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(54) Title: COMPOSITIONS AND METHODS FOR THE TREATMENT OF ANGIOGENESIS-RELATED EYE DISEASES

(57) Abstract: The invention generally relates to compositions and methods of treatment and/or prevention of angiogenesis-related eye diseases using low doses of rhodostomin variants, and in particular, low doses of a fusion protein comprising a rhodostomin variant, wherein the rhodostomin variant is conjugated with a variant of Human Serum Albumin (HSA) where the cysteine residue at position 34 of the HSA amino acid sequence has been replaced with serine.



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Compositions and Methods for the Treatment of Angiogenesis-Related Eye Diseases

Field of the Invention

[001] The present invention generally relates to compositions and methods utilizing low doses of disintegrin variants for the treatment and/or prevention of angiogenesis-related eye diseases.

Background of the Invention

[002] Integrins are heterodimeric matrix receptors that anchor cells to substrates and transmit externally derived signals across the plasma membrane. Integrin $\alpha v \beta 3$ is a type of integrin that is a receptor for vitronectin. Integrin $\alpha v \beta 3$ is expressed at low levels in most normal cells including intestinal, vascular, and smooth muscle cells. The cell types that express high levels of this heterodimer molecular include bone-resorbing osteoclasts, activated macrophages, a small fraction of neutrophils, angiogenic endothelial cells and migrating smooth muscle cells. Integrin $\alpha v \beta 3$ is involved in the osteoclast-mediated bone resorption, both *in vivo* and *in vitro*, as well as new blood vessel formation. This heterodimer molecule recognizes the amino acid motif Arg-Gly-Asp (RGD) contained in bone matrix proteins such as osteopontin and bone sialoprotein.

[003] Disintegrins are a family of low-molecular-weight RGD-containing peptides that bind specifically to integrins $\alpha IIb \beta 3$, $\alpha 5 \beta 1$ and $\alpha v \beta 3$ expressed on platelets and other cells including vascular endothelial cells and some tumor cells.

[004] Various disintegrins are known in the art, including rhodostomin and its variants, including but not limited to ARLDDL.

[005] Angiogenesis-related eye diseases are eye diseases which are related to the growth of new blood vessels from pre-existing vessels. These diseases include but are not limited to, age-related macular degeneration, diabetic retinopathy, corneal neovascularizing diseases, retinal angiomatous proliferation, polypoidal choroidal vasculopathy, ischaemia-induced neovascularizing retinopathy, high myopia and retinopathy of prematurity.

[006] Because existing drugs are not satisfactorily effective in the treatment of angiogenesis-related eye diseases, there is a need for novel treatments of these diseases.

SUMMARY OF THE INVENTION

[007] Generally, the invention relates to compositions and methods for the treatment and/or prevention of an angiogenesis-related eye disease utilizing low doses of rhodostomin variants.

[008] The term "low doses" refers to the doses which are lower than those conventionally used for the treatment of an angiogenesis-related eye disease.

[009] Preferably, "low doses" are from about 0.0001 pg to about 300 ug per eye; more preferably, from about 0.005 pg to about 200 ug per eye, and even more preferably, from about 0.001 pg to about 100 ug per eye of the rhodostomin variants suitable for the purposes of the present invention. The doses can be administered to a subject in need thereof as once weekly, once monthly, once quarterly or once yearly doses.

[010] Preferably, said rhodostomin variants are fused with human serum albumin (HSA) or variants of HSA. As used throughout the application, the term "rhodostomin variant" encompasses a rhodostomin variant fused with HSA or an HSA variant which may be pegylated or otherwise modified.

[011] Rhodostomin variants suitable for use in the present invention comprise an amino acid sequence selected from SEQ ID NO: 1 through SEQ ID NO: 16 or a pharmaceutically acceptable salt of said rhodostomin variant.

[012] SEQ ID NO: 1 represents an amino acid sequence of "ARGDDP" rhodostomin variant, which is a rhodostomin variant having an RGD motif variant ⁴⁸ARGDDP⁵³.

[013] SEQ ID NO: 2 represents an amino acid sequence of "ARGDDV" rhodostomin variant, which is a rhodostomin variant having an RGD motif variant ⁴⁸ARGDDV⁵³.

[014] SEQ ID NO: 3 represents an amino acid sequence of "ARGDDL" rhodostomin variant, which is a rhodostomin variant having an RGD motif variant ⁴⁸ARGDDL⁵³.

[015] SEQ ID NO: 4 represents an amino acid sequence of "PRGDDL" rhodostomin variant, which is a rhodostomin variant having an RGD motif variant ⁴⁸PRGDDL⁵³.

[016] SEQ ID NO: 5 represents an amino acid sequence of "ARGDDM" rhodostomin variant, which is a rhodostomin variant having an RGD motif variant ⁴⁸ARGDDM⁵³.

[017] SEQ ID NO: 6 represents an amino acid sequence of "PRGDDM" rhodostomin variant, which is a rhodostomin variant having an RGD motif variant ⁴⁸PRGDDM⁵³.

[018] SEQ ID NO: 7 represents an amino acid sequence of "PRLDMP" rhodostomin variant, which is a rhodostomin variant having an RGD motif variant ⁴⁸PRLDMP⁵³.

[019] SEQ ID NO: 8 represents an amino acid sequence of "PRLDDL" rhodostomin variant, which is a rhodostomin variant having an RGD motif variant ⁴⁸PRLDDL⁵³.

[020] SEQ ID NO: 9 represents an amino acid sequence of "ARLDDL" rhodostomin variant, which is a rhodostomin variant having an RGD motif variant ⁴⁸ARLDDL⁵³.

[021] SEQ ID NO: 10 represents an amino acid sequence of "PRIDMP" rhodostomin variant, which is a rhodostomin variant having an RGD motif variant ⁴⁸PRIDMP⁵³.

[022] SEQ ID NO: 11 represents an amino acid sequence of "PRHDMP" rhodostomin variant, which is a rhodostomin variant having an RGD motif variant ⁴⁸PRHDMP⁵³.

[023] SEQ ID NO: 12 represents an amino acid sequence of "PRGDNP" rhodostomin variant, which is a rhodostomin variant having an RGD motif variant ⁴⁸PRGDNP⁵³.

[024] SEQ ID NO: 13 represents an amino acid sequence of "PRGDGP" rhodostomin variant, which is a rhodostomin variant having an RGD motif variant ⁴⁸PRGDGP⁵³.

[025] SEQ ID NO: 14 represents an amino acid sequence of "ARLDDL" rhodostomin variant conjugated with HSA.

[026] SEQ ID NO: 15 represents an amino acid sequence of "ARLDDL" rhodostomin variant conjugated with an HSA variant, wherein the cysteine residue at position 34 of wild type HSA amino acid sequence has been replaced with serine. The rhodostomin variant represented by SEQ ID NO: 15 may also be referred to as HSA(C34S)-ARLDDL fusion protein. The protein is a fusion product of: a) an HSA variant wherein the cysteine residue at position 34 of the HSA amino acid sequence has been replaced with serine, b) a linker amino acid sequence and c) ARLDDL rhodostomin variant.

[027] SEQ ID NO: 16 represents an amino acid sequence of "ARLDDL" rhodostomin variant conjugated with an HSA variant, wherein the cysteine residue at position 34 of wild type HSA amino acid sequence has been replaced with alanine.

[028] Accordingly, in one embodiment, the invention relates to a composition for the treatment and/or prevention of an angiogenesis-related eye disease comprising from about 0.0001 pg to about 300 ug of a rhodostomin variant comprising an amino acid sequence selected from SEQ ID NO: 1 through SEQ ID NO: 16, or a pharmaceutically acceptable salt of said rhodostomin variant.

[029] Most preferably, the rhodostomin variants suitable for the purposes of the present invention comprise SEQ ID NO: 9 or SEQ ID NO: 15.

[030] Preferably, the compositions of the invention comprise from about 0.005 pg to about 200 ug, and more preferably, from about 0.001 pg to about 100 ug of the rhodostomin variant suitable for the purposes of the invention.

[031] In one embodiment, the compositions of the invention are formulated as topical compositions. In another embodiment, the compositions of the invention are suitable for an intraocular injection (i.e., into the eye).

[032] In another embodiment, the invention relates to a method for the treatment and/or prevention of an angiogenesis-related eye disease comprising administering to a subject in need thereof from about 0.0001 pg to about 300 ug per eye of said subject of a rhodostomin variant comprising an amino acid sequence selected from SEQ ID NO: 1 through SEQ ID NO: 16, or a pharmaceutically acceptable salt of said rhodostomin variant.

