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(71) Applicant (for all designated States except US): **CHILDREN'S MEDICAL CENTER CORPORATION** [US/US]; 55 Shattuck Street, Boston, Massachusetts 02115 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **ADINI, Irit** [IL/US]; 35G Saint Paul Street, Brookline, Massachusetts 02446 (US). **D'AMATO, Robert** [US/US]; 4 Patriot's Drive, Lexington, Massachusetts 02420 (US).

(74) Agents: **RESNICK, David** et al.; Nixon Peabody LLP, 100 Summer Street, Boston, Massachusetts 02110 (US).

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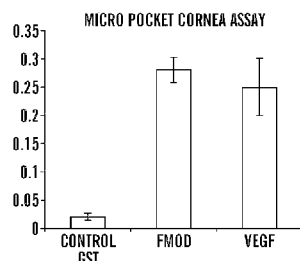
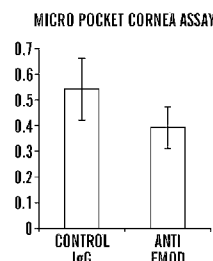
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(54) Title: METHOD FOR MODULATING ANGIOGENESIS USING FIBROMODULIN

**FIG. 7A****FIG. 7B**

(57) Abstract: Described herein are compositions and uses thereof to inhibit or enhance the activity of fibromodulin (FMOD). Such compositions are useful in methods for inhibiting angiogenesis, particularly in subjects having an angiogenesis related disease (e.g., cancer). Also provided herein are fibromodulin compositions for enhancing angiogenesis and are useful in treating disorders of impaired angiogenesis (e.g., wound healing, fertility).

METHOD FOR MODULATING ANGIOGENESIS USING FIBROMODULIN

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This International application claims the benefit of priority under 35 U.S.C. § 119(e) of U.S. Provisional Application No. 61/176,206, filed May 7, 2009, the contents of which are incorporated herein by reference in their entirety.

FIELD OF THE INVENTION

[0002] The present invention relates to the modulation of angiogenesis.

BACKGROUND

[0003] Angiogenesis is the generation of new blood vessels into a tissue or organ. Under normal physiological conditions, humans and animals undergo angiogenesis only in very specific, restricted situations. For example, angiogenesis is normally observed in wound healing, fetal and embryonal development, and formation of the corpus luteum, endometrium and placenta.

[0004] Angiogenesis is controlled through a highly regulated system of angiogenic stimulators and inhibitors. The control of angiogenesis has been found to be altered in certain disease states and, in many cases, pathological damage associated with the diseases is related to uncontrolled angiogenesis. Both controlled and uncontrolled angiogenesis are thought to proceed in a similar manner. Endothelial cells and pericytes, surrounded by a basement membrane, form capillary blood vessels. Angiogenesis begins with the erosion of the basement membrane by enzymes released by endothelial cells and leukocytes. Endothelial cells, lining the lumen of blood vessels, then protrude through the basement membrane. Angiogenic stimulants induce the endothelial cells to migrate through the eroded basement membrane. The migrating cells form a "sprout" off the parent blood vessel where the endothelial cells undergo mitosis and proliferate. The endothelial sprouts merge with each other to form capillary loops, creating a new blood vessel.

[0005] Persistent, unregulated angiogenesis occurs in many disease states, tumor metastases, and abnormal growth by endothelial cells. The diverse pathological disease states in which unregulated angiogenesis is present have been grouped together as angiogenic-dependent or angiogenic-associated diseases.

[0006] Fibromodulin (FMOD) is a member of a family of small interstitial proteoglycans that also includes decorin, biglycan and lumican. The proteoglycans bind to other matrix

macromolecules and thereby help to stabilize the matrix. (Buckwalter et al., 47 Instr. Course Lect 477-86 (1998)). It is thought that they may influence the function of chondrocytes and bind growth factors. Proteoglycan protein cores are structurally related and consist of a central region of leucine-rich repeats flanked by disulfide-bonded terminal domains. Fibromodulin has up to 4 keratin sulfate chains within its leucine-rich domain. It has a wide tissue distribution and is most abundant in articular cartilage, tendon and ligament. It has been suggested that fibromodulin participates in the assembly of the extracellular matrix due to its ability to interact with type I and type II collagen fibrils and to inhibit fibrillogenesis in vitro.

SUMMARY OF THE INVENTION

[0007] Described herein are methods and compositions useful for inhibiting angiogenesis by modulating the activity of fibromodulin (FMOD). Also provided herein are methods for treating an angiogenesis-associated disease by modulating the activity of fibromodulin.

[0008] In one aspect, the methods described herein relate to a fibromodulin activity inhibitor for use in the treatment of an angiogenesis-related disorder.

[0009] In one embodiment of this aspect and all other aspects described herein, the angiogenesis-related disorder is age-related macular degeneration.

[0010] In another embodiment of this aspect and all other aspects described herein, the fibromodulin activity inhibitor comprises an antibody.

[0011] In another aspect, the methods described herein relate to a fibromodulin activity inhibitor for use in the treatment of a disorder of impaired angiogenesis.

[0012] In one embodiment of this aspect and all other aspects described herein, the disorder is an impaired response to wound healing or impaired fertility.

[0013] In another aspect the methods described herein relate to a fibromodulin activity inhibitor for use in the treatment of a wound.

[0014] In another aspect the methods described herein relate to a method for inhibiting endothelial cell growth, the method comprising contacting a cell with a fibromodulin activity inhibitor.

