	TCCCAGGGGGACCCACCTGG (SEQ ID NO: 1036)
EPAS1-Sp393	393	GCCACCAGGTGGGTCCCCCT (SEQ ID NO: 1037)
EPAS1-Sp394	394	TGCCACCAGGTGGGTCCCCC (SEQ ID NO: 1038)
EPAS1-Sp395	395	GTGAGGTGCTGCCACCAGGT (SEQ ID NO: 1039)

EPAS1-Sp396	396	TGTGAGGTGCTGCCACCAGG (SEQ ID NO: 1040)
EPAS1-Sp397	397	AAATGTGAGGTGCTGCCACC (SEQ ID NO: 1041)
EPAS1-Sp398	398	GCAGCACCTCACATTTGATG (SEQ ID NO: 1042)
EPAS1-Sp399	399	CCTCACATTTGATGTGGAAA (SEQ ID NO: 1043)
EPAS1-Sp400	400	CCGTTTCCACATCAAATGTG (SEQ ID NO: 1044)
EPAS1-Sp401	401	GGAAACGGATGAAGAACCTC (SEQ ID NO: 1045)
EPAS1-Sp402	402	GAAACGGATGAAGAACCTCA (SEQ ID NO: 1046)
EPAS1-Sp403	403	AAACGGATGAAGAACCTCAG (SEQ ID NO: 1047)
EPAS1-Sp404	404	CGGATGAAGAACCTCAGGGG (SEQ ID NO: 1048)
EPAS1-Sp405	405	GGATGAAGAACCTCAGGGGT (SEQ ID NO: 1049)
EPAS1-Sp406	406	AAGGGCAGCTCCCACCCCTG (SEQ ID NO: 1050)
EPAS1-Sp407	407	TGGGAGCTGCCCTTTGATGC (SEQ ID NO: 1051)
EPAS1-Sp408	408	GTGGCTTGTCCGGCATCAAA (SEQ ID NO: 1052)
EPAS1-Sp409	409	AGTGGCTTGTCCGGCATCAA (SEQ ID NO: 1053)
EPAS1-Sp410	410	TTTGCGCTCAGTGGCTTGTC (SEQ ID NO: 1054)
EPAS1-Sp411	411	TTGGGTACATTTGCGCTCAG (SEQ ID NO: 1055)
EPAS1-Sp412	412	CTGAGCGCAAATGTACCCAA (SEQ ID NO: 1056)
EPAS1-Sp413	413	TGTGGCCGCTGCTCACCATT (SEQ ID NO: 1057)
EPAS1-Sp414	414	CTGTGGCCGCTGCTCACCAT (SEQ ID NO: 1058)
EPAS1-Sp415	415	AGTTCACCCAAAACCCCATG (SEQ ID NO: 1059)
EPAS1-Sp416	416	GTTCACCCAAAACCCCATGA (SEQ ID NO: 1060)
EPAS1-Sp417	417	TTCACCCAAAACCCCATGAG (SEQ ID NO: 1061)
EPAS1-Sp418	418	CAGGCCCCTCATGGGGTTTT (SEQ ID NO: 1062)
EPAS1-Sp419	419	CCAAAACCCCATGAGGGGCC (SEQ ID NO: 1063)
EPAS1-Sp420	420	CCAGGCCCCTCATGGGGTTT (SEQ ID NO: 1064)
EPAS1-Sp421	421	CAAAACCCCATGAGGGGCCT (SEQ ID NO: 1065)
EPAS1-Sp422	422	GATGGCCCAGGCCCCTCATG (SEQ ID NO: 1066)
EPAS1-Sp423	423	GGATGGCCCAGGCCCCTCAT (SEQ ID NO: 1067)
EPAS1-Sp424	424	GGGATGGCCCAGGCCCCTCA (SEQ ID NO: 1068)
EPAS1-Sp425	425	GATGTCTCAGGGGATGGCCC (SEQ ID NO: 1069)
EPAS1-Sp426	426	GCGGCAGATGTCTCAGGGGA (SEQ ID NO: 1070)
EPAS1-Sp427	427	GGCAGCGGCAGATGTCTCAG (SEQ ID NO: 1071)
EPAS1-Sp428	428	TGGCAGCGGCAGATGTCTCA (SEQ ID NO: 1072)
EPAS1-Sp429	429	GTGGCAGCGGCAGATGTCTC (SEQ ID NO: 1073)
EPAS1-Sp430	430	GCAGATGGAGGCTGTGGCAG (SEQ ID NO: 1074)
EPAS1-Sp431	431	CTGATGGCAGATGGAGGCTG (SEQ ID NO: 1075)

EPAS1-Sp432	432	CCTCCATCTGCCATCAGTCC (SEQ ID NO: 1076)
EPAS1-Sp433	433	CCGGGACTGATGGCAGATGG (SEQ ID NO: 1077)
EPAS1-Sp434	434	CTCCATCTGCCATCAGTCCC (SEQ ID NO: 1078)
EPAS1-Sp435	435	TCCATCTGCCATCAGTCCCG (SEQ ID NO: 1079)
EPAS1-Sp436	436	TCCCCGGGACTGATGGCAGA (SEQ ID NO: 1080)
EPAS1-Sp437	437	GCTGTTCTCCCCGGGACTGA (SEQ ID NO: 1081)
EPAS1-Sp438	438	CTGCTCTTGCTGTTCTCCCC (SEQ ID NO: 1082)
EPAS1-Sp439	439	CCGGGGAGAACAGCAAGAGC (SEQ ID NO: 1083)
EPAS1-Sp440	440	CCTGCTCTTGCTGTTCTCCC (SEQ ID NO: 1084)
EPAS1-Sp441	441	GGGTGGCGTAGCACTGTGGG (SEQ ID NO: 1085)
EPAS1-Sp442	442	TGGGTGGCGTAGCACTGTGG (SEQ ID NO: 1086)
EPAS1-Sp443	443	CTGGGTGGCGTAGCACTGTG (SEQ ID NO: 1087)
EPAS1-Sp444	444	ACTGGGTGGCGTAGCACTGT (SEQ ID NO: 1088)
EPAS1-Sp445	445	TACTGGGTGGCGTAGCACTG (SEQ ID NO: 1089)
EPAS1-Sp446	446	GTGCTACGCCACCCAGTACC (SEQ ID NO: 1090)
EPAS1-Sp447	447	GCTGTAGTCCTGGTACTGGG (SEQ ID NO: 1091)
EPAS1-Sp448	448	CAGGCTGTAGTCCTGGTACT (SEQ ID NO: 1092)
EPAS1-Sp449	449	ACAGGCTGTAGTCCTGGTAC (SEQ ID NO: 1093)
EPAS1-Sp450	450	CTGACGACAGGCTGTAGTCC (SEQ ID NO: 1094)
EPAS1-Sp451	451	CAGCCTGTCGTCAGCCCACA (SEQ ID NO: 1095)
EPAS1-Sp452	452	ACACCTTGTGGGCTGACGAC (SEQ ID NO: 1096)
EPAS1-Sp453	453	TCGTCAGCCCACAAGGTGTC (SEQ ID NO: 1097)
EPAS1-Sp454	454	TCAGCCCACAAGGTGTCAGG (SEQ ID NO: 1098)
EPAS1-Sp455	455	CAGCCCACAAGGTGTCAGGT (SEQ ID NO: 1099)
EPAS1-Sp456	456	ACACCCACCTGACACCTTGT (SEQ ID NO: 1100)
EPAS1-Sp457	457	CACACCCACCTGACACCTTG (SEQ ID NO: 1101)
EPAS1-Sp458	458	ACCAACCCTTCTTTCAGGCA (SEQ ID NO: 1102)
EPAS1-Sp459	459	TTCTTTCAGGCATGGCAAGC (SEQ ID NO: 1103)
EPAS1-Sp460	460	GGCATGGCAAGCCGGCTGCT (SEQ ID NO: 1104)
EPAS1-Sp461	461	GCATGGCAAGCCGGCTGCTC (SEQ ID NO: 1105)
EPAS1-Sp462	462	CAAATGAGGGCCCGAGCAGC (SEQ ID NO: 1106)
EPAS1-Sp463	463	AGCAGGTAGGACTCAAATGA (SEQ ID NO: 1107)
EPAS1-Sp464	464	CAGCAGGTAGGACTCAAATG (SEQ ID NO: 1108)
EPAS1-Sp465	465	GGTCAGTTCGGGCAGCAGGT (SEQ ID NO: 1109)
EPAS1-Sp466	466	ATCTGGTCAGTTCGGGCAGC (SEQ ID NO: 1110)
EPAS1-Sp467	467	CAGTCATATCTGGTCAGTTC (SEQ ID NO: 1111)