[033] In a preferred embodiment, the invention relates to a method for the treatment and/or prevention of an angiogenesis-related eye disease comprising administering to a subject in need thereof from about 0.0001 pg to about 300 ug per eye of said subject of a rhodostomin variant comprising an amino acid sequence selected from SEQ ID NO: 9 and SEQ ID NO: 15, or a pharmaceutically acceptable salt of said rhodostomin variant.

[034] In a more preferred embodiment, the invention relates to a method for the treatment and/or prevention of an angiogenesis-related eye disease comprising administering to a subject in need thereof from about 0.0005 pg to about 200 ug per eye of said subject of a rhodostomin variant comprising an amino acid sequence selected from SEQ ID NO: 1 through SEQ ID NO: 16, or a pharmaceutically acceptable salt of said rhodostomin variant.

[035] In an even more preferred embodiment, the invention relates to a method for the treatment and/or prevention of an angiogenesis-related eye disease comprising administering to a subject in need thereof from about 0.001 pg to about 100 ug per eye of said subject of a rhodostomin variant comprising an amino acid sequence selected from SEQ ID NO: 1 through SEQ ID NO: 16, or a pharmaceutically acceptable salt of said rhodostomin variant.

[036] Preferably, the methods of the invention comprise administering from about 0.005 pg to about 300 ug, and more preferably, from about 0.001 pg to about 100 ug of the rhodostomin variant suitable for the purposes of the invention per eye of said subject.

[037] In one embodiment, the methods of the invention comprise administering the rhodostomin variant suitable for the purposes of the invention through an intravitreal injection to said subject.

[038] In one embodiment, the angiogenesis-related eye disease is selected from the group consisting of age-related macular degeneration (AMD), diabetic retinopathy, corneal neovascularizing diseases, retinal angiomatous proliferation, polypoidal choroidal vasculopathy, age-related ischaemia-induced neovascularizing retinopathy, high myopia and retinopathy of prematurity.

[039] In another embodiment, the methods of the invention further comprise administering a therapeutically effective amount of another active agent. The other active agent may be administered before, during, or after administering the rhodostomin variant suitable for the purposes of the invention.

[040] Preferably, the other active agent is selected from the group consisting of VEGF antagonists, anti-angiogenesis agents, anti-inflammation agents, and steroids.

[041] The compositions of the present invention may further include a pharmaceutically acceptable carrier.

[042] These and other aspects will become apparent from the following description of the various embodiments taken in conjunction with the following drawings, although variations and modifications therein may be affected without departing from the spirit and scope of the novel concepts of the disclosure.

[043] It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention, as claimed.

BRIEF DESCRIPTION OF THE DRAWINGS

[044] FIG. 1A is a set of four photographs showing angiogenesis in a mouse model of oxygen-induced retinopathy (B), reduced angiogenesis in a oxygen-induced retinopathy mouse treated with ARLDDL rhodostomin variant (C) and reduced angiogenesis in a oxygen-induced retinopathy mouse treated with HSA-ARLDDL rhodostomin variant (D). Photograph (A) is normoxia (control group). Arrows indicate blood vessel profiles (BVPs).

[045] FIG. 1B is a graph showing reduced number of vessels per retinal section in a mouse model of oxygen-induced retinopathy treated with ARLDDL rhodostomin variant or HSA-ARLDDL rhodostomin variant.

[046] FIG. 1C is a graph showing reduced number of endothelial cells per retinal section in a mouse model of oxygen-induced retinopathy treated with ARLDDL rhodostomin variant or HSA-ARLDDL rhodostomin variant.

[047] FIG. 2A is a graph showing reduced number of vessels per retina section in a mouse model of oxygen-induced retinopathy treated with HSA-ARLDDL rhodostomin variant at the following amounts: 10 pg, 0.1 pg and 0.001 pg.

[048] FIG. 2B is a graph showing reduced number of endothelial cells per retina section in a mouse model of oxygen-induced retinopathy treated with ARLDDL rhodostomin variant or HSA-ARLDDL rhodostomin variant at the following amounts: 10 pg, 0.1 pg and 0.001 pg.

[049] FIG. 3A is a graph showing reduced number of vessels/retina section in a mouse model of oxygen-induced retinopathy treated with HSA-ARLDDL rhodostomin variant as compared to a oxygen-induced retinopathy mouse treated with Avastin®.

[050] FIG. 3B is a graph showing reduced number of endothelial cells/retina section in a mouse model of oxygen-induced retinopathy treated with HSA-ARLDDL rhodostomin variant as compared to a oxygen-induced retinopathy mouse treated with Avastin®.

[051] FIG. 4A is a graph showing reduced number of vessels/retina section in a mouse model of oxygen-induced retinopathy treated with HSA-ARLDDL rhodostomin variant as compared to a oxygen-induced retinopathy mouse treated with Avastin®.

[052] FIG. 4B is a graph showing reduced number of endothelial cells/retina section in a mouse model of oxygen-induced retinopathy treated with HSA-ARLDDL rhodostomin variant as compared to a oxygen-induced retinopathy mouse treated with Avastin®.

DETAILED DESCRIPTION OF THE INVENTION

[053] Various embodiments of the invention are now described in detail. As used in the description and throughout the claims, the meaning of “a”, “an”, and “the” includes plural reference unless the context clearly dictates otherwise. Also, as used in the description and throughout the claims, the meaning of “in” includes “in” and “on” unless the context clearly dictates otherwise. Additionally, some terms used in this specification are more specifically defined below.

DEFINITIONS

[054] The terms used in this specification generally have their ordinary meanings in the art, within the context of the invention, and in the specific context where each term is used. Certain terms that are used to describe the invention are discussed below, or elsewhere in the specification, to provide additional guidance to the practitioner regarding the description of the invention. Synonyms for certain terms are provided. A recital of one or more synonyms does not exclude the use of other synonyms. The use of examples anywhere in this specification including examples of any terms discussed herein is illustrative only, and in no way limits the scope and meaning of the invention or of any exemplified term. The invention is not limited to the various embodiments given in this specification.

[055] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to

which this invention pertains. In the case of conflict, the present document, including definitions will control.

[056] The terms “around,” “about” or “approximately” shall generally mean within 20 percent, within 10 percent, within 5, 4, 3, 2 or 1 percent of a given value or range. Numerical quantities given are approximate, meaning that the term “around,” “about” or “approximately” can be inferred if not expressly stated.

[057] The terms “peptide” and “protein” are used interchangeably throughout the application.

[058] The terms “low doses” and “low amounts” are used interchangeably throughout the application.

[059] The terms “subject” and “mammal” include, but are not limited to, a human.

[060] The term “treatment” refers to any administration or application of remedies for disease in a mammal and includes inhibiting the disease, arresting its development, relieving the disease, for example, by causing regression, or restoring or repairing a lost, missing, or defective function; or stimulating an inefficient process. The term includes obtaining a desired pharmacologic and/or physiologic effect, covering any treatment of a pathological condition or disorder in a mammal. The effect may be prophylactic in terms of completely or partially preventing a disorder or symptom thereof and/or may be therapeutic in terms of a partial or complete cure for a disorder and/or adverse affect attributable to the disorder. It includes (1) preventing the disorder from occurring or recurring in a subject who may be predisposed to the disorder but is not yet symptomatic, (2) inhibiting the disorder, such as arresting its development, (3) stopping or terminating the disorder or at least its associated symptoms, so that the host no longer suffers from the disorder or its symptoms, such as causing regression of the disorder or its symptoms, for example, by restoring or repairing a lost, missing or defective function, or stimulating an inefficient process, or (4) relieving, alleviating or ameliorating the disorder, or symptoms associated therewith, where ameliorating is used in a broad sense to refer to at least a reduction in the magnitude of a parameter, such as inflammation, pain and/or tumor size.

[061] The term “pharmaceutically acceptable carrier” refers to a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material, formulation auxiliary, or excipient of any conventional type. A pharmaceutically acceptable carrier is non-toxic to recipients at the dosages and concentrations employed in the formulation and is compatible with other ingredients of the formulation.

[062] The term “composition” refers to a mixture that usually contains a carrier, such as a pharmaceutically acceptable carrier or excipient that is conventional in the art and which is suitable for administration into a subject for therapeutic, diagnostic, or prophylactic purposes. It may include a cell culture in which the rhodostomin variant is present in the cells or in the culture medium. For example, compositions for oral administration can form solutions, suspensions, tablets, pills, capsules, sustained release formulations, oral rinses or powders.