[0015] In one embodiment of this aspect and all other aspects described herein, the cell is selected from the group consisting of a primary cell or a cell of a cell line.

[0016] In another embodiment of this aspect and all other aspects described herein, the cell is human.

[0017] In another aspect the methods described herein relate to a method of treating an angiogenesis-related disease, the method comprising administering a therapeutically effective amount of a fibromodulin activity inhibitor to a mammal having an angiogenesis-related disease.

[0018] In one embodiment of this aspect and all other aspects described herein, the angiogenesis-related disease is age-related macular degeneration.

[0019] In another embodiment of this aspect and all other aspects described herein, the fibromodulin activity inhibitor is selected from the group consisting of an antibody, an RNA interference molecule, a small molecule, a peptide and an aptamer.

[0020] In another embodiment of this aspect and all other aspects described herein, the fibromodulin activity inhibitor comprises an antibody.

[0021] In another embodiment of this aspect and all other aspects described herein, the mammal is a human.

[0022] Another aspect described herein relates to a method for inhibiting angiogenesis, the method comprising administering a therapeutically effective amount of a fibromodulin activity inhibitor to a mammal in need thereof.

[0023] Another aspect described herein relates to a method for inhibiting fibromodulin activity in a mammal, the method comprising administering a therapeutically effective amount of a fibromodulin activity inhibitor to a mammal in need thereof.

[0024] Further described herein is the use of a fibromodulin activity inhibitor in the manufacture of a medicament for the treatment of an angiogenesis-related disease.

[0025] Another aspect described herein is a method for promoting endothelial cell proliferation and/or migration, the method comprising: contacting an endothelial cell with an agent that activates fibromodulin activity.

[0026] In one embodiment of this aspect and all other aspects described herein, the agent is a fibromodulin polypeptide, or a fragment thereof.

[0027] In another embodiment of this aspect and all other aspects described herein, the cell is selected from the group consisting of a primary cell or a cell of a cell line.

[0028] In another embodiment of this aspect and all other aspects described herein, the cell is human.

[0029] Another aspect described herein relates to a method for promoting angiogenesis in a subject, the method comprising: administering a therapeutically effective amount of an agent that activates fibromodulin activity to a subject in need thereof.

[0030] In one embodiment of this aspect and all other aspects described herein, the agent is a fibromodulin polypeptide, or a fragment thereof.

[0031] In another embodiment of this aspect and all other aspects described herein, the subject is a mammal.

[0032] In another embodiment of this aspect and all other aspects described herein, said mammal is a human.

[0033] Another aspect described herein relates to the use of a fibromodulin polypeptide or fragment thereof in the manufacture of a medicament for the treatment of a disorder of impaired angiogenesis.

[0034] In one embodiment of this aspect and all other aspects described herein, the disorder is impaired response to wound healing.

[0035] In another embodiment of this aspect and all other aspects described herein, the disorder is impaired fertility.

[0036] Also described herein is a method for promoting wound healing in a subject, the method comprising: administering an agent that activates fibromodulin activity.

[0037] Also described herein is the use of a fibromodulin polypeptide or fragment thereof in the manufacture of a medicament for the treatment of a wound.

Definitions

[0038] As used herein, the terms "angiogenesis-related disease" and "angiogenesis-associated disease" are used interchangeably herein and refer to any pathological state that is characterized by or involves uncontrolled or undesired growth of blood vessels.

[0039] As used herein, the term "therapeutically effective amount" refers to the amount of an agent that is effective, at dosages and for periods of time necessary to achieve the desired therapeutic result, e.g., a diminishment or prevention of angiogenesis. A therapeutically effective amount of the agents, factors, or inhibitors described herein, or functional derivatives thereof, may vary according to factors such as disease state, age, sex, and weight of the subject, and the ability of the therapeutic compound to elicit a desired response in the subject. The effective amount of a given therapeutic agent will also vary with factors such as the nature of the agent, the route of administration, the size and species of the mammal to receive the therapeutic agent, and the purpose of the administration. A therapeutically effective amount is also one in which any toxic or detrimental effects of the therapeutic agent are outweighed by the therapeutically beneficial effects. The effective amount in each individual case may be determined empirically by a skilled artisan according to established

methods in the art and without undue experimentation. In general, an inhibitor is determined to be "therapeutically effective" in the methods described herein if (a) measurable symptom(s) of angiogenesis or an angiogenesis-related disease, (e.g., capillary density, tumor growth, rate of vessel formation) are decreased by at least 10% compared to the measurement prior to treatment onset, (b) the progression of the disease is halted (e.g., patients do not worsen, new vessels do not form, or the tumor does not continue to grow, or (c) symptoms are reduced or even ameliorated, for example, by measuring a reduction in tumor size or a reduction in vessel infiltration in the eye or elsewhere. Efficacy of treatment can be judged by an ordinarily skilled practitioner. Where promotion of angiogenesis is desired, e.g., in promotion of wound healing, an agent, e.g., a FMOD polypeptide is "therapeutically effect" if angiogenesis or one or more markers of angiogenesis or wound healing are increased by at least 10% relative to angiogenesis or the marker measured in the absence of that agent. Efficacy can be assessed in animal models of angiogenesis, cancer and tumor, for example treatment of a rodent with an experimental cancer, and any treatment or administration of the compositions or formulations that leads to a decrease of at least one symptom of the cancer, for example a reduction in the size of the tumor or a cessation or slowing of the rate of growth of the tumor indicates effective treatment. Alternatively, anti-angiogenesis efficacy can be assessed in an animal model of angiogenesis, such as e.g., hindlimb ischemia, wherein a treatment is considered efficacious if there is a reduction in new vessel formation or re-perfusion of the hindlimb compared to untreated animals. As yet another alternative, a corneal pocket assay, aortic ring assay or CAM assay can be used to predict treatment efficacy for a given agent.