EPAS1-Sp468	468	ACAGTCATATCTGGTCAGTT (SEQ ID NO: 1112)
EPAS1-Sp469	469	ACTGACCAGATATGACTGTG (SEQ ID NO: 1113)
EPAS1-Sp470	470	GTTCACCTCACAGTCATATC (SEQ ID NO: 1114)
EPAS1-Sp471	471	TGAGGTGAACGTGCCCGTGC (SEQ ID NO: 1115)
EPAS1-Sp472	472	GAGGTGAACGTGCCCGTGCT (SEQ ID NO: 1116)
EPAS1-Sp473	473	AGCGTGGAGCTTCCCAGCAC (SEQ ID NO: 1117)
EPAS1-Sp474	474	GAGCGTGGAGCTTCCCAGCA (SEQ ID NO: 1118)
EPAS1-Sp475	475	GGAAGCTCCACGCTCCTGCA (SEQ ID NO: 1119)
EPAS1-Sp476	476	AGCTCCACGCTCCTGCAAGG (SEQ ID NO: 1120)
EPAS1-Sp477	477	GCTCCACGCTCCTGCAAGGA (SEQ ID NO: 1121)
EPAS1-Sp478	478	CTCCACGCTCCTGCAAGGAG (SEQ ID NO: 1122)
EPAS1-Sp479	479	GTCCCCTCCTTGCAGGAGCG (SEQ ID NO: 1123)
EPAS1-Sp480	480	TGAGGAGGTCCCCTCCTTGC (SEQ ID NO: 1124)
EPAS1-Sp481	481	AGGGGACCTCCTCAGAGCCC (SEQ ID NO: 1125)
EPAS1-Sp482	482	CCTCCTCAGAGCCCTGGACC (SEQ ID NO: 1126)
EPAS1-Sp483	483	CCTGGTCCAGGGCTCTGAGG (SEQ ID NO: 1127)
EPAS1-Sp484	484	TGGCCTGGTCCAGGGCTCTG (SEQ ID NO: 1128)
EPAS1-Sp485	485	GGCTCAGGTGGCCTGGTCCA (SEQ ID NO: 1129)
EPAS1-Sp486	486	TGGCTCAGGTGGCCTGGTCC (SEQ ID NO: 1130)
EPAS1-Sp487	487	TGGACCAGGCCACCTGAGCC (SEQ ID NO: 1131)
EPAS1-Sp488	488	AAGGCCTGGCTCAGGTGGCC (SEQ ID NO: 1132)
EPAS1-Sp489	489	GGTAGAAGGCCTGGCTCAGG (SEQ ID NO: 1133)

[Table 13]

Target sequences of ANGPT2 gene for SpCas9

Gene	No.	Target sequence
ANGPT2-Sp1	1	TCTGAGCTGTGATCTTGTCT (SEQ ID NO: 1134)
ANGPT2-Sp2	2	CCGCAGCCTATAACAACTTT (SEQ ID NO: 1135)
ANGPT2-Sp3	3	CCGAAAGTTGTTATAGGCTG (SEQ ID NO: 1136)
ANGPT2-Sp4	4	GCTCTTCCGAAAGTTGTTAT (SEQ ID NO: 1137)
ANGPT2-Sp5	5	TAACAACTTTCGGAAGAGCA (SEQ ID NO: 1138)
ANGPT2-Sp6	6	CGGAAGAGCATGGACAGCAT (SEQ ID NO: 1139)
ANGPT2-Sp7	7	CATAGGAAAGAAGCAATATC (SEQ ID NO: 1140)
ANGPT2-Sp8	8	AAGCAATATCAGGTCCAGCA (SEQ ID NO: 1141)
ANGPT2-Sp9	9	AGCAATATCAGGTCCAGCAT (SEQ ID NO: 1142)
ANGPT2-Sp10	10	TGTAGCTGCAGGACCCATGC (SEQ ID NO: 1143)
ANGPT2-Sp11	11	CAGGAGGAAAGTGTAGCTGC (SEQ ID NO: 1144)
ANGPT2-Sp12	12	CACTTTCCTCCTGCCAGAGA (SEQ ID NO: 1145)
ANGPT2-Sp13	13	AGTTGTCCATCTCTGGCAGG (SEQ ID NO: 1146)
ANGPT2-Sp14	14	GGCAGTTGTCCATCTCTGGC (SEQ ID NO: 1147)
ANGPT2-Sp15	15	GAGCGGCAGTTGTCCATCTC (SEQ ID NO: 1148)
ANGPT2-Sp16	16	CGTAGGGGCTGGAGGAAGAG (SEQ ID NO: 1149)
ANGPT2-Sp17	17	ATTGGACACGTAGGGGCTGG (SEQ ID NO: 1150)
ANGPT2-Sp18	18	AGCATTGGACACGTAGGGGC (SEQ ID NO: 1151)
ANGPT2-Sp19	19	GCACAGCATTGGACACGTAG (SEQ ID NO: 1152)
ANGPT2-Sp20	20	TGCACAGCATTGGACACGTA (SEQ ID NO: 1153)
ANGPT2-Sp21	21	CTGCACAGCATTGGACACGT (SEQ ID NO: 1154)
ANGPT2-Sp22	22	ACGTGTCCAATGCTGTGCAG (SEQ ID NO: 1155)
ANGPT2-Sp23	23	CGTGTCCAATGCTGTGCAGA (SEQ ID NO: 1156)
ANGPT2-Sp24	24	CGCGTCCCTCTGCACAGCAT (SEQ ID NO: 1157)
ANGPT2-Sp25	25	GCCGCTCGAATACGATGACT (SEQ ID NO: 1158)
ANGPT2-Sp26	26	ACCGAGTCATCGTATTCGAG (SEQ ID NO: 1159)
ANGPT2-Sp27	27	AATACGATGACTCGGTGCAG (SEQ ID NO: 1160)
ANGPT2-Sp28	28	GGTGCAGAGGCTGCAAGTGC (SEQ ID NO: 1161)
ANGPT2-Sp29	29	GCAAGTGCTGGAGAACATCA (SEQ ID NO: 1162)
ANGPT2-Sp30	30	TCATGGAAAACAACACTCAG (SEQ ID NO: 1163)
ANGPT2-Sp31	31	CAACACTCAGTGGCTAATGA (SEQ ID NO: 1164)
ANGPT2-Sp32	32	ACTCAGTGGCTAATGAAGGT (SEQ ID NO: 1165)
ANGPT2-Sp33	33	CTAGCTTGAGAATTATATCC (SEQ ID NO: 1166)
ANGPT2-Sp34	34	TTTCTTTCTTCATGTTGTCC (SEQ ID NO: 1167)
ANGPT2-Sp35	35	GGACAACATGAAGAAAGAAA (SEQ ID NO: 1168)

ANGPT2-Sp36	36	GAATGCAGTACAGAACCAGA (SEQ ID NO: 1169)
ANGPT2-Sp37	37	TTTCTATCATCACAGCCGTC (SEQ ID NO: 1170)
ANGPT2-Sp38	38	ACGGCTGTGATGATAGAAAT (SEQ ID NO: 1171)
ANGPT2-Sp39	39	CGGCTGTGATGATAGAAATA (SEQ ID NO: 1172)
ANGPT2-Sp40	40	AAACCTGTTGAACCAAACAG (SEQ ID NO: 1173)
ANGPT2-Sp41	41	GCTCCGCTGTTTGGTTCAAC (SEQ ID NO: 1174)
ANGPT2-Sp42	42	ACCAAACAGCGGAGCAAACG (SEQ ID NO: 1175)
ANGPT2-Sp43	43	TCCGCGTTTGCTCCGCTGTT (SEQ ID NO: 1176)
ANGPT2-Sp44	44	AACGCGGAAGTTAACTGATG (SEQ ID NO: 1177)
ANGPT2-Sp45	45	CTAGCTTGAGAATTATATCC (SEQ ID NO: 1178)
ANGPT2-Sp46	46	TTTCTTTCTTCATGTTGTCC (SEQ ID NO: 1179)
ANGPT2-Sp47	47	GGACAACATGAAGAAAGAAA (SEQ ID NO: 1180)
ANGPT2-Sp48	48	GAATGCAGTACAGAACCAGA (SEQ ID NO: 1181)
ANGPT2-Sp49	49	TTTCTATCATCACAGCCGTC (SEQ ID NO: 1182)
ANGPT2-Sp50	50	ACGGCTGTGATGATAGAAAT (SEQ ID NO: 1183)
ANGPT2-Sp51	51	CGGCTGTGATGATAGAAATA (SEQ ID NO: 1184)
ANGPT2-Sp52	52	AAACCTGTTGAACCAAACAG (SEQ ID NO: 1185)
ANGPT2-Sp53	53	GCTCCGCTGTTTGGTTCAAC (SEQ ID NO: 1186)
ANGPT2-Sp54	54	ACCAAACAGCGGAGCAAACG (SEQ ID NO: 1187)
ANGPT2-Sp55	55	TCCGCGTTTGCTCCGCTGTT (SEQ ID NO: 1188)
ANGPT2-Sp56	56	AACGCGGAAGTTAACTGATG (SEQ ID NO: 1189)
ANGPT2-Sp57	57	TACAAGTTTCCTAGAAAAGA (SEQ ID NO: 1190)
ANGPT2-Sp58	58	TAGCTAGCACCTTCTTTTCT (SEQ ID NO: 1191)
ANGPT2-Sp59	59	AGAAAAGAAGGTGCTAGCTA (SEQ ID NO: 1192)
ANGPT2-Sp60	60	CTTCTTTTATTGACTGTAGT (SEQ ID NO: 1193)
ANGPT2-Sp61	61	AGAAGAGAAAGATCAGCTAC (SEQ ID NO: 1194)
ANGPT2-Sp62	62	TTCAATGATGGAATTTTGCT (SEQ ID NO: 1195)
ANGPT2-Sp63	63	TTTTTCTAGTTCTTCAATGA (SEQ ID NO: 1196)
ANGPT2-Sp64	64	AAAAAAAATAGTGACTGCCA (SEQ ID NO: 1197)
ANGPT2-Sp65	65	AAGAACTGAATTATTCACCG (SEQ ID NO: 1198)
ANGPT2-Sp66	66	GAAGCAGCAACATGATCTCA (SEQ ID NO: 1199)
ANGPT2-Sp67	67	TGTAAACTTACAGTTTGATG (SEQ ID NO: 1200)
ANGPT2-Sp68	68	CTATTTTTTAAAAGCAGCTA (SEQ ID NO: 1201)
ANGPT2-Sp69	69	GTTCTTCTTTAGCAACAGTG (SEQ ID NO: 1202)
ANGPT2-Sp70	70	TGTTCTTCTTTAGCAACAGT (SEQ ID NO: 1203)
ANGPT2-Sp71	71	TTGTTCTTCTTTAGCAACAG (SEQ ID NO: 1204)