[063] The term “angiogenesis-related eye disease” refers to any ocular disease that is related to the growth of new blood vessels from pre-existing vessels. These diseases include but are not limited to, age-related macular degeneration, diabetic retinopathy, corneal neovascularizing diseases, retinal angiomatous proliferation, polypoidal choroidal vasculopathy, ischaemia-induced neovascularizing retinopathy, high myopia and retinopathy of prematurity.

[064] The term “therapeutically effective amount” refers to an amount which, when administered to a living subject, achieves a desired effect on the living subject. The exact amount will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques. As is known in the art, adjustments for systemic versus localized delivery, age, body weight, general health, sex, diet, time of administration, drug interaction and the severity of the condition may be necessary, and will be ascertainable with routine experimentation by those skilled in the art.

[065] The term “RGD motif variant” refers to a peptide comprising a modification in the amino acid sequence that spans the RGD sequence of a corresponding wild type sequence, such as the sequence comprising RGD in Rhodostomin.

[066] The term “ARGDDP” refers to a rhodostomin variant having an RGD motif variant ⁴⁸ARGDDP⁵³. The numbers “48” and “53” refer to positions of these amino

acids in the amino acid sequence of wild type rhodostomin. ARGDDP is represented by SEQ ID NO: 1.

[067] The term "ARGDDV" refers to a rhodostomin variant having an RGD motif variant ⁴⁸ARGDDV⁵³. The numbers "48" and "53" refer to positions of these amino acids in the amino acid sequence of wild type rhodostomin. ARGDDV is represented by SEQ ID NO: 2.

[068] The term "ARGDDL" refers to a rhodostomin variant having an RGD motif variant ⁴⁸ARGDDL⁵³. The numbers "48" and "53" refer to positions of these amino acids in the amino acid sequence of wild type rhodostomin. ARGDDL is represented by SEQ ID NO: 3.

[069] The term "PRGDDL" refers to a rhodostomin variant having an RGD motif variant ⁴⁸PRGDDL⁵³. The numbers "48" and "53" refer to positions of these amino acids in the amino acid sequence of wild type rhodostomin. PRGDDL is represented by SEQ ID NO: 4.

[070] The term "ARGDDM" refers to a rhodostomin variant having an RGD motif variant ⁴⁸ARGDDM⁵³. The numbers "48" and "53" refer to positions of these amino acids in the amino acid sequence of wild type rhodostomin. ARGDDM is represented by SEQ ID NO: 5.

[071] The term "PRGDDM" refers to a rhodostomin variant having an RGD motif variant ⁴⁸PRGDDM⁵³. The numbers "48" and "53" refer to positions of these amino acids in the amino acid sequence of wild type rhodostomin. PRGDDM is represented by SEQ ID NO: 6.

[072] The term "PRLDMP" refers to a rhodostomin variant having an RGD motif variant ⁴⁸PRLDMP⁵³. The numbers "48" and "53" refer to positions of these amino acids in the amino acid sequence of wild type rhodostomin. PRLDMP is represented by SEQ ID NO: 7.

[073] The term "PRLDDL" refers to a rhodostomin variant having an RGD motif variant ⁴⁸PRLDDL⁵³. The numbers "48" and "53" refer to positions of these amino

acids in the amino acid sequence of wild type rhodostomin. PRLDDL is represented by SEQ ID NO: 8.

[074] The term "ARLDDL" refers to a rhodostomin variant having an RGD motif variant ⁴⁸ARLDDL⁵³. The numbers "48" and "53" refer to positions of these amino acids in the amino acid sequence of wild type rhodostomin. ARLDDL is represented by SEQ ID NO: 9.

[075] The term "PRIDMP" refers to a rhodostomin variant having an RGD motif variant ⁴⁸PRIDMP⁵³. The numbers "48" and "53" refer to positions of these amino acids in the amino acid sequence of wild type rhodostomin. PRIDMP is represented by SEQ ID NO: 10.

[076] The term "PRHDMP" refers to a rhodostomin variant having an RGD motif variant ⁴⁸PRHDMP⁵³. The numbers "48" and "53" refer to positions of these amino acids in the amino acid sequence of wild type rhodostomin. PRHDMP is represented by SEQ ID NO: 11.

[077] The term "PRGDNP" refers to a rhodostomin variant having an RGD motif variant ⁴⁸PRGDNP⁵³. The numbers "48" and "53" refer to positions of these amino acids in the amino acid sequence of wild type rhodostomin. PRGDNP is represented by SEQ ID NO: 12.

[078] The term "PRGDGP" refers to a rhodostomin variant having an RGD motif variant ⁴⁸PRGDGP⁵³. The numbers "48" and "53" refer to positions of these amino acids in the amino acid sequence of wild type rhodostomin. PRGDGP is represented by SEQ ID NO: 13.

[079] The term "HSA-ARLDDL" refers to a fusion protein which comprises a) a human serum albumin (HSA) variant, b) a linker amino acid sequence and c) a rhodostomin variant having an RGD motif variant ⁴⁸ARLDDL⁵³. It is represented by SEQ ID NO: 14.

[080] The term "HSA(C34S)-ARLDDL" refers to a fusion protein which comprises a) a human serum albumin (HSA) variant wherein the cysteine residue at position 34 of wild type HSA amino acid sequence has been replaced with serine, b) a linker

amino acid sequence and c) a rhodostomin variant having an RGD motif variant ⁴⁸ARLDDL⁵³. It is represented by SEQ ID NO: 15.

[081] The term "HSA(C34A)-ARLDDL" refers to a fusion protein which comprises a) a human serum albumin (HSA) variant wherein the cysteine residue at position 34 of wild type HSA amino acid sequence has been replaced with alanine, b) a linker amino acid sequence, and a rhodostomin variant having an RGD motif variant ⁴⁸ARLDDL⁵³. It is represented by SEQ ID NO: 16.

[082] It is to be understood that the invention comprises compositions and methods utilizing fusion proteins wherein rhodostomin variants other than ARLDDL are fused to an HSA variant through a linker amino acid sequence. For example, fusion proteins suitable for the purposes of the invention include, but are not limited to, HSA-ARGDDP, HSA(C34S)-ARGDDP, HSA(C34A)-ARGDDP, HSA-ARGDDV, HSA(C34S)-ARGDDV, HSA(C34A)-ARGDDV, and etc.

COMPOSITIONS AND METHODS OF THE INVENTION

[083] The inventors expressly incorporate by reference all of the methods and rhodostomin variants disclosed in the patent application U.S. Serial No. 61/226,945.

[084] Generally, the invention relates to compositions and methods for the treatment of an angiogenesis-related eye disease utilizing low doses of rhodostomin variants.

[085] The term "low doses" refers to the doses which are lower than those conventionally used for the treatment of an angiogenesis-related eye disease.

[086] Preferably, "low doses" are from about 0.0001 pg to about 300 ug; more preferably, from about 0.0005 pg to about 200 ug, and even more preferably, from about 0.001 pg to about 100 ug of the rhodostomin variants suitable for the purposes of the present invention. The doses can be administered to a subject in need thereof as either single doses or divided doses as long as the total administered dose is within the provided range.

[087] Preferably, said rhodostomin variants are fused with human serum albumin (HSA) or variants of HSA which may be pegylated or otherwise modified.

[088] Even more preferably, a rhodostomin variant suitable for the purposes of the present invention is selected from a rhodostomin variant comprising an amino acid sequence selected from SEQ ID NO: 1 through SEQ ID NO: 16, or a pharmaceutically acceptable salt of said rhodostomin variant.

[089] In a preferred embodiment, the rhodostomin variants are conjugated with albumin or pegylated.

[090] Even more preferably, the rhodostomin variants suitable for the purposes of the present invention comprise SEQ ID NO: 9 or SEQ ID NO: 15.

[091] The most preferred rhodostomin variant for the purposes of the present invention is HSA(C34S)-ARLDDL, which is represented by SEQ ID NO: 15. HSA-ARLDDL C34S is a recombinant protein comprising a rhodostomin variant with an RGD motif ⁴⁸ARLDDL⁵³, wherein the rhodostomin variant is conjugated with a variant of Human Serum Albumin (HSA). HSA(C34S)-ARLDDL is selective for $\alpha v \beta 3$ integrin and exhibits reduced binding to $\alpha I I b \beta 3$ and/or $\alpha 5 \beta 1$ integrin as compared to a wild-type rhodostomin.