[0040] As used herein, the term "inhibiting angiogenesis" refers to a decrease in a measurable marker of angiogenesis by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 99%, or even 100% (i.e., absent) in the presence of a fibromodulin activity inhibitor compared to the level of the measurable marker in the absence of an inhibitor. Some non-limiting examples of measurable markers of angiogenesis include capillary density, endothelial cell proliferation, endothelial cell migration, and vessel ingrowth.

[0041] As used herein, the terms "increasing angiogenesis" or "promoting angiogenesis" refer to an increase in at least one measurable marker of angiogenesis by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 99%, at least 1-fold, at least 2-fold, at least 5-fold, at least 10-fold,

at least 100-fold, at least 1000-fold or more, in the presence of a pro-angiogenic agent relative to that marker in the absence of such agent.

[0042] As used herein, the term "fibromodulin activity" refers to the pro-angiogenic effect of fibromodulin. Fibromodulin angiogenic activity and its inhibition can be assessed by measuring endothelial cell growth and migration in vitro. Endothelial cell growth can be determined, for example, by measuring cell proliferation using an MTS assay commercially available from a variety of companies including RnD Systems, and Promega, among others. Endothelial cell migration can be assessed, for example, by measuring the migration of cells through a porous membrane using a commercially available kit such as BD BioCoat Angiogenesis System or through a Boyden chamber apparatus. As used herein, the term "inhibition of migration" refers to a decrease in the migration of endothelial cells through a porous membrane (e.g., using a commercially available migration assay kit such as BD BioCoat Angiogenesis System) of at least 10% in the presence of a fibromodulin inhibitor, preferably the decrease is at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 99% decrease in the migration of endothelial cells through a porous membrane, or even 100% (i.e., no migration) in the presence of a fibromodulin inhibitor. As used herein, the term "promoting migration" refers to an increase in the migration of endothelial cells through a porous membrane of at least 10% in the presence of an agent that enhances fibromodulin activity, preferably the increase is at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 99%, at least 1-fold, at least 2-fold, at least 5-fold, at least 10-fold, at least 100-fold, at least 1000-fold or more in the presence of an agent that enhances fibromodulin activity, as that term is used herein.

[0043] The anti-angiogenic activity of a fibromodulin inhibitor can also be assessed in vivo by a decrease in capillary density or neovascular infiltration using a Matrigel plug assay as described by e.g., Kragh M, et al., (2003) (Kragh M, Hjarnaa PJ, Bramm E, Kristjansen PE, Rygaard J, and Binderup L. *Int J Oncol.* (2003) 22(2):305-11, which is herein incorporated by reference in its entirety) in a mammal treated with a fibromodulin inhibitor, compared to capillary density or neovascular infiltration observed in the absence of a fibromodulin inhibitor. A "decrease in capillary density" means a decrease of at least 5% in the presence of a fibromodulin inhibitor compared to untreated subjects; preferably a decrease in capillary density is at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 99% lower,

or even 100% (i.e., absent) in the presence of a fibromodulin inhibitor compared to that measured in the absence of fibromodulin inhibitor administration.

[0044] Pro-angiogenic activity of a fibromodulin polypeptide can be measured in similar angiogenesis assay as described herein or known in the art. An "increase in capillary density" means an increase of at least 5% in the presence of an exogenous fibromodulin polypeptide relative to the absence of exogenous fibromodulin polypeptide, preferably at least 10% at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 99%, at least 1-fold, at least 2-fold, at least 5-fold, at least 10-fold, at least 100-fold, at least 1000-fold or more, in the presence of a pro-angiogenic agent relative to that marker in the absence of such agent.

[0045] As used herein, the term "inhibiting fibromodulin activity" refers to a decrease in the pro-angiogenic activity of fibromodulin by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 99%, or even 100% (i.e., no activity) in the presence of a fibromodulin activity inhibitor compared to the pro-angiogenic activity of fibromodulin in the absence of an inhibitor. As used herein, the term "inhibiting endothelial cell proliferation and/or migration" refers to a decrease in the proliferation and/or migration of endothelial cells of at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 99%, or even 100% (i.e., no growth) in the presence of a fibromodulin activity inhibitor compared to the level of proliferation and/or migration in the absence of an inhibitor.

[0046] As used herein, the terms "increasing fibromodulin activity" or "promoting fibromodulin activity" refers to an increase in the pro-angiogenic activity of fibromodulin by at least 10% at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 99%, at least 1-fold, at least 2-fold, at least 5-fold, at least 10-fold, at least 100-fold, at least 1000-fold or more, in the presence of a pro-angiogenic agent relative to fibromodulin activity in the absence of such agent.

[0047] As used herein, the term "fibromodulin polypeptide" refers to a polypeptide of SEQ ID NO. 001 (Genbank Accession No. NM_002023) or to a conservative substitution variant or fragment thereof that retains fibromodulin activity as that term is defined herein. It should be understood that the carbohydrate moieties of fibromodulin can be involved in fibromodulin pro-angiogenic activity, including, e.g., N-linked keratin sulfate chains. The leucine-rich repeats in the C-terminal domain of the fibromodulin polypeptide have been implicated in the binding of fibromodulin to type I collagen and can play a role in

fibromodulin pro-angiogenic activity. See e.g., Kalamajski and Oldberg, (2007) *J Biol Chem* 282:26740-26745, which highlights the role of leucine-rich repeats in type-I collagen binding. By "retaining fibromodulin activity" is meant that a polypeptide retains at least 50% of the fibromodulin activity of a polypeptide of SEQ ID NO. 001. Also encompassed by the term "fibromodulin polypeptide" are mammalian homologs of human fibromodulin and conservative substitution variants or fragments thereof that retain fibromodulin activity. In one aspect, such homologs or conservative variants thereof stimulate human endothelial cell growth and/or migration as measured, for example, as described herein.