ANGPT2-Sp72	72	TGTGCTGAAGTATTCAAATC (SEQ ID NO: 1205)
ANGPT2-Sp73	73	AAATCAGGACACACCACGAA (SEQ ID NO: 1206)
ANGPT2-Sp74	74	TAACGTGTAGATGCCATTCG (SEQ ID NO: 1207)
ANGPT2-Sp75	75	TGATCTCTTCTGTAGAATTA (SEQ ID NO: 1208)
ANGPT2-Sp76	76	TTGATCTCTTCTGTAGAATT (SEQ ID NO: 1209)
ANGPT2-Sp77	77	TAATTCTACAGAAGAGATCA (SEQ ID NO: 1210)
ANGPT2-Sp78	78	CTACAGAAGAGATCAAGGTG (SEQ ID NO: 1211)
ANGPT2-Sp79	79	TTTGCAGGCCTACTGTGACA (SEQ ID NO: 1212)
ANGPT2-Sp80	80	GCCTACTGTGACATGGAAGC (SEQ ID NO: 1213)
ANGPT2-Sp81	81	TCCAGCTTCCATGTCACAGT (SEQ ID NO: 1214)
ANGPT2-Sp82	82	TACTGTGACATGGAAGCTGG (SEQ ID NO: 1215)
ANGPT2-Sp83	83	TGTGACATGGAAGCTGGAGG (SEQ ID NO: 1216)
ANGPT2-Sp84	84	GACATGGAAGCTGGAGGAGG (SEQ ID NO: 1217)
ANGPT2-Sp85	85	ACATGGAAGCTGGAGGAGGC (SEQ ID NO: 1218)
ANGPT2-Sp86	86	TGGAAGCTGGAGGAGGCGGG (SEQ ID NO: 1219)
ANGPT2-Sp87	87	GACAATTATTCAGCGACGTG (SEQ ID NO: 1220)
ANGPT2-Sp88	88	ATTATTCAGCGACGTGAGGA (SEQ ID NO: 1221)
ANGPT2-Sp89	89	ATGGCAGCGTTGATTTTCAG (SEQ ID NO: 1222)
ANGPT2-Sp90	90	GCGTTGATTTTCAGAGGACT (SEQ ID NO: 1223)
ANGPT2-Sp91	91	GACTTGGAAAGAATATAAAG (SEQ ID NO: 1224)
ANGPT2-Sp92	92	GGAAAGAATATAAAGTGGTA (SEQ ID NO: 1225)
ANGPT2-Sp93	93	CAGGGATTTGGTAACCCTTC (SEQ ID NO: 1226)
ANGPT2-Sp94	94	GTAACCCTTCAGGAGAATAT (SEQ ID NO: 1227)
ANGPT2-Sp95	95	CCCTTCAGGAGAATATTGGC (SEQ ID NO: 1228)
ANGPT2-Sp96	96	CCAGCCAATATTCTCCTGAA (SEQ ID NO: 1229)
ANGPT2-Sp97	97	CCTTCAGGAGAATATTGGCT (SEQ ID NO: 1230)
ANGPT2-Sp98	98	CCCAGCCAATATTCTCCTGA (SEQ ID NO: 1231)
ANGPT2-Sp99	99	TTAAAATACACCTTAAAGAC (SEQ ID NO: 1232)
ANGPT2-Sp100	100	TAAAATACACCTTAAAGACT (SEQ ID NO: 1233)
ANGPT2-Sp101	101	ATACACCTTAAAGACTGGGA (SEQ ID NO: 1234)
ANGPT2-Sp102	102	TACACCTTAAAGACTGGGAA (SEQ ID NO: 1235)
ANGPT2-Sp103	103	CATTCCCTTCCCAGTCTTTA (SEQ ID NO: 1236)
ANGPT2-Sp104	104	TAAAGACTGGGAAGGGAATG (SEQ ID NO: 1237)
ANGPT2-Sp105	105	CAAGTGAAGAACTCAATTAT (SEQ ID NO: 1238)
ANGPT2-Sp106	106	GCTTACAGGATTCACCTTAA (SEQ ID NO: 1239)
ANGPT2-Sp107	107	ATTCACCTTAAAGGACTTAC (SEQ ID NO: 1240)

ANGPT2-Sp108	108	TTCACCTTAAAGGACTTACA (SEQ ID NO: 1241)
ANGPT2-Sp109	109	CTGTCCCTGTAAGTCCTTTA (SEQ ID NO: 1242)
ANGPT2-Sp110	110	AAAGGACTTACAGGGACAGC (SEQ ID NO: 1243)
ANGPT2-Sp111	111	GCTGATGCTGCTTATTTTGC (SEQ ID NO: 1244)
ANGPT2-Sp112	112	ATAAGCAGCATCAGCCAACC (SEQ ID NO: 1245)
ANGPT2-Sp113	113	TGCTAAAATCATTTCCTGGT (SEQ ID NO: 1246)
ANGPT2-Sp114	114	TTTGTGCTAAAATCATTTCC (SEQ ID NO: 1247)
ANGPT2-Sp115	115	AGGAAATGATTTTAGCACAA (SEQ ID NO: 1248)
ANGPT2-Sp116	116	AATGATTTTAGCACAAAGGA (SEQ ID NO: 1249)
ANGPT2-Sp117	117	AAATGTTCACAAATGCTAAC (SEQ ID NO: 1250)
ANGPT2-Sp118	118	TGTTCACAAATGCTAACAGG (SEQ ID NO: 1251)
ANGPT2-Sp119	119	CACAAATGCTAACAGGAGGT (SEQ ID NO: 1252)
ANGPT2-Sp120	120	ACAAATGCTAACAGGAGGTA (SEQ ID NO: 1253)
ANGPT2-Sp121	121	GGCTGGTGGTTTGATGCATG (SEQ ID NO: 1254)
ANGPT2-Sp122	122	TGTGGTCCTTCCAACTTGAA (SEQ ID NO: 1255)
ANGPT2-Sp123	123	TACATTCCGTTCAAGTTGGA (SEQ ID NO: 1256)
ANGPT2-Sp124	124	ATAGTACATTCCGTTCAAGT (SEQ ID NO: 1257)
ANGPT2-Sp125	125	ACGGAATGTACTATCCACAG (SEQ ID NO: 1258)
ANGPT2-Sp126	126	TTATTTGTGTTCTGCCTCTG (SEQ ID NO: 1259)
ANGPT2-Sp127	127	CAGAACACAAATAAGTTCAA (SEQ ID NO: 1260)
ANGPT2-Sp128	128	ATAAGTTCAACGGCATTAAA (SEQ ID NO: 1261)
ANGPT2-Sp129	129	ACGGCATTAAATGGTACTAC (SEQ ID NO: 1262)
ANGPT2-Sp130	130	ATTAAATGGTACTACTGGAA (SEQ ID NO: 1263)
ANGPT2-Sp131	131	TGGTACTACTGGAAAGGCTC (SEQ ID NO: 1264)
ANGPT2-Sp132	132	AGGCTCAGGCTATTCGCTCA (SEQ ID NO: 1265)
ANGPT2-Sp133	133	TGGTCGGATCATCATGGTTG (SEQ ID NO: 1266)
ANGPT2-Sp134	134	ATCTGCTGGTCGGATCATCA (SEQ ID NO: 1267)
ANGPT2-Sp135	135	ATGTTTAGAAATCTGCTGGT (SEQ ID NO: 1268)
ANGPT2-Sp136	136	TGGGATGTTTAGAAATCTGC (SEQ ID NO: 1269)

[Table 14]

Target sequences of ANGPTL4 gene for SpCas9

Gene	No.	Target sequence
ANGPTL4-Sp1	1	AGGCTACCTAAGAGGATGAG (SEQ ID NO: 1270)
ANGPTL4-Sp2	2	GAGGATGAGCGGTGCTCCGA (SEQ ID NO: 1271)
ANGPTL4-Sp3	3	ATGAGCGGTGCTCCGACGGC (SEQ ID NO: 1272)
ANGPTL4-Sp4	4	TGAGCGGTGCTCCGACGGCC (SEQ ID NO: 1273)
ANGPTL4-Sp5	5	GAGCGGTGCTCCGACGGCCG (SEQ ID NO: 1274)
ANGPTL4-Sp6	6	ATCAGGGCTGCCCCGGCCGT (SEQ ID NO: 1275)
ANGPTL4-Sp7	7	GCAGAGCATCAGGGCTGCCC (SEQ ID NO: 1276)
ANGPTL4-Sp8	8	GGTGGCGGCGCAGAGCATCA (SEQ ID NO: 1277)
ANGPTL4-Sp9	9	CGGTGGCGGCGCAGAGCATC (SEQ ID NO: 1278)
ANGPTL4-Sp10	10	GCTCAGTAGCACGGCGGTGG (SEQ ID NO: 1279)
ANGPTL4-Sp11	11	AGCGCTCAGTAGCACGGCGG (SEQ ID NO: 1280)
ANGPTL4-Sp12	12	CTGAGCGCTCAGTAGCACGG (SEQ ID NO: 1281)
ANGPTL4-Sp13	13	CGCCGTGCTACTGAGCGCTC (SEQ ID NO: 1282)
ANGPTL4-Sp14	14	GCCGTGCTACTGAGCGCTCA (SEQ ID NO: 1283)
ANGPTL4-Sp15	15	GCCCTGAGCGCTCAGTAGCA (SEQ ID NO: 1284)
ANGPTL4-Sp16	16	GTGCTACTGAGCGCTCAGGG (SEQ ID NO: 1285)
ANGPTL4-Sp17	17	CGCGGCGACTTGGACTGCAC (SEQ ID NO: 1286)
ANGPTL4-Sp18	18	GCGCGGCGACTTGGACTGCA (SEQ ID NO: 1287)
ANGPTL4-Sp19	19	GGACGCAAAGCGCGGCGACT (SEQ ID NO: 1288)
ANGPTL4-Sp20	20	AGTCGCCGCGCTTTGCGTCC (SEQ ID NO: 1289)
ANGPTL4-Sp21	21	GTCGCCGCGCTTTGCGTCCT (SEQ ID NO: 1290)
ANGPTL4-Sp22	22	TCGTCCCAGGACGCAAAGCG (SEQ ID NO: 1291)
ANGPTL4-Sp23	23	CAGGACATTCATCTCGTCCC (SEQ ID NO: 1292)
ANGPTL4-Sp24	24	CTGGGACGAGATGAATGTCC (SEQ ID NO: 1293)
ANGPTL4-Sp25	25	GAGATGAATGTCCTGGCGCA (SEQ ID NO: 1294)
ANGPTL4-Sp26	26	GCTGCAGGAGTCCGTGCGCC (SEQ ID NO: 1295)
ANGPTL4-Sp27	27	GCGCACGGACTCCTGCAGCT (SEQ ID NO: 1296)
ANGPTL4-Sp28	28	CGGACTCCTGCAGCTCGGCC (SEQ ID NO: 1297)
ANGPTL4-Sp29	29	GGACTCCTGCAGCTCGGCCA (SEQ ID NO: 1298)
ANGPTL4-Sp30	30	GACTCCTGCAGCTCGGCCAG (SEQ ID NO: 1299)
ANGPTL4-Sp31	31	GCAGCCCCTGGCCGAGCTGC (SEQ ID NO: 1300)
ANGPTL4-Sp32	32	CCAGGGGCTGCGCGAACACG (SEQ ID NO: 1301)
ANGPTL4-Sp33	33	CCGCGTGTTCGCGCAGCCCC (SEQ ID NO: 1302)
ANGPTL4-Sp34	34	CAGCGCGCTCAGCTGACTGC (SEQ ID NO: 1303)
ANGPTL4-Sp35	35	CCGCAGTCAGCTGAGCGCGC (SEQ ID NO: 1304)