[092] In one embodiment, the invention relates to a composition for the treatment and/or prevention of an angiogenesis-related eye disease comprising from about 0.0001 pg to about 300 ug of a rhodostomin variant comprising an amino acid sequence selected from SEQ ID NO: 1 through SEQ ID NO: 16, or a pharmaceutically acceptable salt of said rhodostomin variant.

[093] Preferably, the compositions of the invention comprise from about 0.0005 pg to about 200 ug, and more preferably, from about 0.001 pg to about 100 ug of the rhodostomin variant suitable for the purposes of the invention.

[094] In one embodiment, the compositions of the invention are formulated as topical compositions. In another embodiment, the compositions of the invention are suitable for an intravitreal injection (i.e., into the eye).

[095] In another embodiment, the invention relates to a method for the treatment and/or prevention of an angiogenesis-related eye disease comprising administering to a subject in need thereof from about 0.0001 pg to about 300 ug per eye of said subject of a rhodostomin variant comprising an amino acid sequence selected from SEQ ID NO: 1 through SEQ ID NO: 16, or a pharmaceutically acceptable salt of said rhodostomin variant.

[096] In a preferred embodiment, the invention relates to a method for the treatment and/or prevention of an angiogenesis-related eye disease comprising administering to a subject in need thereof from about 0.0001 pg to about 300 ug per eye of said subject of a rhodostomin variant comprising an amino acid sequence selected from SEQ ID NO: 9 and SEQ ID NO: 15, or a pharmaceutically acceptable salt of said rhodostomin variant.

[097] In a more preferred embodiment, the invention relates to a method for the treatment and/or prevention of an angiogenesis-related eye disease comprising administering to a subject in need thereof from about 0.0005 pg to about 200 ug per eye of said subject of a rhodostomin variant comprising an amino acid sequence selected from SEQ ID NO: 1 through SEQ ID NO: 16, or a pharmaceutically acceptable salt of said rhodostomin variant.

[098] In an even more preferred embodiment, the invention relates to a method for the treatment and/or prevention of an angiogenesis-related eye disease comprising administering to a subject in need thereof from about 0.001 pg to about 100 ug per eye of said subject of a rhodostomin variant comprising an amino acid sequence selected from SEQ ID NO: 1 through SEQ ID NO: 16, or a pharmaceutically acceptable salt of said rhodostomin variant.

[099] Preferably, the methods of the invention comprise administering from about 0.005 pg to about 200 ug, and more preferably, from about 0.001 pg to about 100 ug of a rhodostomin variant suitable for the purposes of the invention per eye of said subject.

[0100] In one embodiment, the methods of the invention comprise administering a rhodostomin variant suitable for the purposes of the invention through an intraocular injection to said subject.

[0101] In one embodiment, the angiogenesis-related eye disease is selected from the group consisting of age-related macular degeneration, diabetic retinopathy, corneal neovascularizing diseases, retinal angiomatous proliferation, polypoidal choroidal vasculopathy, age-related ischaemia-induced neovascularizing retinopathy, high myopia and retinopathy of prematurity.

[0102] The compositions of the invention may be administered to a subject in need of treatment by injection systemically, such as by intravitreous injection or intravenous injection; or by injection or application to the relevant site, such as by direct injection, or direct application to the site when the site is exposed in surgery; or by topical application.

[0103] The compositions of the invention can be used in combination with another active agent to treat angiogenesis-related eye diseases.

[0104] Thus, in one embodiment, the method of treatment and/or prevention of an angiogenesis-related eye disease further comprises administering to a subject another active agent.

[0105] Preferably, the other active agent is selected from the group consisting of VEGF antagonists, anti-angiogenesis agents, anti-inflammation agents, and steroids.

[0106] Administration of the active agents can be achieved in various ways, including oral, buccal, nasal, rectal, parenteral, intraperitoneal, intradermal, transdermal, subcutaneous, intravenous, intra-arterial, intracardiac, intraventricular, intracranial, intratracheal, and intrathecal administration, intramuscular injection, intravitreous injection (i.e., into the eye), topical application, including but not limited to eye drops, creams, and emulsions, implantation and inhalation.

[0107] Pharmaceutical compositions of the invention may be provided as formulations with pharmaceutically acceptable carriers, excipients and diluents, which are known in the art. These pharmaceutical carriers, excipients and diluents include those listed in the USP pharmaceutical excipients listing. USP and NF Excipients, Listed by Categories, p. 2404-2406, USP 24 NF 19, United States Pharmacopeial Convention Inc., Rockville, Md. (ISBN 1-889788-03-1).

Pharmaceutically acceptable excipients, such as vehicles, adjuvants, carriers or diluents, are readily available to the public. Moreover, pharmaceutically acceptable auxiliary substances, such as pH adjusting and buffering agents, tonicity adjusting agents, stabilizers, wetting agents and the like, are readily available to the public.

[0108] Suitable carriers include, but are not limited to, water, dextrose, glycerol, saline, ethanol, and combinations thereof. The carrier can contain additional agents such as wetting or emulsifying agents, pH buffering agents, or adjuvants which enhance the effectiveness of the formulation. Topical carriers include liquid petroleum, isopropyl palmitate, polyethylene glycol, ethanol (95%), polyoxyethylene monolaurate (5%) in water, or sodium lauryl sulfate (5%) in water. Other materials such as antioxidants, humectants, viscosity stabilizers, and similar agents can be added as necessary. Percutaneous penetration enhancers such as Azone can also be included.

[0109] The rhodostomin variants suitable for the purposes of the invention can be formulated into preparations for injection by dissolving, suspending or emulsifying them in an aqueous or nonaqueous solvent, such as vegetable or other similar oils, synthetic aliphatic acid glycerides, esters of higher aliphatic acids or propylene glycol; and if desired, with conventional additives such as solubilizers, isotonic agents, suspending agents, emulsifying agents, stabilizers and preservatives. Other formulations for oral or parenteral delivery can also be used, as conventional in the art.

[0110] The pharmaceutical compositions of the invention can be formulated into preparations in solid, semi-solid, liquid or gaseous forms, such as tablets, capsules, powders, granules, ointments, solutions, suppositories, injections, inhalants and aerosols.

[0111] In pharmaceutical dosage forms, the compositions of the invention can be administered in the form of their pharmaceutically acceptable salts, or they can also be used alone or in appropriate association, as well as in combination, with other pharmaceutically active compounds. The subject compositions are formulated in accordance to the mode of potential administration.

[0112] The present invention is more particularly described in the following examples that are intended as illustrative only, since many modifications and variations therein will be apparent to those skilled in the art.

EXAMPLE 1

Inhibition of Angiogenesis in Retina by ARLDDL and HSA-ARLDDL in a mouse model of oxygen-induced retinopathy

[087] An animal model for oxygen-induced retinopathy in mice was generated as follows. 7-day-old mice with at least two nursing dams per group were assigned to an oxygen chamber containing 75% oxygen (Hyperoxia) or to room air (Normoxia). The mice were exposed to less than 300 lux of 12-hour cyclical broad spectrum light. Surrogate dams were substituted if nursing dams died. The oxygen-treated mice were housed in an incubator connected to a Bird 3-M oxygen blender (Palm Springs, CA) with oxygen and nitrogen, allowing adjustment of oxygen concentration to 75% \pm 2%. A flow rate of 1.5L/min was checked twice daily. Oxygen concentration was monitored with a Beckman oxygen analyzer (Model D2, Irvine, CA). The cage temperature was maintained at 23°C \pm 2°C. The mice were placed in the oxygen chamber with enough food and water to sustain them for 5 days. The chamber was not opened during hyperoxia exposure from Day1 to Day 5. On Day5, the animals were returned to room air for 7 days. Once the oxygen-treated mice returned to room air, normal saline (2 μ l/eye), ARLDDL (2ng/eye or 20pg/eye), HSA-ARLDDL (2ng/eye), or anti-VEGF antibody (2ng/eye or 20pg/eye) was administered via intravitreal injection on Day 5 and the mice were sacrificed on Day 12. Sections from one of the eyes of each animal were made, deparaffinized, and stained with hematoxylin and eosin. Vessel number per retinal section was counted in the inner retina, and included vessels adherent to the inner limiting membrane. Counting was performed on a photomicroscope (Leica) at a magnification of 100x. The results are shown in FIG. 1B.