[0048] The term "variant" as used herein refers to a polypeptide or nucleic acid that is "substantially similar" to a wild-type fibromodulin polypeptide or polynucleic acid. A molecule is said to be "substantially similar" to another molecule if both molecules have substantially similar structures (i.e., they are at least 50% similar in amino acid sequence as determined by BLASTp alignment set at default parameters) and are substantially similar in at least one relevant function (e.g., effect on cell migration). A variant differs from the naturally occurring polypeptide or nucleic acid by one or more amino acid or nucleic acid deletions, additions, substitutions or side-chain modifications, yet retains one or more specific functions or biological activities of the naturally occurring molecule. Amino acid substitutions include alterations in which an amino acid is replaced with a different naturally-occurring or a non-conventional amino acid residue. Some substitutions can be classified as "conservative," in which case an amino acid residue contained in a polypeptide is replaced with another naturally occurring amino acid of similar character either in relation to polarity, side chain functionality or size. Substitutions encompassed by variants as described herein can also be "non-conservative," in which an amino acid residue which is present in a peptide is substituted with an amino acid having different properties (e.g., substituting a charged or hydrophobic amino acid with an uncharged or hydrophilic amino acid), or alternatively, in which a naturally-occurring amino acid is substituted with a non-conventional amino acid. Also encompassed within the term "variant," when used with reference to a polynucleotide or polypeptide, are variations in primary, secondary, or tertiary structure, as compared to a reference polynucleotide or polypeptide, respectively (e.g., as compared to a wild-type polynucleotide or polypeptide). Polynucleotide changes can result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence. Variants can also include insertions, deletions or substitutions of amino acids, including insertions and substitutions of amino acids and other molecules) that do not

normally occur in the peptide sequence that is the basis of the variant, including but not limited to insertion of ornithine which does not normally occur in human proteins.

[0049] The term “derivative” as used herein refers to peptides which have been chemically modified, for example by ubiquitination, labeling, pegylation (derivatization with polyethylene glycol) or addition of other molecules. A molecule is also a “derivative” of another molecule when it contains additional chemical moieties not normally a part of the molecule. Such moieties can improve the molecule's solubility, absorption, biological half life, etc. The moieties can alternatively decrease the toxicity of the molecule, or eliminate or attenuate an undesirable side effect of the molecule, etc. Moieties capable of mediating such effects are disclosed in Remington's Pharmaceutical Sciences, 18th edition, A. R. Gennaro, Ed., MackPubl., Easton, PA (1990).

[0050] The term “functional” when used in conjunction with “derivative” or “variant” refers to a polypeptide which possess a biological activity that is substantially similar to a biological activity of the entity or molecule of which it is a derivative or variant. By “substantially similar” in this context is meant that at least 50% of the relevant or desired biological activity of a corresponding wild-type peptide is retained. In the instance of promotion of angiogenesis, for example, an activity retained would be promotion of endothelial cell migration; preferably the variant retains at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 100% or even higher (i.e., the variant or derivative has greater activity than the wild-type), e.g., at least 110%, at least 120%, or more compared to a measurable activity (i.e., promotion or inhibition of endothelial cell migration) of the wild-type polypeptide.

[0051] As used herein the term “comprising” or “comprises” is used in reference to compositions, methods, and respective component(s) thereof, that are essential to the invention, yet open to the inclusion of unspecified elements, whether essential or not.

[0052] As used herein the term “consisting essentially of” refers to those elements required for a given embodiment. The term permits the presence of elements that do not materially affect the basic and novel or functional characteristic(s) of that embodiment of the invention.

[0053] The term “consisting of” refers to compositions, methods, and respective components thereof as described herein, which are exclusive of any element not recited in that description of the embodiment.

[0054] As used in this specification and the appended claims, the singular forms “a,” “an,” and “the” include plural references unless the context clearly dictates otherwise. Thus

for example, references to "the method" includes one or more methods, and/or steps of the type described herein and/or which will become apparent to those persons skilled in the art upon reading this disclosure and so forth.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1. Fibromodulin (FMOD) increases human microvessel endothelial cell (HMVEC) migration. 8 replicate samples per condition of cells (100,000/ml) were seeded into the upper chamber of transwells in the presence of full medium as a positive control. Negative control received starving medium, 40nM recombinant protein or GST was added to the starving condition in order to determine their effect on migration. After 4 hours, cells on the lower surface were detached, lysed, incubated with the dye and read with fluorescence reader using 480/520 nm filter. Significant differences in migration are indicated where $P < 0.001$ using Student's t-test.

Figure 2. Fibromodulin increases human microvessel endothelial cell (HMVEC) proliferation. A) 5000 cells are plated in each well of a 96 well plate overnight. All wells were rinsed with phosphate-buffered saline. Negative control wells received starvation medium, and positive control wells received full medium. 40nM recombinant protein or GST as a control was added to the starving condition in order to determine their effect on proliferation. Cells were allowed to incubate for 24 h. At this time WST-1 reagent was applied for 4 h to measure cell proliferation. The cells were read using a 450 nm filter. Significant differences in proliferation are indicated where $P < 0.001$ using Student's t-test.