ANGPTL4-Sp36	36	CCAGCGCGCTCAGCTGACTG (SEQ ID NO: 1305)
ANGPTL4-Sp37	37	GTCAGCTGAGCGCGCTGGAG (SEQ ID NO: 1306)
ANGPTL4-Sp38	38	GAGCGGCGCCTGAGCGCGTG (SEQ ID NO: 1307)
ANGPTL4-Sp39	39	AGCGGCGCCTGAGCGCGTGC (SEQ ID NO: 1308)
ANGPTL4-Sp40	40	AGGCGGACCCGCACGCGCTC (SEQ ID NO: 1309)
ANGPTL4-Sp41	41	CGCGTGCGGGTCCGCCTGTC (SEQ ID NO: 1310)
ANGPTL4-Sp42	42	GCGTGCGGGTCCGCCTGTCA (SEQ ID NO: 1311)
ANGPTL4-Sp43	43	GTCCGCCTGTCAGGGAACCG (SEQ ID NO: 1312)
ANGPTL4-Sp44	44	TCCGCCTGTCAGGGAACCGA (SEQ ID NO: 1313)
ANGPTL4-Sp45	45	CCGCCTGTCAGGGAACCGAG (SEQ ID NO: 1314)
ANGPTL4-Sp46	46	CCCCTCGGTTCCCTGACAGG (SEQ ID NO: 1315)
ANGPTL4-Sp47	47	GGACCCCTCGGTTCCCTGAC (SEQ ID NO: 1316)
ANGPTL4-Sp48	48	CGGGAGGTCGGTGGACCCCT (SEQ ID NO: 1317)
ANGPTL4-Sp49	49	AGGGGCTAACGGGAGGTCGG (SEQ ID NO: 1318)
ANGPTL4-Sp50	50	CTCAGGGGCTAACGGGAGGT (SEQ ID NO: 1319)
ANGPTL4-Sp51	51	GGCTCTCAGGGGCTAACGGG (SEQ ID NO: 1320)
ANGPTL4-Sp52	52	TCCCGTTAGCCCCTGAGAGC (SEQ ID NO: 1321)
ANGPTL4-Sp53	53	CCCGTTAGCCCCTGAGAGCC (SEQ ID NO: 1322)
ANGPTL4-Sp54	54	CCCGGCTCTCAGGGGCTAAC (SEQ ID NO: 1323)
ANGPTL4-Sp55	55	ACCCGGCTCTCAGGGGCTAA (SEQ ID NO: 1324)
ANGPTL4-Sp56	56	GTTAGCCCCTGAGAGCCGGG (SEQ ID NO: 1325)
ANGPTL4-Sp57	57	AGGGTCCACCCGGCTCTCAG (SEQ ID NO: 1326)
ANGPTL4-Sp58	58	CAGGGTCCACCCGGCTCTCA (SEQ ID NO: 1327)
ANGPTL4-Sp59	59	TCAGGGTCCACCCGGCTCTC (SEQ ID NO: 1328)
ANGPTL4-Sp60	60	TGAGAGCCGGGTGGACCCTG (SEQ ID NO: 1329)
ANGPTL4-Sp61	61	GAAGGACCTCAGGGTCCACC (SEQ ID NO: 1330)
ANGPTL4-Sp62	62	GCAGGCTGTGAAGGACCTCA (SEQ ID NO: 1331)
ANGPTL4-Sp63	63	TGCAGGCTGTGAAGGACCTC (SEQ ID NO: 1332)
ANGPTL4-Sp64	64	TGAGGTCCTTCACAGCCTGC (SEQ ID NO: 1333)
ANGPTL4-Sp65	65	CACGTACCTGCAGGCTGTGA (SEQ ID NO: 1334)
ANGPTL4-Sp66	66	CCCTGGGGACACGTACCTGC (SEQ ID NO: 1335)
ANGPTL4-Sp67	67	CCCTCCCCAGACACAACTCA (SEQ ID NO: 1336)
ANGPTL4-Sp68	68	TGAGCCTTGAGTTGTGTCTG (SEQ ID NO: 1337)
ANGPTL4-Sp69	69	CTGAGCCTTGAGTTGTGTCT (SEQ ID NO: 1338)
ANGPTL4-Sp70	70	TCTGAGCCTTGAGTTGTGTC (SEQ ID NO: 1339)
ANGPTL4-Sp71	71	AACTCAAGGCTCAGAACAGC (SEQ ID NO: 1340)

ANGPTL4-Sp72	72	GATCCAGCAACTCTTCCACA (SEQ ID NO: 1341)
ANGPTL4-Sp73	73	CCAGCAACTCTTCCACAAGG (SEQ ID NO: 1342)
ANGPTL4-Sp74	74	CCACCTTGTGGAAGAGTTGC (SEQ ID NO: 1343)
ANGPTL4-Sp75	75	GCTGCTGCTGGGCCACCTTG (SEQ ID NO: 1344)
ANGPTL4-Sp76	76	ACAAGGTGGCCCAGCAGCAG (SEQ ID NO: 1345)
ANGPTL4-Sp77	77	GGCCCAGCAGCAGCGGCACC (SEQ ID NO: 1346)
ANGPTL4-Sp78	78	CTCCAGGTGCCGCTGCTGCT (SEQ ID NO: 1347)
ANGPTL4-Sp79	79	TCTCCAGGTGCCGCTGCTGC (SEQ ID NO: 1348)
ANGPTL4-Sp80	80	TTCGCAGGTGCTGCTTCTCC (SEQ ID NO: 1349)
ANGPTL4-Sp81	81	TTTGCAGATGCTGAATTCGC (SEQ ID NO: 1350)
ANGPTL4-Sp82	82	AATTCAGCATCTGCAAAGCC (SEQ ID NO: 1351)
ANGPTL4-Sp83	83	CCCTTGATCCTAGGGTTACC (SEQ ID NO: 1352)
ANGPTL4-Sp84	84	CCCATCCTAGTTTGGCCTCC (SEQ ID NO: 1353)
ANGPTL4-Sp85	85	GTGGTCCAGGAGGCCAAACT (SEQ ID NO: 1354)
ANGPTL4-Sp86	86	CTAGGTGCTTGTGGTCCAGG (SEQ ID NO: 1355)
ANGPTL4-Sp87	87	GGTCTAGGTGCTTGTGGTCC (SEQ ID NO: 1356)
ANGPTL4-Sp88	88	CCACAAGCACCTAGACCATG (SEQ ID NO: 1357)
ANGPTL4-Sp89	89	CCTCATGGTCTAGGTGCTTG (SEQ ID NO: 1358)
ANGPTL4-Sp90	90	CAAGCACCTAGACCATGAGG (SEQ ID NO: 1359)
ANGPTL4-Sp91	91	GCTTGGCCACCTCATGGTCT (SEQ ID NO: 1360)
ANGPTL4-Sp92	92	GGGCAGGCTTGGCCACCTCA (SEQ ID NO: 1361)
ANGPTL4-Sp93	93	CCAAGCCTGCCCGAAGAAAG (SEQ ID NO: 1362)
ANGPTL4-Sp94	94	CCTCTTTCTTCGGGCAGGCT (SEQ ID NO: 1363)
ANGPTL4-Sp95	95	GGCAGCCTCTTTCTTCGGGC (SEQ ID NO: 1364)
ANGPTL4-Sp96	96	CTCGGGCAGCCTCTTTCTTC (SEQ ID NO: 1365)
ANGPTL4-Sp97	97	TCTCGGGCAGCCTCTTTCTT (SEQ ID NO: 1366)
ANGPTL4-Sp98	98	AAGAAAGAGGCTGCCCGAGA (SEQ ID NO: 1367)
ANGPTL4-Sp99	99	TCAACTGGCTGGGCCATCTC (SEQ ID NO: 1368)
ANGPTL4-Sp100	100	GTCAACTGGCTGGGCCATCT (SEQ ID NO: 1369)
ANGPTL4-Sp101	101	GATGGCCCAGCCAGTTGACC (SEQ ID NO: 1370)
ANGPTL4-Sp102	102	GTGAGCCGGGTCAACTGGCT (SEQ ID NO: 1371)
ANGPTL4-Sp103	103	TGTGAGCCGGGTCAACTGGC (SEQ ID NO: 1372)
ANGPTL4-Sp104	104	ACATTGTGAGCCGGGTCAAC (SEQ ID NO: 1373)
ANGPTL4-Sp105	105	GGCGGCTGACATTGTGAGCC (SEQ ID NO: 1374)
ANGPTL4-Sp106	106	AGGCGGCTGACATTGTGAGC (SEQ ID NO: 1375)
ANGPTL4-Sp107	107	GCAGACACTCACGGTGCAGG (SEQ ID NO: 1376)