[088] The results demonstrate that ARLDDL and HSA-ARLDDL at doses of 2ng/eye and 20pg/eye reduced the vessel number per retinal section as compared to normal saline treated group. Further, even at a very low dose of 20pg/eye, ARLDDL resulted in a greater reduction of the vessel number per retinal section as

compared to 2ng/eye of ARLDDL, 2ng/eye of HSA-ARLDDL and 20pg/eye of an anti-VEGF antibody.

[089] Endothelial cells were counted in the anterior part of the ganglion cell layer and on inner limiting membrane of the retina by a person blinded to the same identity. The results are shown in FIG. 1C.

[090] The results demonstrate that ARLDDL and HSA-ARLDDL at both doses of 2ng/eye and 20pg/eye reduced the endothelial cell number per retinal section.

EXAMPLE 2

Inhibition of Angiogenesis in Retina by Very Low Doses of ARLDDL and HSA-

ARLDDL in a mouse model of oxygen-induced retinopathy

[091] An animal model for oxygen-induced retinopathy in mice was generated as described in EXAMPLE 1. The following amounts of HSA-ARLDDL: 10pg/eye, 0.1pg/eye and 0.001pg/eye were administered via intravitreal injection and the treated mice were sacrificed on Day 12. Sections from one of the eyes of each animal were made, deparaffinized, and stained with hematoxylin and eosin. Vessel number per retinal section was counted in the inner retina, and included vessels adherent to the inner limiting membrane. Counting was performed on a photomicroscope (Leica) at a magnification of 100x. The results are shown in FIG. 2A.

[092] The results demonstrate that HSA-ARLDDL at 10 pg, 0.1 pg and 0.001 pg per eye reduced the vessel number per retinal section as compared to the normal saline treated group.

[093] Endothelial cells were counted in the anterior part of the ganglion cell layer and on inner limiting membrane of the retina by a person blinded to the same identity. The results are shown in FIG. 2B.

[094] The results demonstrate that HSA-ARLDDL at 10 pg, 0.1 pg and 0.001 pg per eye reduced the endothelial cell number per retinal section as compared to the normal saline treated group.

EXAMPLE 3

Inhibition of Angiogenesis in Retina by HSA-ARLDDL versus Avastin® in a mouse model of oxygen-induced retinopathy

[095] An animal model for hyperoxia/normoxia in mice was generated as described in EXAMPLE 1. ARLDDL (10pg/eye or 0.1pg/eye) or Avastin® (20pg/eye or 0.2pg/eye) was administered via intravitreal injection and the treated mice were sacrificed on Day 12.

[096] Sections from one of the eyes of each animal were made, deparaffinized, and stained with hematoxylin and eosin. Vessel number per retinal section was counted in the inner retina, and included vessels adherent to the inner limiting membrane. Counting was performed on a photomicroscope (Leica) at a magnification of 100x. The results are shown in FIGs. 3A and 4A.

[097] Endothelial cells were counted in the anterior part of the ganglion cell layer and on the inner limiting membrane of the retina by a person blinded to the same identity. The results are shown in FIG. 3B and 4B.

[098] The results demonstrate that HSA-ARLDDL at all administered dosages was significantly more effective than Avastin® in inhibiting of angiogenesis in retina.

Amino Acid and Nucleotide Sequences Used in the Application

[099] SEQ ID NO: 1 represents an amino acid sequence of "ARGDDP" rhodostomin variant.

[0100] SEQ ID NO: 2 represents an amino acid sequence of "ARGDDV" rhodostomin variant.

[0101] SEQ ID NO: 3 represents an amino acid sequence of "ARGDDL" rhodostomin variant.

[0102] SEQ ID NO: 4 represents an amino acid sequence of "PRGDDL" rhodostomin variant.

[0103] SEQ ID NO: 5 represents an amino acid sequence of "ARGDDM" rhodostomin variant.

[0104] SEQ ID NO: 6 represents an amino acid sequence of "PRGDDM" rhodostomin variant.

[0105] SEQ ID NO: 7 represents an amino acid sequence of "PRLDMP" rhodostomin variant.

[0106] SEQ ID NO: 8 represents an amino acid sequence of "PRLDDL" rhodostomin variant.

[0107] SEQ ID NO: 9 represents an amino acid sequence of "ARLDDL" rhodostomin variant.

[0108] SEQ ID NO: 10 represents an amino acid sequence of "PRIDMP" rhodostomin variant.

[0109] SEQ ID NO: 11 represents an amino acid sequence of "PRHDMP" rhodostomin variant.

[0110] SEQ ID NO: 12 represents an amino acid sequence of "PRGDNP" rhodostomin variant.

[0111] SEQ ID NO: 13 represents an amino acid sequence of "PRGDGP" rhodostomin variant.

[0112] SEQ ID NO: 14 represents an amino acid sequence of "HSA-ARLDDL" rhodostomin variant..

[0113] SEQ ID NO: 15 represents an amino acid sequence of "HSA(C34S)-ARLDDL" rhodostomin variant.

[0114] SEQ ID NO: 16 represents an amino acid sequence of "HSA(C34A)-ARLDDL" rhodostomin variant.

[0115] The foregoing description of the exemplary embodiments of the invention has been presented only for the purposes of illustration and description and is not intended to be exhaustive or to limit the invention to the precise forms disclosed. Many modifications and variations are possible in light of the above teaching.

[0116] Other embodiments of the invention will be apparent to those skilled in the art from consideration of the specification and practice of the invention disclosed herein. It is intended that the specification and examples be considered as exemplary only, with a true scope and spirit of the invention being indicated by the following claims.

WHAT IS CLAIMED IS:

1. A composition for the treatment and/or prevention of an angiogenesis-related eye disease comprising a low dose of a rhodostomin variant comprising an amino acid sequence selected from SEQ ID NO: 1 through SEQ ID NO: 16, or a pharmaceutically acceptable salt of said rhodostomin variant.
2. The composition of claim 1, wherein said amino acid sequence is selected from SEQ ID NO: 9 and SEQ ID NO: 15.
3. The composition of claim 1, wherein said amino acid sequence is SEQ ID NO: 15.
4. The composition of claim 1, wherein said rhodostomin variant is conjugated with albumin or pegylated.
5. The composition of claim 1 wherein the low dose is from about 0.0001 pg to about 300 ug of said rhodostomin variant.
6. The composition of claim 1 wherein the low dose is from about 0.0005 pg to about 200 ug of said rhodostomin variant.
7. The composition of claim 1 wherein the low dose is from about 0.001 pg to about 100 ug of said rhodostomin variant.
8. The composition of claim 1, wherein said composition is formulated as a topical composition.
9. The composition of claim 1, wherein said composition is formulated as an injectable composition.
10. A composition for the treatment and/or prevention of an angiogenesis-related eye disease comprising a low dose of a rhodostomin variant comprising an amino acid sequence of SEQ ID NO: 15, or a pharmaceutically acceptable salt of said rhodostomin variant.

11. A method for the treatment and/or prevention of an angiogenesis-related eye disease comprising administering to a subject in need thereof the composition of claim 1.
12. A method for the treatment and/or prevention of an angiogenesis-related eye disease comprising administering to a subject in need thereof a low dose per eye of said subject of a rhodostomin variant comprising an amino acid sequence selected from SEQ ID NO: 1 through SEQ ID NO: 16, or a pharmaceutically acceptable salt of said rhodostomin variant.
13. The method of claim 12, wherein said angiogenesis-related eye disease is selected from the group consisting of age-related macular degeneration, diabetic retinopathy, corneal neovascularizing diseases, retinal angiomatous proliferation, polypoidal choroidal vasculopathy, age-related ischaemia-induced neovascularizing retinopathy, high myopia and retinopathy of prematurity.
14. The method of claim 13, wherein said angiogenesis-related eye disease is age-related macular degeneration.
15. The method of claim 13, wherein said angiogenesis-related eye disease is diabetic retinopathy.
16. The method of claim 12, wherein said rhodostomin variant is conjugated with albumin or pegylated.
17. The method of claim 12, wherein said subject is human and the rhodostomin variant is administered to an eye of said subject.
18. The method of claim 12, wherein said rhodostomin variant is applied topically to an eye of said subject.
19. The method of claim 12, wherein said rhodostomin variant is applied via an intravitreal injection to an eye of said subject.

20. The method of claim 11, further comprising administering to said subject a therapeutically effective amount of another active agent.