Figure 3. 3A. In order to evaluate wound neovascularization, mice ears were subjected to full thickness skin punch and then treated with GST as control or FMOD (P is for the Punch) . To visualize the blood vessels mice were injected with dextran-FITC. **3B.** the vessels pixels as measured by ImageJ. The ear/wound was observed and is shown in Figure 3A. The angiogenesis around the circular wound among mice treated with recombinant FMOD was higher than among mice treated with recombinant FMOD ($p < 0.001$).

Figure 4. Effect of FMOD on Choroidal neovascularization (CNV).

Experiments were performed to investigate the effect of intravitreal injections of GST (5.25nM) and FMOD (5.25nM) on laser-induced CNV. Five mice were injected with the

fibromodulin or GST polypeptide into the vitreous humor after three laser burns to the choroid were placed in each eye ($n = 15\text{--}18$ successful burns in each group). One week later, mice were anesthetized and perfused with fluorescein-labeled dextran. Eyes were enucleated and labeled with anti Lectin or anti-CD31PE. Choroidal flatmounts were prepared, and the CNV area was measured by using ImageJ, perimeter, area, and mean diameter (pixels) were calculated for each region. Significant differences in CNV are indicated where $P < 0.001$ using Student's t-test

Figure 5. siRNA-fibromodulin reduces migration of human microvessel endothelial cells (HMVEC). 48hr before the migration assay non-pigments melanocytes transected with pre-designed siRNA (Ambion) mediated reduction of fibromodulin expression and scrambled siRNA without significant homology to human gene sequences was used as a control. 8 replicate samples per condition of cells (100,000/mL) were seeded into the upper chamber of transwells in the presence of conditioned medium from the transfected dye and read with fluorescence reader using 480/520 nm filter. Significant differences in migration are indicated where $P < 0.001$ using Student's t-test.

Figure 6. An anti-fibromodulin antibody decreases endothelial cell migration.

FACS analysis of matrigel liberated cells from three groups of treated mice. Groups were injected with non-pigment cell (melanocytes) mixed with matrigel containing an antibody against fibromodulin or IgG. The liberated cells were incubated with antibodies specific for endothelial cells (i.e., CD31) and hematopoietic cells (i.e., CD45). The left upper panel reflects the percentage of endothelial cells that can contribute to vessel formation.

Figure 7. 7A. The corneal micropocket assay was performed as described, using pellets containing 4 μ M of FMOD or 4 μ M GST or 4 μ M carrier-free human recombinant VEGF 165. The area of vascular response was assessed on the sixth postoperative day using a slit lamp. Vessel area was calculated using the equation $0.2 \times VL \times CH$, where VL is vessel length from the limbus in millimeters and CH is clock hours around the cornea. **7B.** Corneal micropocket assay was performed as described herein using pellets containing only 4 μ M FMOD; in order to inhibit the angiogenesis, mice were injected intraperitoneally everyday with 1mg/kg antibody against FMOD or IgG as control.

Figure 8. Quantification of retinal neovascularization was performed 7 days after birth. Mosaic images covering the entire retina at 5x magnification were taken on a fluorescence microscope. **8A.** 4 day old pups were injected intravitreally with recombinant FMOD (0.5 μ M) versus GST (control); fluorescence micrographs (Axio Observer Z1; Zeiss) on day 7 show projected images of vessels stained with Alexa-594-isolectin in the retina. Neovascular tuft formation was quantified by comparing the number of pixels in the affected areas with the total number of pixels in the retina (Image J). **8B.** Digitized images of the total retinal area and peripheral avascular areas were measured using the freeware ImageJ. The peripheral avascular area was expressed as a percentage of the total retinal area. **8C.** Neovascularization density was quantified by summing capillary junctions within four equal areas, in each of the four quadrants of the vascularized retina determined by ImageJ. Areas of VO and NV were quantified as percentages of total retina area. P7, n = 10; ***P 0.001.

Figure 9. Fibromodulin siRNA reduces tumor growth in vivo. To confirm that fibromodulin plays a role in the microenvironment during tumor development, fibromodulin expression was systemically inhibited by inoculation of mice with fibromodulin siRNA or a control siRNA. In vivo experiments were obtained by intradermal injection of 1x10⁶ melanoma cells (B16Luc) on the rear dorsum of 6 week-old male C57BL/6J mice. Mice received 2 tail vein injections of either 5nmol siRNA-FMOD or 5nmol siRNA scramble on day 8 and day 11. The siRNA was complexed with a polymer from TRANSIT™ in vivo gene delivery system according to the manufacturer's recommendations (TRANSIT-QR™ Hydrodynamic Delivery System - Mirus). Mice were treated once the tumors reached a volume of 100-150 mm³.

DETAILED DESCRIPTION

[0055] Described herein are methods based, at least in part, on the discovery that fibromodulin inhibitors can inhibit angiogenesis. Thus, for example, described herein are methods for inhibiting endothelial cell growth, migration, and/or angiogenesis by administering an inhibitor of a fibromodulin's pro-angiogenic activity. Similarly described are methods of treatment of e.g., diseases or disorders involving or characterized by inappropriate angiogenesis. Such treatment methods rely upon the administration of an inhibitor of fibromodulin's pro-angiogenic activity. Also described herein are methods based,

at least in part, on the discovery that fibromodulin polypeptides can promote angiogenesis. Described below are the various elements and considerations necessary for one of skill in the art to practice these and related aspects described and encompassed herein.