ANGPTL4-Sp108	108	GGGGCAGACACTCACGGTGC (SEQ ID NO: 1377)
ANGPTL4-Sp109	109	ATCTCCCTTCAGGGCTGCCC (SEQ ID NO: 1378)
ANGPTL4-Sp110	110	TCTCCCTTCAGGGCTGCCCA (SEQ ID NO: 1379)
ANGPTL4-Sp111	111	AGGGCTGCCCAGGGATTGCC (SEQ ID NO: 1380)
ANGPTL4-Sp112	112	AACAGCTCCTGGCAATCCCT (SEQ ID NO: 1381)
ANGPTL4-Sp113	113	GAACAGCTCCTGGCAATCCC (SEQ ID NO: 1382)
ANGPTL4-Sp114	114	GGATTGCCAGGAGCTGTTCC (SEQ ID NO: 1383)
ANGPTL4-Sp115	115	TGCCAGGAGCTGTTCCAGGT (SEQ ID NO: 1384)
ANGPTL4-Sp116	116	CCAGGAGCTGTTCCAGGTTG (SEQ ID NO: 1385)
ANGPTL4-Sp117	117	CCCCAACCTGGAACAGCTCC (SEQ ID NO: 1386)
ANGPTL4-Sp118	118	AGCTGTTCCAGGTTGGGGAG (SEQ ID NO: 1387)
ANGPTL4-Sp119	119	CACTCTGCCTCTCCCCAACC (SEQ ID NO: 1388)
ANGPTL4-Sp120	120	CAGGTTGGGGAGAGGCAGAG (SEQ ID NO: 1389)
ANGPTL4-Sp121	121	ACTATTTGAAATCCAGCCTC (SEQ ID NO: 1390)
ANGPTL4-Sp122	122	CTATTTGAAATCCAGCCTCA (SEQ ID NO: 1391)
ANGPTL4-Sp123	123	TATTTGAAATCCAGCCTCAG (SEQ ID NO: 1392)
ANGPTL4-Sp124	124	ATGGCGGAGACCCCTGAGGC (SEQ ID NO: 1393)
ANGPTL4-Sp125	125	AAAAATGGCGGAGACCCCTG (SEQ ID NO: 1394)
ANGPTL4-Sp126	126	TCAGGGGTCTCCGCCATTTT (SEQ ID NO: 1395)
ANGPTL4-Sp127	127	TTGCAGTTCACCAAAAATGG (SEQ ID NO: 1396)
ANGPTL4-Sp128	128	ATCTTGCAGTTCACCAAAAA (SEQ ID NO: 1397)
ANGPTL4-Sp129	129	GTGAACTGCAAGATGACCTC (SEQ ID NO: 1398)
ANGPTL4-Sp130	130	ACTGCAAGATGACCTCAGGT (SEQ ID NO: 1399)
ANGPTL4-Sp131	131	CTGCAAGATGACCTCAGGTA (SEQ ID NO: 1400)
ANGPTL4-Sp132	132	GGACTAACACACCCTACCTG (SEQ ID NO: 1401)
ANGPTL4-Sp133	133	GTACCTTTCTGGGCAGATGG (SEQ ID NO: 1402)
ANGPTL4-Sp134	134	CTTTCTGGGCAGATGGAGGC (SEQ ID NO: 1403)
ANGPTL4-Sp135	135	GAGGCTGGACAGTAATTCAG (SEQ ID NO: 1404)
ANGPTL4-Sp136	136	GTAATTCAGAGGCGCCACGA (SEQ ID NO: 1405)
ANGPTL4-Sp137	137	GAGGCGCCACGATGGCTCAG (SEQ ID NO: 1406)
ANGPTL4-Sp138	138	TGAAGTCCACTGAGCCATCG (SEQ ID NO: 1407)
ANGPTL4-Sp139	139	ATGGCTCAGTGGACTTCAAC (SEQ ID NO: 1408)
ANGPTL4-Sp140	140	CAGTGGACTTCAACCGGCCC (SEQ ID NO: 1409)
ANGPTL4-Sp141	141	AGTGGACTTCAACCGGCCCT (SEQ ID NO: 1410)
ANGPTL4-Sp142	142	CCGGCCCTGGGAAGCCTACA (SEQ ID NO: 1411)
ANGPTL4-Sp143	143	CCTTGTAGGCTTCCCAGGGC (SEQ ID NO: 1412)

ANGPTL4-Sp144	144	GCCCTGGGAAGCCTACAAGG (SEQ ID NO: 1413)
ANGPTL4-Sp145	145	CCCTGGGAAGCCTACAAGGC (SEQ ID NO: 1414)
ANGPTL4-Sp146	146	CCCGCCTTGTAGGCTTCCCA (SEQ ID NO: 1415)
ANGPTL4-Sp147	147	CCTGGGAAGCCTACAAGGCG (SEQ ID NO: 1416)
ANGPTL4-Sp148	148	CCCCGCCTTGTAGGCTTCCC (SEQ ID NO: 1417)
ANGPTL4-Sp149	149	GAAGCCTACAAGGCGGGGTT (SEQ ID NO: 1418)
ANGPTL4-Sp150	150	AAGCCTACAAGGCGGGGTTT (SEQ ID NO: 1419)
ANGPTL4-Sp151	151	AGCCTACAAGGCGGGGTTTG (SEQ ID NO: 1420)
ANGPTL4-Sp152	152	ATCCCCAAACCCCGCCTTGT (SEQ ID NO: 1421)
ANGPTL4-Sp153	153	GCGGGGTTTGGGGATCCCCA (SEQ ID NO: 1422)
ANGPTL4-Sp154	154	GGTTTGGGGATCCCCACGGT (SEQ ID NO: 1423)
ANGPTL4-Sp155	155	CACTAGAAACACCTACCGTG (SEQ ID NO: 1424)
ANGPTL4-Sp156	156	CCACTAGAAACACCTACCGT (SEQ ID NO: 1425)
ANGPTL4-Sp157	157	CTCCCACTCCAGGCGAGTTC (SEQ ID NO: 1426)
ANGPTL4-Sp158	158	CACTCCAGGCGAGTTCTGGC (SEQ ID NO: 1427)
ANGPTL4-Sp159	159	ACTCCAGGCGAGTTCTGGCT (SEQ ID NO: 1428)
ANGPTL4-Sp160	160	AGACCCAGCCAGAACTCGCC (SEQ ID NO: 1429)
ANGPTL4-Sp161	161	AGGCGAGTTCTGGCTGGGTC (SEQ ID NO: 1430)
ANGPTL4-Sp162	162	GTTCTGGCTGGGTCTGGAGA (SEQ ID NO: 1431)
ANGPTL4-Sp163	163	GGAGAAGGTGCATAGCATCA (SEQ ID NO: 1432)
ANGPTL4-Sp164	164	GAGAAGGTGCATAGCATCAC (SEQ ID NO: 1433)
ANGPTL4-Sp165	165	AGAAGGTGCATAGCATCACG (SEQ ID NO: 1434)
ANGPTL4-Sp166	166	GAAGGTGCATAGCATCACGG (SEQ ID NO: 1435)
ANGPTL4-Sp167	167	GGGGGACCGCAACAGCCGCC (SEQ ID NO: 1436)
ANGPTL4-Sp168	168	GCACGGCCAGGCGGCTGTTG (SEQ ID NO: 1437)
ANGPTL4-Sp169	169	GCCGCCTGGCCGTGCAGCTG (SEQ ID NO: 1438)
ANGPTL4-Sp170	170	CCGCCTGGCCGTGCAGCTGC (SEQ ID NO: 1439)
ANGPTL4-Sp171	171	CCCGCAGCTGCACGGCCAGG (SEQ ID NO: 1440)
ANGPTL4-Sp172	172	AGTCCCGCAGCTGCACGGCC (SEQ ID NO: 1441)
ANGPTL4-Sp173	173	TGGCCGTGCAGCTGCGGGAC (SEQ ID NO: 1442)
ANGPTL4-Sp174	174	GGCCGTGCAGCTGCGGGACT (SEQ ID NO: 1443)
ANGPTL4-Sp175	175	ATCCCAGTCCCGCAGCTGCA (SEQ ID NO: 1444)
ANGPTL4-Sp176	176	GTGCAGCTGCGGGACTGGGA (SEQ ID NO: 1445)
ANGPTL4-Sp177	177	CACGGAGAACTGCAGCAACT (SEQ ID NO: 1446)
ANGPTL4-Sp178	178	GCTGCAGTTCTCCGTGCACC (SEQ ID NO: 1447)
ANGPTL4-Sp179	179	CTGCAGTTCTCCGTGCACCT (SEQ ID NO: 1448)