21. The method of claim 20, wherein said another active agent is selected from the group consisting of VEGF antagonists, anti-angiogenesis agents, anti-inflammation agents, and steroids.

22. The method of claim 20, wherein said other active agent is administered concurrently with said rhodostomin variant.

23. The method of claim 20, wherein said other active agent is administered prior to administering said rhodostomin variant.

24. The method of claim 20, wherein said other active agent is administered after administering said rhodostomin variant.

25. A method for the treatment and/or prevention of an angiogenesis-related eye disease comprising administering to a subject in need thereof a low dose per eye of said subject of a rhodostomin variant comprising an amino acid sequence of SEQ ID NO: 15, or a pharmaceutically acceptable salt of said rhodostomin variant.

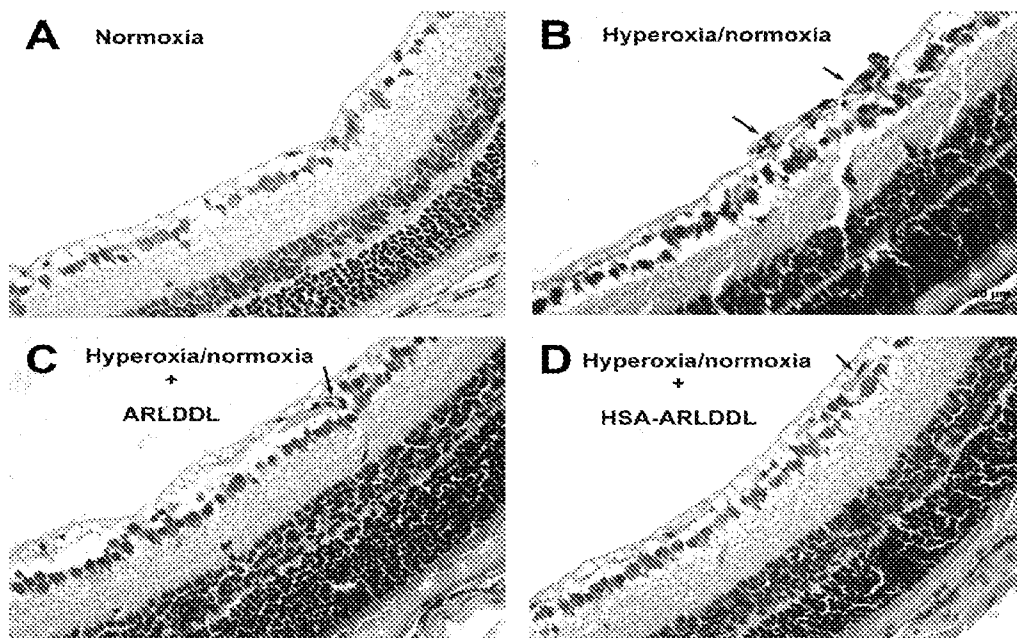
FIG. 1A**Inhibition of Angiogenesis in Retina by ARLDDL and HSA-ARLDDL**