Fibromodulin

[0056] Fibromodulin (FMOD), also called SLRR2E, is a member of a family of small interstitial proteoglycans. The protein is 59 kDa with leucine-rich repeats flanked by disulfide-bonded terminal domains, possessing up to 4 keratan sulfate chains (Takahashi, T., Cho, H. I., Kublin, C. L. & Cintron, C. (1993) *J Histochem Cytochem* 41:1447-57). Fibromodulin exhibits a wide tissue distribution with the highest concentration found in articular cartilage, tendon, and ligament. The subcellular location of Fibromodulin is within the cytosolic proteins with a secretory sequence but no transmembrane or extracellular domain.

[0057] While it is not wished to indicate that such activity is critical to the pro-angiogenic activity of fibromodulin, several activities of fibromodulin are worth noting here. A characteristic feature of this protein is its participation in the assembly of the extracellular matrix by virtue of its ability to interact with type I, type II and XII collagen fibrils to form collagen fibrils network (Hedbom, E. & Heinegard, D. (1993) *J Biol Chem* 268: 27307-12; Font, B., Eichenberger, D., Goldschmidt, D., Boutillon, M. M. & Hulmes, D. J. (1998) *Eur J Biochem* 254:580-7) and to inhibit fibrillogenesis in vitro (Antonsson, P., Heinegard, D. & Oldberg, A. (1991) *J Biol Chem* 266:16859-61; Hedlund, H., Mengarelli-Widholm, S., Heinegard, D., Reinholt, F. P. & Svensson, O. (1994) *Matrix Biol* 14:227-32; Ezura, Y., Chakravarti, S., Oldberg, A., Chervoneva, I. & Birk, D. E. (2000) *J Cell Biol* 151:779-88; Gori, F., Schipani, E. & Demay, M. B. (2001) *J Cell Biochem* 82:46-57; Ameye, L. et al. (2002) *Faseb J* 16: 673-80; Ameye, L. & Young, M. F. (2002) *Glycobiology* 12:107R-16R; Chakravarti, S. (2002) *Glycoconj J* 19:287-93) FMOD interaction with transforming growth factor (TGF)- β , a key profibrotic cytokine, is considered to enhance the retention of this growth factor within the ECM, thus regulating TGF- β local action (Burton-Wurster, N. et al. (2003) *Osteoarthritis Cartilage* 11:167-76; San Martin, S. et al. (2003) *Reproduction* 125:585-95; Fukushima, D., Butzow, R., Hildebrand, A. & Ruoslahti, E. (1993) *J Biol Chem* 268:22710-5; Hildebrand, A. et al. (1994) *Biochem J* 302 (Pt 2):527-34). The protein is involved in a variety of adhesion processes of connective tissue, and with immunoglobulins activating both the classical and the alternative pathways of complement. Further studies revealed that fibromodulin binds directly to the globular heads of C1q, leading to activation

of C1. Fibromodulin also binds complement inhibitor factor H (Sjoberg, A. P. et al. (2007) *J Biol Chem* 282:10894-900; Sjoberg, A., Onnerfjord, P., Morgelin, M., Heinegard, D. & Blom, A. M. (2005) *J Biol Chem* 280:32301-8)

[0058] The fibromodulin gene has been found to be an overexpressed gene in B-cell chronic lymphocytic leukemia and chronic lymphocytic leukemia (CLL). It may serve as a potential tumor-associated antigen (TAA) in CLL (Mayr, C. et al. (2005) *Blood* 105:1566-73; Mayr, C. et al. (2005) *Blood* 106:3223-6). The amino acid sequences of human and bovine, rat and murine fibromodulin show an overall homology of 90%, allowing for close translation between human and murine experimental models (Antonsson, P., Heinegard, D. & Oldberg, A. (1993) *Biochim Biophys Acta* 1174:204-6).

Fibromodulin Activity Inhibitors

[0059] Essentially any agent that inhibits fibromodulin activity, as that term is defined herein, can be used with the methods described herein. It is preferred, however, that an inhibitor of fibromodulin activity is specific, or substantially specific, for fibromodulin activity inhibition. Further, it is noted that fibromodulin activity can be inhibited by agents that specifically inhibit the expression of the fibromodulin proteoglycan as well as by agents that specifically either bind to, or cleave, the fibromodulin proteoglycan molecule. Some non-limiting examples of agents include antibodies, small molecules, RNA interference molecules, aptamers, ligands, peptides, nucleic acids, or a combination thereof. In addition, expression of a dominant negative mutant of a fibromodulin polypeptide can also be used to inhibit fibromodulin activity. Competitive mutants and/or competitive peptides of a fibromodulin polypeptide are also contemplated for use herein for inhibiting fibromodulin activity.

[0060] Inhibitors of fibromodulin can be screened for efficacy by measuring fibromodulin's pro-angiogenic activity in the presence and absence of the inhibitor, using for example, a fibromodulin activity assay performed using methods e.g., as described in the Examples herein. To avoid doubt, an agent that inhibits fibromodulin activity will, at a minimum, reduce the pro-angiogenic activity of fibromodulin, as that term is used herein.

Small Molecule Inhibitors

[0061] As used herein, the term "small molecule" refers to a chemical agent including, but not limited to, peptides, peptidomimetics, amino acids, amino acid analogs, polynucleotides, polynucleotide analogs, , nucleotides, nucleotide analogs, organic or inorganic compounds (i.e., including heteroorganic and organometallic compounds) having a

molecular weight less than about 10,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 5,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 1,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 500 grams per mole, and salts, esters, and other pharmaceutically acceptable forms of such compounds.