ANGPTL4-Sp180	180	CAGTTCTCCGTGCACCTGGG (SEQ ID NO: 1449)
ANGPTL4-Sp181	181	CTCCGTGCACCTGGGTGGCG (SEQ ID NO: 1450)
ANGPTL4-Sp182	182	GTCCTCGCCACCCAGGTGCA (SEQ ID NO: 1451)
ANGPTL4-Sp183	183	GCACCTGGGTGGCGAGGACA (SEQ ID NO: 1452)
ANGPTL4-Sp184	184	AGGCCGTGTCCTCGCCACCC (SEQ ID NO: 1453)
ANGPTL4-Sp185	185	TGCAGTGAGCTGCAGGCTAT (SEQ ID NO: 1454)
ANGPTL4-Sp186	186	CCTGCAGCTCACTGCACCCG (SEQ ID NO: 1455)
ANGPTL4-Sp187	187	CCACGGGTGCAGTGAGCTGC (SEQ ID NO: 1456)
ANGPTL4-Sp188	188	CAGCTCACTGCACCCGTGGC (SEQ ID NO: 1457)
ANGPTL4-Sp189	189	TGCACCCGTGGCCGGCCAGC (SEQ ID NO: 1458)
ANGPTL4-Sp190	190	GCACCCGTGGCCGGCCAGCT (SEQ ID NO: 1459)
ANGPTL4-Sp191	191	GCGCCCAGCTGGCCGGCCAC (SEQ ID NO: 1460)
ANGPTL4-Sp192	192	GGCGCCCAGCTGGCCGGCCA (SEQ ID NO: 1461)
ANGPTL4-Sp193	193	GGTGGTGGCGCCCAGCTGGC (SEQ ID NO: 1462)
ANGPTL4-Sp194	194	GGACGGTGGTGGCGCCCAGC (SEQ ID NO: 1463)
ANGPTL4-Sp195	195	GCCACCACCGTCCCACCCAG (SEQ ID NO: 1464)
ANGPTL4-Sp196	196	GCCGCTGGGTGGGACGGTGG (SEQ ID NO: 1465)
ANGPTL4-Sp197	197	GAGGCCGCTGGGTGGGACGG (SEQ ID NO: 1466)
ANGPTL4-Sp198	198	GGAGAGGCCGCTGGGTGGGA (SEQ ID NO: 1467)
ANGPTL4-Sp199	199	GTACGGAGAGGCCGCTGGGT (SEQ ID NO: 1468)
ANGPTL4-Sp200	200	GGTACGGAGAGGCCGCTGGG (SEQ ID NO: 1469)
ANGPTL4-Sp201	201	AAGGGTACGGAGAGGCCGCT (SEQ ID NO: 1470)
ANGPTL4-Sp202	202	GAAGGGTACGGAGAGGCCGC (SEQ ID NO: 1471)
ANGPTL4-Sp203	203	AAGTGGAGAAGGGTACGGAG (SEQ ID NO: 1472)
ANGPTL4-Sp204	204	TCTCCGTACCCTTCTCCACT (SEQ ID NO: 1473)
ANGPTL4-Sp205	205	CTCCGTACCCTTCTCCACTT (SEQ ID NO: 1474)
ANGPTL4-Sp206	206	GTCCCAAGTGGAGAAGGGTA (SEQ ID NO: 1475)
ANGPTL4-Sp207	207	ACCCTTCTCCACTTGGGACC (SEQ ID NO: 1476)
ANGPTL4-Sp208	208	TCCTGGTCCCAAGTGGAGAA (SEQ ID NO: 1477)
ANGPTL4-Sp209	209	ATCCTGGTCCCAAGTGGAGA (SEQ ID NO: 1478)
ANGPTL4-Sp210	210	GTCGTGATCCTGGTCCCAAG (SEQ ID NO: 1479)
ANGPTL4-Sp211	211	ACCAGGATCACGACCTCCGC (SEQ ID NO: 1480)
ANGPTL4-Sp212	212	CCAGGATCACGACCTCCGCA (SEQ ID NO: 1481)
ANGPTL4-Sp213	213	CCCTGCGGAGGTCGTGATCC (SEQ ID NO: 1482)
ANGPTL4-Sp214	214	CGCAGTTCTTGTCCCTGCGG (SEQ ID NO: 1483)
ANGPTL4-Sp215	215	TGGCGCAGTTCTTGTCCCTG (SEQ ID NO: 1484)

ANGPTL4-Sp216	216	AACTGCGCCAAGAGCCTCTC (SEQ ID NO: 1485)
ANGPTL4-Sp217	217	CTGCTCACCAGAGAGGCTCT (SEQ ID NO: 1486)
ANGPTL4-Sp218	218	GCAGGGCCTGCTCACCAGAG (SEQ ID NO: 1487)
ANGPTL4-Sp219	219	CCCTGACCCCGGCAGGAGGC (SEQ ID NO: 1488)
ANGPTL4-Sp220	220	TGACCCCGGCAGGAGGCTGG (SEQ ID NO: 1489)
ANGPTL4-Sp221	221	CCGGCAGGAGGCTGGTGGTT (SEQ ID NO: 1490)
ANGPTL4-Sp222	222	GTTGAGGTTGGAATGGCTGC (SEQ ID NO: 1491)
ANGPTL4-Sp223	223	TGCAGCCATTCCAACCTCAA (SEQ ID NO: 1492)
ANGPTL4-Sp224	224	ACTGGCCGTTGAGGTTGGAA (SEQ ID NO: 1493)
ANGPTL4-Sp225	225	GAAGTACTGGCCGTTGAGGT (SEQ ID NO: 1494)
ANGPTL4-Sp226	226	AGCGGAAGTACTGGCCGTTG (SEQ ID NO: 1495)
ANGPTL4-Sp227	227	GTGGGATGGAGCGGAAGTAC (SEQ ID NO: 1496)
ANGPTL4-Sp228	228	TCCGCTCCATCCCACAGCAG (SEQ ID NO: 1497)
ANGPTL4-Sp229	229	GCCGCTGCTGTGGGATGGAG (SEQ ID NO: 1498)
ANGPTL4-Sp230	230	CTTCTGCCGCTGCTGTGGGA (SEQ ID NO: 1499)
ANGPTL4-Sp231	231	TAAGCTTCTGCCGCTGCTGT (SEQ ID NO: 1500)
ANGPTL4-Sp232	232	TTAAGCTTCTGCCGCTGCTG (SEQ ID NO: 1501)
ANGPTL4-Sp233	233	GCAGCGGCAGAAGCTTAAGA (SEQ ID NO: 1502)
ANGPTL4-Sp234	234	CAGCGGCAGAAGCTTAAGAA (SEQ ID NO: 1503)
ANGPTL4-Sp235	235	AGCTTAAGAAGGGAATCTTC (SEQ ID NO: 1504)
ANGPTL4-Sp236	236	AGGGAATCTTCTGGAAGACC (SEQ ID NO: 1505)
ANGPTL4-Sp237	237	GAATCTTCTGGAAGACCTGG (SEQ ID NO: 1506)
ANGPTL4-Sp238	238	AATCTTCTGGAAGACCTGGC (SEQ ID NO: 1507)
ANGPTL4-Sp239	239	ATCTTCTGGAAGACCTGGCG (SEQ ID NO: 1508)
ANGPTL4-Sp240	240	CGGGTAGTAGCGGCCCCGCC (SEQ ID NO: 1509)
ANGPTL4-Sp241	241	GGGCCGCTACTACCCGCTGC (SEQ ID NO: 1510)
ANGPTL4-Sp242	242	TGGCCTGCAGCGGGTAGTAG (SEQ ID NO: 1511)
ANGPTL4-Sp243	243	ACATGGTGGTGGCCTGCAGC (SEQ ID NO: 1512)
ANGPTL4-Sp244	244	AACATGGTGGTGGCCTGCAG (SEQ ID NO: 1513)
ANGPTL4-Sp245	245	GGGCTGGATCAACATGGTGG (SEQ ID NO: 1514)
ANGPTL4-Sp246	246	CATGGGCTGGATCAACATGG (SEQ ID NO: 1515)
ANGPTL4-Sp247	247	CACCATGTTGATCCAGCCCA (SEQ ID NO: 1516)
ANGPTL4-Sp248	248	TGCCATGGGCTGGATCAACA (SEQ ID NO: 1517)
ANGPTL4-Sp249	249	GATCCAGCCCATGGCAGCAG (SEQ ID NO: 1518)
ANGPTL4-Sp250	250	CTGCCTCTGCTGCCATGGGC (SEQ ID NO: 1519)
ANGPTL4-Sp251	251	GAGGCTGCCTCTGCTGCCAT (SEQ ID NO: 1520)

ANGPTL4-Sp252	252	GGAGGCTGCCTCTGCTGCCA (SEQ ID NO: 1521)
ANGPTL4-Sp253	253	GGCCCAGCCAGGACGCTAGG (SEQ ID NO: 1522)

2. Construction of CjCas9 and sgRNA plasmids

A sequence encoding human codon-optimized CjCas9 (derived from *Campylobacter jejuni* subsp. *Jejuni* NCTC 11168) was synthesized to have a nuclear localization signal (NLS) and an HA epitope at the C-terminus (GeneArtTM Gene Synthesis, Thermo Fisher Scientific), and the synthesized nucleic acid sequence was replicated using a p3s plasmid described in previous research (Cho, S.W., Kim, S., Kim, J.M. & Kim, J.S. Targeted genome engineering in human cells with the Cas9 RNA-guided endonuclease. Nature Biotechnology 31, 230-232 (2013)).

A tracrRNA (transactivating crRNA) sequence and a pre-crRNA (precursor CRISPR RNA) sequence were connected using a GAAA or TGAA linker, thereby producing sgRNA. The sgRNA regulated transcription with an U6 promoter.

3. PAM characterization using cell-based reporter analysis

An AAVS1 target site (AAVS1-TS1) having a variable PAM sequence (5'-NNNNXCAC-3', 5'-NNNNAXAC-3', 5'-NNNNACXC-3', and 5'-NNNNACAX-3') including a random sequence at the X site was synthesized (Macrogen, Inc.), and the synthesized nucleic acid sequence may be replicated using a surrogate reporter plasmid encoding RFP and GFP.