FIG. 1B

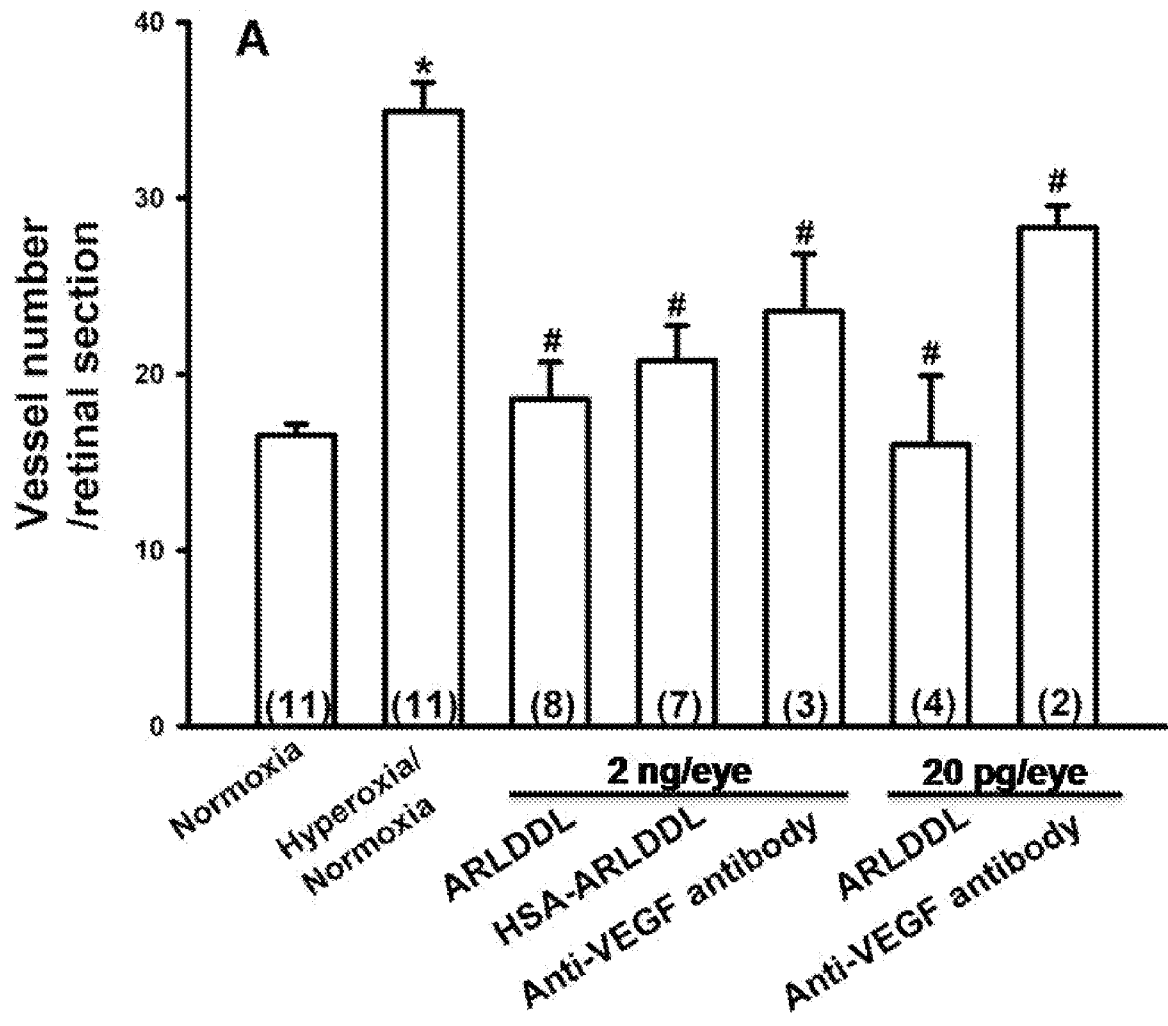


FIG. 1C

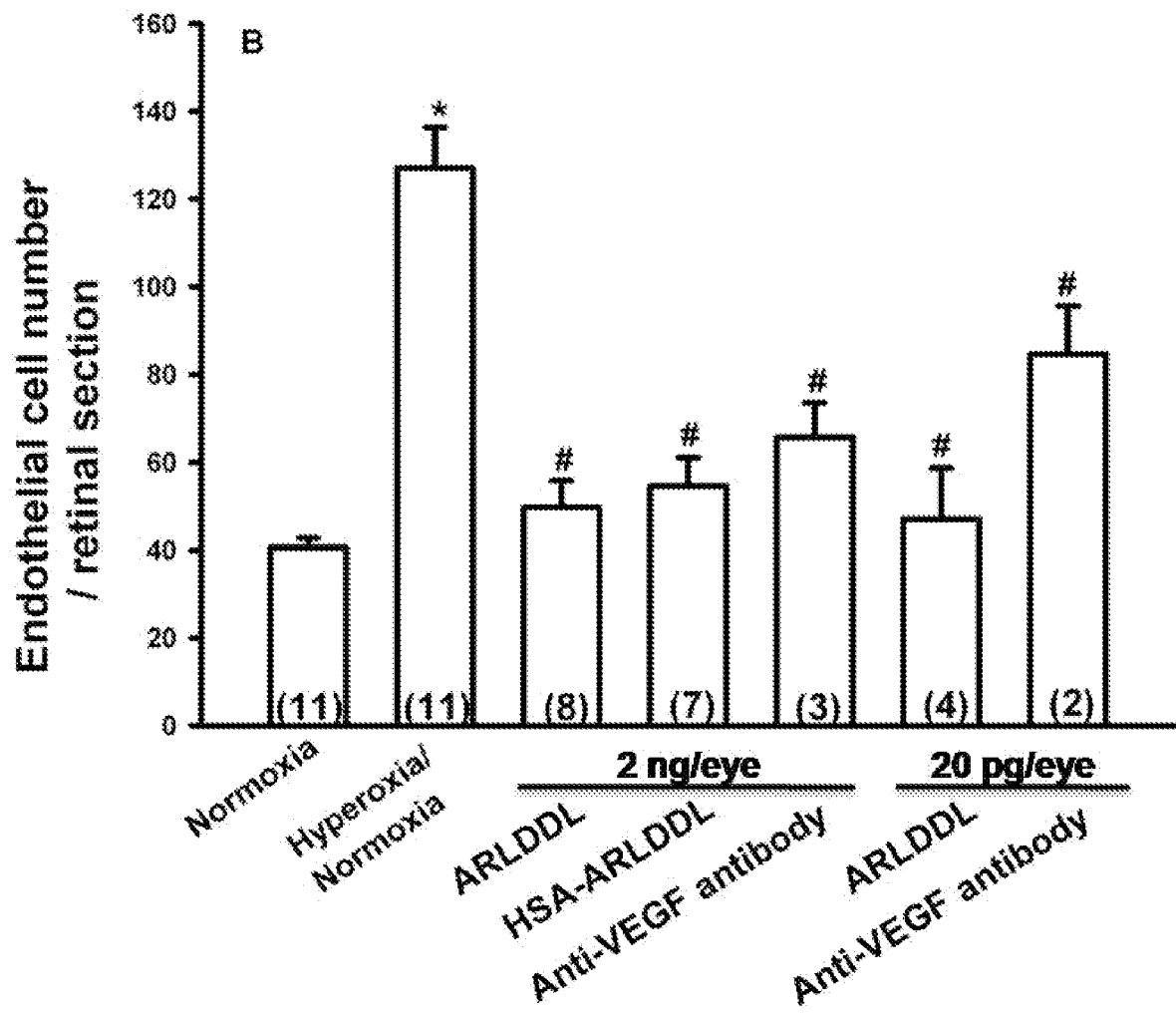


FIG. 2A

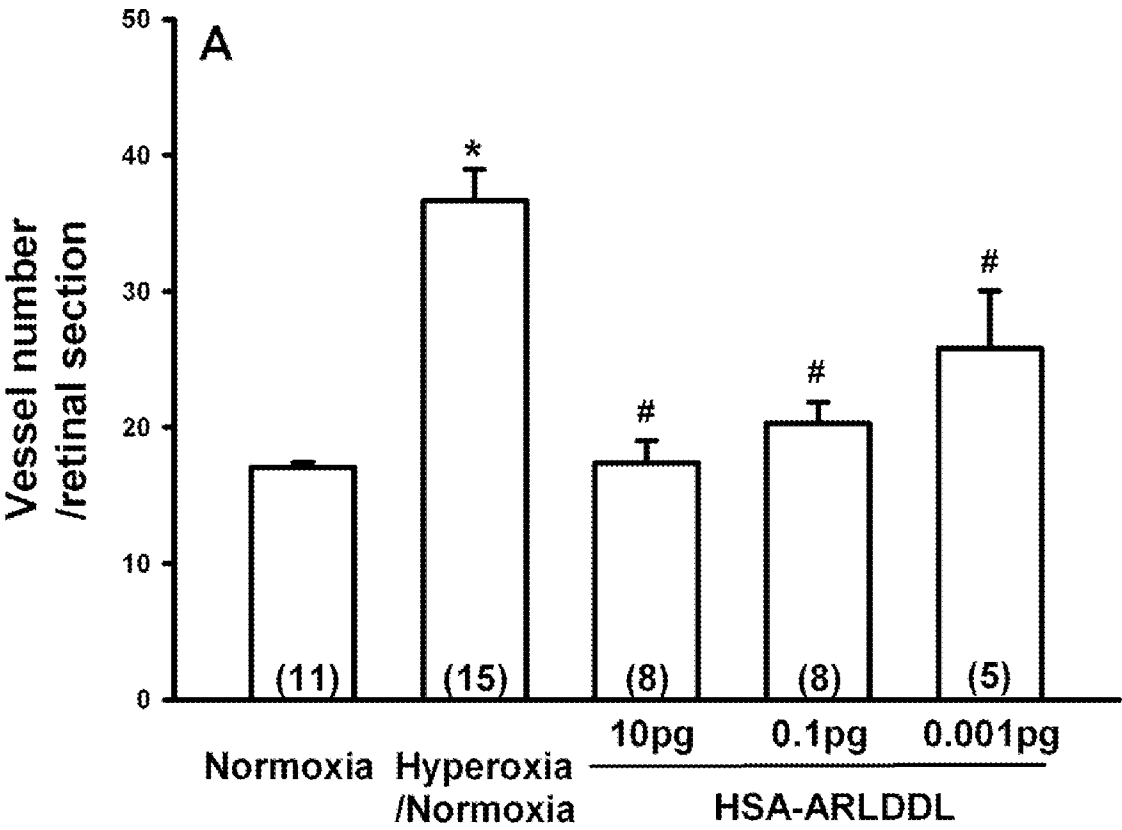


FIG. 2B

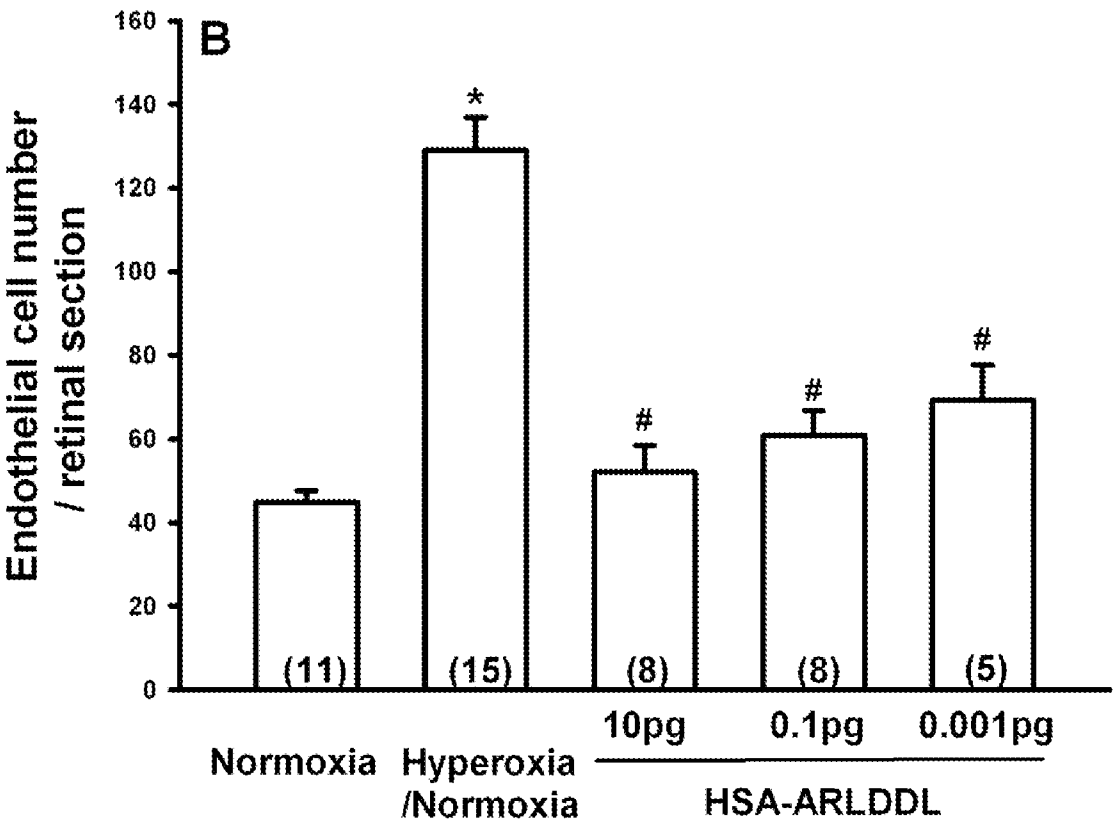


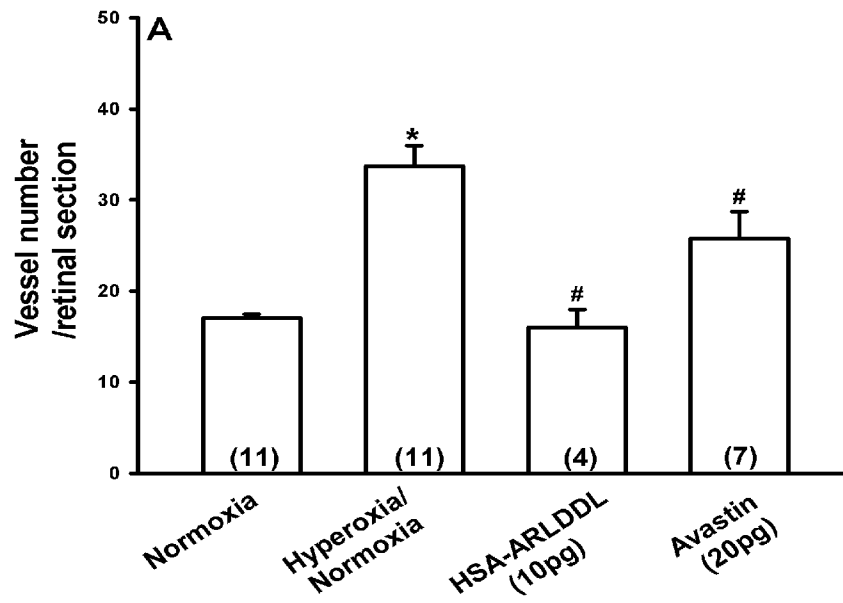
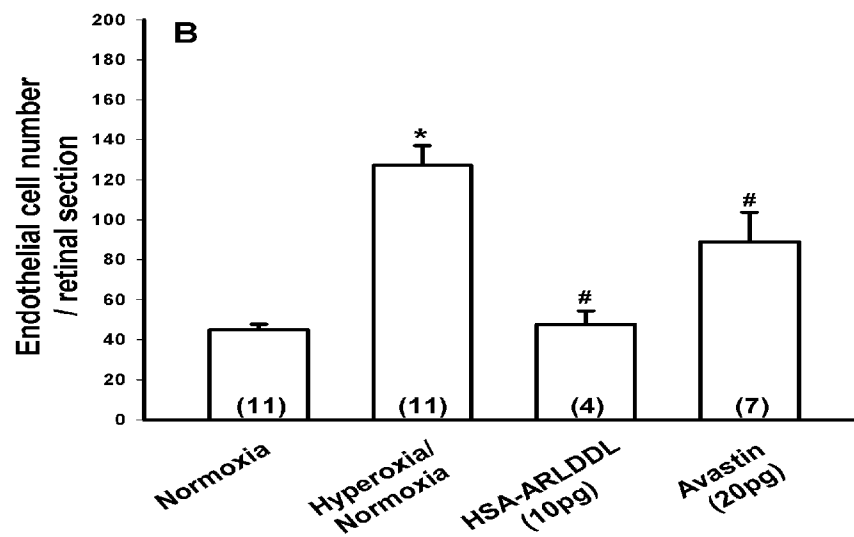
FIG. 3A**Inhibition of Angiogenesis in Retina by HSA-ARLDDL****FIG. 3 B**

FIG. 4A

Inhibition of Angiogenesis in Retina by HSA-ARLDDL

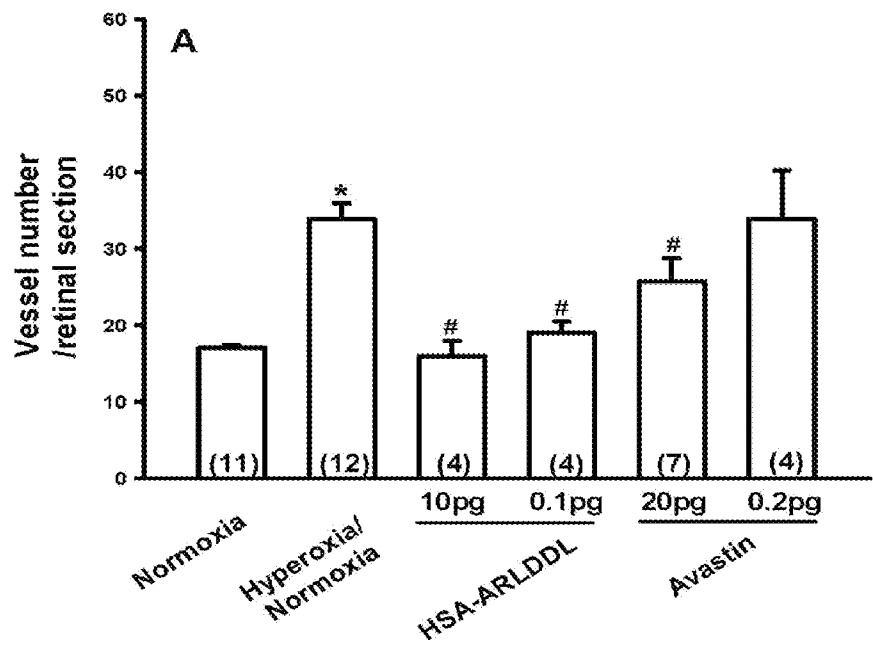
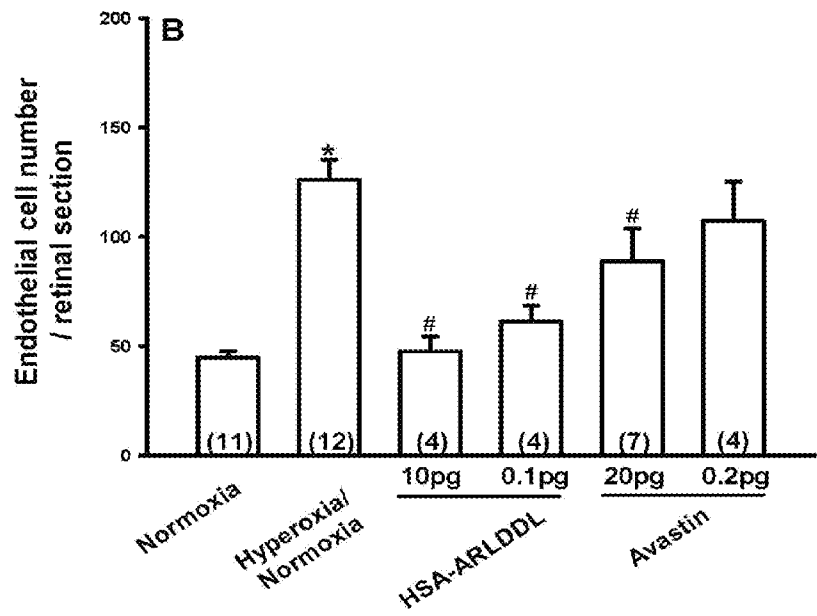


FIG. 4B



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 10/61738

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 38/16, C07K 14/00 (2011.01)

USPC - 514/13.3

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8): A61K 38/16, C07K 14/00 (2011.01)

USPC: 514/13.3

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

USPC: 435/69.6, 530/363, 530/350, 530/300

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PubWest, Google Scholar: albumin, conjugat\$4, pegylat\$4, low, dos\$4, angiogenesis, rhodostomin, VEGF antagonist\$2, anti-angiogenesis agent\$2, antiinflammation agent\$2, steroid\$2, age-related macular degeneration, diabetic retinopathy, corneal neovascularizing diseases, retinal angiomatous proliferation, polypoidal choroidal vasculopathy, age-rela

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y --- A	US 2008/0188413 A1 (CHUANG et al.) 07 August 2008 (07.08.2008); abstract, SEQ ID NO: 57, SEQ ID NO: 65; para [0010], [0030], [0105], [0107], [0216]	1-2, 4-9, 11-24 ----- 3, 10, 25
Y	US 5,659,018 A (BERNDT et al.) 19 August 1997 (19.08.1997); abstract; col 3, ln 45-61; col 9, ln 51-65	1-2, 4-9, 11-24
A	US 2003/0228298 A1 (NESBIT et al.) 11 December 2003 (11.12.2003); SEQ ID NO: 18	3, 10, 25

☐ Further documents are listed in the continuation of Box C.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

03 March 2011 (03.03.2011)

Date of mailing of the international search report

22 MAR 2011

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