[0062] Small molecules inhibitors of fibromodulin activity can be identified from within a small molecule library, which can be obtained from commercial sources such as AMRI (Albany, NY), AsisChem Inc. (Cambridge, MA), TimTec (Newark, DE), among others, or from libraries as known in the art.

Aptamers

[0063] Aptamers are relatively short RNA or DNA oligonucleotides, which bind ligands and are isolated in vitro using, for example, the selection procedure known as SELEX (systematic evolution of ligands by exponential enrichment) (Tuerk & Gold, 1990; Ellington & Szostak, 1990, U.S. Patent Nos. 5,475,096 and 5,270,163, which are incorporated herein by reference in their entirety). Because the selection procedure is driven by binding of ligands, aptamers bind their ligands with high affinity and fold into secondary structures which are optimized for ligand binding (Herman & Patel, 2000, incorporated herein by reference in its entirety). In this respect aptamers resemble antibodies by selectively binding corresponding ligand from complex chemical or biological mixtures.

[0064] The aptamer oligonucleotide of such an embodiment can be any useful aptamer now known or later developed. Methods to design and synthesize aptamers and aptamer binding sequences are known to those of skill in the art.

[0065] It is contemplated herein that aptamers directed at binding fibromodulin and inhibiting its pro-angiogenic activity can be used in the methods described herein.

Antibodies

[0066] Antibodies can be used to inhibit fibromodulin by e.g., recognition of an epitope such that a bound antibody inhibits fibromodulin's pro-angiogenic activity. Production of antibodies useful for the methods described herein are known to those of skill in the art.

[0067] The production of non-human monoclonal antibodies, e.g., murine or rat, can be accomplished by, for example, immunizing the animal with a desired target peptide or polypeptide and preparing hybridomas of spleen cells from the immunized animals, according to well established methods (e.g., See Harlow & Lane, Antibodies, A Laboratory

Manual (CSHP NY, 1988, which is herein incorporated by reference in its entirety). Immunogen can be obtained from a natural source, by peptide synthesis or by recombinant expression. Humanized forms of mouse antibodies (e.g., as produced by a hybridoma) can be generated by cloning and linking the CDR regions of the murine antibodies to human constant regions by recombinant DNA techniques. See Queen et al., Proc. Natl. Acad. Sci. USA 86, 10029-10033 (1989) and WO 90/07861 (incorporated by reference herein in their entirety). Human antibodies can be obtained using phage-display methods. See, e.g., Dower et al., WO 91/17271; McCafferty et al., WO 92/01047, which are incorporated herein by reference in their entirety. In these methods, libraries of phage are produced in which members display different antibodies on their outer surfaces. Antibodies are usually displayed as Fv or Fab fragments. Phage displaying antibodies with a desired specificity are selected by binding to a fibromodulin polypeptide or fragments thereof. Increased affinity can be selected by successive rounds of affinity enrichment by binding to the same fragment. Human antibodies against fibromodulin can also be produced from non-human transgenic mammals having transgenes encoding at least a segment of the human immunoglobulin locus and an inactivated endogenous immunoglobulin locus. See, e.g., Lonberg et al., WO93/12227 (1993); Kucherlapati, WO 91/10741 (1991) (each of which is incorporated by reference in its entirety). Human antibodies can be selected by competitive binding experiments, or otherwise, to have the same epitope specificity as a particular mouse antibody. Such antibodies are particularly likely to share the useful functional properties of the mouse antibodies. Human polyclonal antibodies can also be provided in the form of serum from humans immunized with an immunogenic agent. Optionally, such polyclonal antibodies can be concentrated by affinity purification using fibromodulin as an affinity reagent. Human or humanized antibodies can be designed to have IgG, IgD, IgA and IgE constant region, and any isotype, including IgG1, IgG2, IgG3 and IgG4. Antibodies can be expressed as tetramers containing two light and two heavy chains, as separate heavy chains, light chains, as Fab, Fab'F(ab')₂, and Fv, or as single chain antibodies in which heavy and light chain variable domains are linked through a spacer.

[0068] Fibromodulin antibodies can be obtained from commercial sources such as e.g., Santa Cruz Biotechnology Inc. (Santa Cruz, CA), Millipore (Billerica, MA), Novus Biologicals (Littleton, CO), AbNova Corporation (Walnut, CA), and AbCam (Cambridge, MA), among others. A mouse monoclonal antibody against human fibromodulin can be obtained from Acris Antibodies (Herford, Germany), which is distributed in the U.S. by Novus Biologicals (Littleton, CO). In addition, the genes encoding, for example, murine or

goat anti fibromodulin antibodies provide candidates for humanization for therapeutic purposes.

RNA interference

[0069] RNA interference agents can be used with the methods described herein, to inhibit the expression and/or activity of a fibromodulin polypeptide. "RNA interference (RNAi)" is an evolutionarily conserved process whereby the expression or introduction of RNA of a sequence that is identical or highly similar to a target gene results in the sequence specific degradation or specific post-transcriptional gene silencing (PTGS) of messenger RNA (mRNA) transcribed from that targeted gene (see Coburn, G. and Cullen, B., J. of Virology 76(18):9225 (2002), herein incorporated by reference in its entirety), thereby inhibiting expression of the target gene. As used herein, "inhibition of target gene expression" includes any decrease in expression or protein activity or level of the target gene or protein encoded by the target gene as compared to a situation wherein no RNA interference has been induced. The decrease can be of at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or 99% or more as compared to the expression of a target gene or the activity or level of the protein encoded by a target gene which has not been targeted by an RNA interfering agent. RNA interfering agents contemplated for use with the methods described herein include, but are not limited to, siRNA, shRNA, miRNA, and dsRNAi.