To determine a suitable PAM sequence, the constructed reporter plasmid (100 ng), and plasmids encoding CjCas9 (225 ng) and sgRNA (675 ng) were co-transfected into HEK293 cells ($1x10^5$) using lipofectamine 2000 (Invitrogen). Two days after the transfection, a fractionation of the GFP and RFP-positive cells was measured by flow cytometry (BD Accuri^{TM} C6, BD).

4. Cell culture and mutation analysis

HEK293 (ATCC, CRL-1573) cells and mouse NIH 3T3 (ATCC, CRL-1658) cells were cultured in a Dulbecco's modified Eagle's medium (DMEM) supplemented with 100 units/mL penicillin, 100 mg/mL streptomycin, and 10% fetal bovine serum (FBS).

A sgRNA plasmid (750 ng) and a CjCas9 plasmid (250 ng) were transfected into cells ($0.5\sim1x10^5$) using lipofectamine 2000 (Invitrogen). 48 hours after the transfection, genome DNA was separated using a DNeasy blood & tissue kit (Qiagen), and an on-target or off-target site was amplified for targeted deep sequencing. Deep sequencing libraries were generated by PCR. TruSeq HT Dual Index primers were used to label respective samples. Mixed libraries were subjected to paired-end sequencing (LAS, Inc.), and indel frequencies were calculated.

5. Construction of AAV vectors encoding CjCas9 and sgRNA sequences

An AAV inverted terminal repeat (ITR)-based vector plasmid containing an sgRNA sequence and a CjCas9 gene having NLS and HA tags at the C-terminus was constructed. sgRNA transcription was induced by an U6 promoter, and CjCas9 expression was regulated by an EFS promoter in C2C12 myoblasts, or by a Spc512 promoter in the TA muscle of C57BL/6 mice.

For retinal delivery, an AAV vector encoding the U6 promoter-induced sgRNA and CjCas9 under the control of the EFS promoter, specific to the Vegfa gene and Hif1a gene, was constructed, wherein CjCas9 had eGFP linked to the C-terminus using a self-cleaved T2A peptide.

6. Production, purification and characterization of AAV vector

To produce the AAV vector, a pseudotype of AAVDJ or AAV9 capsids was used. The HEK293T cells were transfected with pAAV-ITR-CjCas9-sgRNA, pAAVED2/9, and a

helper plasmid. The HEK293T cells were cultured in a 2% FBS-containing DMEM. Recombinant AAV vector stocks were produced using PEI coprecipitation by mixing polyplus-transfection (PEIpro), triple-transfection and the plasmid in a molar ratio of 1:1:1 in the HEK293T cells. After 72 hours of culturing, the cells were lysed, and particles were isolated and purified with iodixanol (Sigma-Aldrich) by step-gradient ultracentrifugation. The number of vector genomes was quantified through quantitative PCR.

7. AAV transduction in mouse myoblasts

Mouse myoblasts were infected with various viral amounts of AAVDJ-CjCas9 (multiplicity of infection (MOI): 1, 5, 10, 50, and 100 (determined by quantitative PCR)), and cultured in 2% FBS-containing DMEM. For the target deep sequencing, cells were obtained at different points of time. MOI 1 is considered as infection by one virus particle among a total of 100 virus particles determined by quantitative PCR.

8. Animals

Management, use and treatment of all animals used in this research were performed under the guidance provided by the Seoul National University Animal Care and Use Committee, ophthalmology and security research, and the strict agreement according to the ARVO statement on animal use in veterinary medicine. In this research, specific male pathogen free (SPF)-6-week old C57BL/6J mice were used. The mice were maintained in a 12-hour light/dark cycle.

Diabetic model mice were induced by injecting streptozotocin (STZ, Sigma-Aldrich, St. Louis, MO, USA) intraperitonially once. As a control, a citrate buffer was injected. 4 days after the STZ injection, when a blood glucose level of the mouse was 300 mg/dl or more, diabetes was considered to be induced.

9. Injection of AAV into vitreous body

A mixture of tiletamine and zolazepam in a ratio of 1:1 (2.25 mg/kg (body weight) each), and 0.7 mg/kg (body weight) of xylazine hydrochloride was injected into a vitreous body of a 6-week old mouse for anesthetization. 2 μl (2 x 10^{10} viral genome) of AAV9-CjCas9 was injected into the vitreous body using a Nanofil syringe (World Precision Instruments Inc.) having a 33G blunt needle under a surgical microscope (Leica Microsystems Ltd.).

In the case of diabetic mouse models, the mice were injected with STZ to induce diabetes, anesthetized after 1 or 7 weeks, and 2 μl of the mixture containing $0.8x10^{8}$ vg/μl of CjCas9:Vegfa or $1.5x10^{9}$ vg/μl of Rosa26 was injected in the vitreous body.

10. Immunofluorescence staining and imaging of retinal tissue

42 days after the injection, the sample was fixed with formalin and embedded in paraffin (n=4). A sample obtained by cross-section of the sample-embedded paraffin was immunostained with an anti-HA antibody (Roche, 3F10, 1:1000), an anti-opsin antibody (Millipore, AB5405, 1:1000), and an Alexa Fluor 488 or 594 antibody (Thermo Fisher Scientific, 1:500). An opsin-positive site was detected in RPE cells expressing HA-tagged CjCas9 using Image J software (1.47v, NIH). The distribution of CjCas9 and eGFP was visualized on RPE flat-mounts using a confocal microscope (LSM 710, Carl Zeiss).

11. Extraction of genome DNA

To extract DNA from RPE and the retina, after obtaining images of RPE and the retina flat-mounts, the tissue samples were washed with PBS. RPE cells were separated from the choroid/sclera by vortexing for 30 seconds in a lysis buffer (NucleoSpin Tissue, Macherey-Nagel). Genome DNA was analyzed to identify complete isolation of the RPE

cells from remaining choroid/sclera tissue. The genome DNA was analyzed by target deep sequencing.

12. Mouse Vegfa ELISA

42 days after the injection, a total RPE mixture was isolated from neural retina tissue, and two kinds of tissue were frozen for subsequent analysis. The sample tissue was lysed in 120 μl of a cell lysis buffer (CST #9803), and the amount of a Vegfa protein was measured using a mouse VEGF Quantikine ELISA kit (MMV00, R&D Systems).

13. Laser-induced CNV model

After anesthetization of a mouse, eye drops containing 0.5% phenylephrine and 0.5% tropicamide were injected to dilate a pupil. Laser photocoagulation was performed using an indirect head set delivery system (Iridex) and a laser system (Ilooda). Parameters of the laser are a wavelength of 532 nm, a spot size of 200 μm, a power of 800 mW and exposure time of 70 ms. A laser burn was induced in the proximity of the optical nerve three to four times. Burns in which bubbles are generated without bleeding of the vitreous body were only used for the research. After 7 days, an eyeball was fixed with 4% paraformaldehyde at room temperature for one hour. An RPE mixture (RPE/choroid/sclera) was immunostained overnight at 4 ℃ using isolectin-B4 (Thermo Fisher Scientific, cat. no. I21413, 1:100) and an anti-GFP antibody (Abcam, ab6556, 1:100). The RPE mixture was flat-mounted, and visualized using a fluorescent microscope (Eclipse 90i, Nikon) or a confocal microscope (LSM 710, Carl Zeiss) at a 100x magnification. A CNV site was detected using Image J software (1.47v, NIH). An average of three to four CNV sites per eyeball was analyzed. Each group consists of 17 to 18 eyeballs.

14. Quantitative and qualitative analyses for rupturing of retinal vessels

To detect vascular leakage, STZ-induced diabetic mouse models were used. 200 μl of an Evans blue dye (20 mg/ml) dissolved in PBS was intravenously injected into an anesthetized mouse. Two hours after perfusion, an eyeball was extracted to be fixed with 4% paraformaldehyde for 1 hour. The retina was excised in 2X PBS and flat-mounted, and images of the retina were obtained at 40x and 100x magnifications using a fluorescent microscope (Eclipse 90i, Nikon).

To quantitatively analyze the vascular leakage, representative four sites of the vascular leakage in the mid-peripheral retina (0.5 μm × 0.5 μm) of each mouse were selected. The mid-peripheral retina was designated as the middle 1/3 of the retina from the optic nerve head to the ciliary body, images were modulated according to color threshold values based on the automatic isodata algorithm using the Image J software (1.47v, NIH), and regions of interest containing the Evans blue dye were marked in red. Afterward, the regions marked in red were detected. The data were normalized with data of control mice, and represented as vascular leakage (%).

15. Data analysis

To previously determine a sample size *in vitro* or *in vivo*, a statistical method was not used. For statistical analysis, one-way ANOVA and Tukey post-hoc tests were used.

Example 1. Confirmation of CjCas9 expression through AAV in mouse retinal tissue

Since CjCas9 consisting of 984 amino acids has a considerably smaller size (2.95kbp) than SpCas9, both CjCas9 gene and sgRNA are able to be packaged in one AAV vector.

Therefore, in this example, to confirm the possibility of gene manipulation using CjCas9 as a method for treating AMD, CjCas9-expressing AAV was used.

To confirm the expression of CjCas9 through AAV in tissue such as the retina in a mouse, under the control of U6 promoter-induced sgRNA and an EFS promoter specific to a choroidal neovascularization (CNV)-associated Vegfa gene and an Hif1a gene, a CjCas9-coding AAV9 vector was constructed, and here, CjCas9 was linked to eGFP at the C-terminus using a self-cleaved T2A peptide (FIGS. 1A and 1B). The constructed virus was injected into an eyeball through injection into the vitreous body, and after 6 weeks, CjCas9 expression in the eyeball was confirmed, an indel frequency was measured using target deep sequencing, and the amount of Vegfa protein was measured through ELISA (FIG. 1D).

The expression of the CjCas9-linked eGFP was confirmed in retinal pigment epithelial (RPE) cells (FIG. 2).

In addition, indels induced by CjCas9 were observed at Rosa26, Vegfa, and Hif1a target sites of the RPE cells, indel frequencies of 14 ± 5%, 22 ± 3%, and 31 ± 2% were confirmed, respectively (FIG. 1C). In addition, noticeable off-target indels were not induced by Vegfa- or Hif1a-specific sgRNA in the cells (FIG. 1E).

As expected, the expression level of the Vegfa protein was decreased in AAV-treated RPE cells encoding Vegfa-specific CjCas9 (AAV-CjCas9: Vegfa), but was not when Hif1a- or Rosa26-specific CjCas9 (AAV-CjCas9: Hif1a or Rosa26)-coding AAV was treated (FIG. 1D). Particularly, since a Hif1a protein is degraded under a normoxia condition, the expression level of the protein was not able to be measured.

Example 2. Effect of Vegfa- or Hif1a-specific AAV-CjCas9 using CNV-induced mouse

To induce CNV, an eyeball was injected with AAV, and after 6 weeks, subjected to laser treatment, followed by detecting the CNV sites one week after the laser treatment (FIG. 1F). When AAV-CjCas9: Vegfa and AAV-CjCas9: Hif1a were injected onto respective eyeballs, it was confirmed that, compared with an AAV-free negative control, CNV sites were decreased 24 ± 4% and 20 ± 4%, respectively (FIGS. 1F and 1G). In the case of another negative control, that is, Rosa26-specific CjCas9, no therapeutic effect was confirmed, either.

Example 3. Effect of AAV-CjCas9 for AMD treatment

Since cone dysfunction is caused by conditional knockout of a Vegfa gene in the mouse RPE cells, the cause of side effects by the AAV-induced gene knockout in the RPE cells was investigated. To this end, the size of a cone function-related opsin-positive site in the retina was measured. As a result, the Vegfa-specific CjCas9 was decreased in size by approximately 30 ± 10% the AAV-free control (FIG. 3). However, as expected, the Hif1a- or Rosa26-specific CjCas9 did not induce cone dysfunction. Such a result may suggest that Hifla inactivation for treatment of AMD inhibits neovascularization and does not cause cone dysfunction.

HIF1A is a hypoxia-inducible transcription factor, and serves to activate VEGFA transcription. Unlike VEGFA, which is a primary therapeutic target for AMD treatment and a secretory protein, HIF1A may not be considered as a drug target, and generally, a transcription factor such as HIF1A may not be directly targeted by an antibody or aptamer, or small molecules. In this research, as the Hif1a gene was effectively inactivated in the RPE cells using CjCas9 targeting the Hif1a gene on the eyeball of a mouse, it was confirmed that the CNV sites were reduced in the AMD mouse models (FIG. 3).

Example 4. Effect of AAV-CjCas9 for treatment of retinal disease

For extended application to retinal diseases such as diabetic retinopathy (DR), retinopathy of prematurity, etc., the constructed virus was injected into the eyeball through injection into the vitreous body, and 6 weeks later, the *in vivo* genome editing effect caused by the indel frequency in retinal tissue was observed by target deep sequencing, followed by confirming an expression level of the Vegfa protein through ELISA (FIG. 1B). In the retinal tissue, the indels induced by CjCas9 were observed at the Rosa26, Vegfa, and Hif1a target sites in the retinal cells, indel frequencies of 44 ± 8%, 20 ± 2%, and 58 ± 5% were confirmed, respectively (FIGS. 4A, 4B, and 4C). As expected, an expression level of the Vegfa protein was decreased in retinal cells when treated with AAV encoding Vegfa- or Hif1a-specific CjCas9 (AAV-CjCas9: Vegfa or Hif1a), but did not when AAV encoding Rosa26-specific CjCas9 (AAV-CjCas9: Rosa26) was treated (FIG. 4D).

Example 5. Effect of AAV-CjCas9 for treatment of diabetic retinopathy

Diabetic retinopathy is characterized by the symptoms of vascular leakage and blood leakage. Therefore, in this example, the vascular leakage or blood leakage symptom was confirmed using diabetes-induced mice using STZ, and an improvement or treatment effect caused by CjCas9 was confirmed. As a result, it was confirmed that the blood leakage caused by vascular leakage and rupturing was decreased in the Vegfa-specific CjCas9 (AAV-CjCas9: Vegfa)-injected retina. Compared with Rosa26-specific CjCas9 (AAV-CjCas9: Rosa26)-injected mice, vascular leakage and blood leakage in Vegfa-specific CjCas9 (AAV-CjCas9: Vegfa)-injected mice were decreased and thus recovered to a level similar as in normal mice of the same age. Such a result was similarly shown in both of an experiment in which STZ was injected, AAV-CjCas9 was injected after 7 weeks and observation was performed after 6 weeks (FIG. 5), and an experiment in which STZ was injected, AAV-CjCas9 was injected

after 14 weeks and observation was performed after 7 weeks (FIG. 6). According to the above results, it was confirmed that Vegfa-specific CjCas9 (AAV-CjCas9: Vegfa) has an effect of reducing vascular leakage and blood leakage, and thus it can be expected that the Vegfa-specific CjCas9 (AAV-CjCas9: Vegfa) can effectively treat diabetic retinopathy.

Example 6. Screening of target site of human neovascularization-associated factor

To extend the application of the previously-described example, in addition to human VEGFA (FIG. 7) and human HIF1A (FIG. 8A), human ANGPT2 (FIG. 9), human EPAS1 (FIG. 10) and human ANGPTL4 (FIG. 11) were selected as potential targets of genome editing for AMD and DR treatments to screen the target site of each gene capable of being effectively edited in human cells using a CjCas9 system. Particularly, the CjCas9 target site of the mouse Hif1a gene was completely conserved in a human or a different mammal (FIG. 8B). Additionally, the high editing efficiency of the conserved target site was observed in human cells (FIG. 8A, sgRNA #7). Therefore, it is expected that AAV for Hifla suggested in this research or a mutant thereof is able to be used in treatment of a future human patient.

【Industrial Applicability】

An artificially manipulated neovascularization-associated factor and a neovascularization system artificially modified in function thereby can be effectively used in treatment of an angiogenic disease, for example, an angiogenesis-associated ocular disease.

Efficiency of the neovascularization system can be improved by regulating characteristics such as survival, proliferation, persistency, cytotoxicity, and cytokine-release of various neovascularization-associated factors.

【CLAIMS】

【Claim 1】

A guide nucleic acid, which comprises a guide domain capable of targeting a target sequence for artificially manipulating one or more neovascularization-associated factors wherein the neovascularization-associated factor is a VEGFA gene,

wherein the guide domain comprises a homologous RNA sequence to one strand of DNA double strand including the target sequence,

wherein the target sequence is selected from the group consisting of SEQ ID NOs: 4, 7, 9 and 10 in nucleic acid sequences of the VEGFA gene,

wherein the guide nucleic acid further includes at least one domain selected from the group consisting of a first complementary domain, a linker domain, a second complementary domain, a proximal domain and a tail domain, and

wherein the guide nucleic acid is capable of complexing with an editor protein for artificially manipulating the gene, in which the editor protein is a *Campylobacter jejuni*-derived Cas9 protein.

【Claim 2】

A composition for gene manipulation, comprising:

a guide nucleic acid comprising a guide domain capable of targeting a target sequence of a neovascularization-associated factor, or a nucleic acid sequence encoding the same, wherein the neovascularization-associated factor is a VEGFA gene,

wherein the guide domain comprises a homologous RNA sequence to one strand of DNA double strand including the target sequence,

wherein the target sequence is selected from the group consisting of SEQ ID NOs: 4, 7, 9 and 10 in nucleic acid sequences of the VEGFA gene,

wherein the guide nucleic acid further includes at least one domain selected from the group consisting of a first complementary domain, a linker domain, a second complementary domain, a proximal domain and a tail domain; and

an editor protein or a nucleic acid sequence encoding the same,

wherein the editor protein is a *Campylobacter jejuni*-derived Cas9 protein, and

wherein the gene manipulation is artificial manipulation including one or more modification in nucleic acid of the neovascularization-associated factor.

【Claim 3】

The composition for gene manipulation of claim 2, wherein the one or more modifications are at least one of a deletion or insertion of one or more nucleotides, a substitution with one or more nucleotides different from a wild-type gene, and an insertion of one or more foreign nucleotide,

wherein the one or more modifications are positioned in a proto-spacer-adjacent motif (PAM) sequence in a nucleic acid of the neovascularization-associated factor or in a continuous 1 bp to 50 bp region adjacent to the 5’ end and/or 3’ end of the PAM.

【Claim 4】

The composition for gene manipulation of claim 3, wherein the PAM sequence is NNNNRYAC (each N is independently A, T, C or G, R is A or G, and Y is C or T).

【Claim 5】

The composition for gene manipulation of claim 2, wherein the composition for gene manipulation is formed in a viral vector system.

【Claim 6】

The composition for gene manipulation of claim 5, wherein the viral vector includes one or more selected from a retrovirus, a lentivirus, an adenovirus, adeno-associated virus (AAV), vaccinia virus, a poxvirus and a herpes simplex virus.

【Claim 7】

The composition for gene manipulation of claim 2, wherein the composition is formed in a ribonucleoprotein which is a complex of a guide nucleic acid and an editor protein,

wherein the guide nucleic acid is guide RNA.

【Claim 8】

The composition of claims 2 to 7 for use in treating ocular neovascularization disease,

wherein the ocular neovascularization disease is diabetic retinopathy, macular degeneration, ischemic retinopathy or retinopathy of prematurity.