[0070] The target gene or sequence of the RNA interfering agent can be a cellular gene or genomic sequence. An siRNA can be substantially homologous to the target gene or genomic sequence, or a fragment thereof. As used in this context, the term "homologous" is defined as being substantially identical, sufficiently complementary, or similar to the target mRNA, or a fragment thereof, to effect RNA interference of the target. Preferably, the siRNA is identical in sequence to its target and targets only one sequence. Each of the RNA interfering agents, such as siRNAs, can be screened for potential off-target effects by, for example, expression profiling. Such methods are known to one skilled in the art and are described, for example, in Jackson et al., Nature Biotechnology 6:635-637 (2003), herein incorporated by reference in its entirety.

[0071] It is well within the ability of one skilled in the art to design and test for siRNAs that are useful for inhibiting fibromodulin expression and/or activity. It is important to note that double-stranded siRNA or shRNA molecules that are cleaved by Dicer in the cell can be up to 100 times more potent than a 21-mer siRNA or shRNA molecule supplied exogenously (Kim, DH., et al (2005) *Nature Biotechnology* 23(2):222-226). Thus, an RNAi molecule can

be designed to be more effective by providing a sequence for Dicer cleavage. Methods for effective siRNA design for use *in vivo* can be found in U.S. Patent No. 7, 427, 605, which is herein incorporated by reference in its entirety.

[0072] Commercially available RNA interference molecules that target fibromodulin can be obtained from e.g., Santa Cruz Biotechnology Inc. (Santa Cruz, CA), Cell Signaling Technologies (Danvers, MA), Sigma-Aldrich (St. Louis, MO), and Dharmacon Inc. (Lafayette, CO), among others.

In Vivo Delivery of RNA interference (RNAi) molecules

[0073] In general, any method of delivering a nucleic acid molecule can be adapted for use with an RNAi interference molecule (see e.g., Akhtar S. and Julian RL. (1992) Trends Cell. Biol. 2(5):139-144; WO94/02595, which are incorporated herein by reference in their entirety). However, there are three factors that are important to consider in order to successfully deliver an RNAi molecule *in vivo*: (a) biological stability of the RNAi molecule, (2) preventing non-specific effects, and (3) accumulation of the RNAi molecule in the target tissue. The non-specific effects of an RNAi molecule can be minimized by local administration by e.g., direct injection into a tissue including, for example, a tumor or topically administering the molecule.

[0074] Local administration of an RNAi molecule to a treatment site limits the exposure of the e.g., siRNA to systemic tissues and permits a lower dose of the RNAi molecule to be administered. Several studies have shown successful knockdown of gene products when an RNAi molecule is administered locally. For example, intraocular delivery of a VEGF siRNA by intravitreal injection in cynomolgus monkeys (Tolentino, MJ., et al (2004) Retina 24:132-138) and subretinal injections in mice (Reich, SJ., et al (2003) Mol. Vis. 9:210-216) were both shown to prevent neovascularization in an experimental model of age-related macular degeneration. In addition, direct intratumoral injection of an siRNA in mice reduces tumor volume (Pille, J., et al (2005) Mol. Ther. 11:267-274) and can prolong survival of tumor-bearing mice (Kim, WJ., et al (2006) Mol. Ther. 14:343-350; Li, S., et al (2007) Mol. Ther. 15:515-523). RNA interference has also shown success with local delivery to the CNS by direct injection (Dorn, G., et al. (2004) Nucleic Acids 32:e49; Tan, PH., et al (2005) Gene Ther. 12:59-66; Makimura, H., et al (2002) BMC Neurosci. 3:18; Shishkina, GT., et al (2004) Neuroscience 129:521-528; Thakker, ER., et al (2004) Proc. Natl. Acad. Sci. U.S.A. 101:17270-17275; Akaneya, Y., et al (2005) J. Neurophysiol. 93:594-602) and to the lungs by

intranasal administration (Howard, KA., et al (2006) *Mol. Ther.* 14:476-484; Zhang, X., et al (2004) *J. Biol. Chem.* 279:10677-10684; Bitko, V., et al (2005) *Nat. Med.* 11:50-55).

[0075] For administering an RNAi molecule systemically for the treatment of a disease, the RNAi molecule can be either be modified or alternatively delivered using a drug delivery system; both methods act to prevent the rapid degradation of the RNAi molecule by endo- and exo-nucleases in vivo. Modification of the RNAi molecule or the pharmaceutical carrier can also permit targeting of the RNAi molecule to the target tissue and avoid undesirable off-target effects.

[0076] RNA interference molecules can be modified by chemical conjugation to lipophilic groups such as cholesterol to enhance cellular uptake and prevent degradation. For example, an siRNA directed against ApoB conjugated to a lipophilic cholesterol moiety was injected systemically into mice and resulted in knockdown of apoB mRNA in both the liver and jejunum (Soutschek, J., et al (2004) *Nature* 432:173-178). Conjugation of an RNAi molecule to an aptamer has been shown to inhibit tumor growth and mediate tumor regression in a mouse model of prostate cancer (McNamara, JO., et al (2006) *Nat. Biotechnol.* 24:1005-1015).

[0077] In an alternative embodiment, the RNAi molecules can be delivered using drug delivery systems such as e.g., a nanoparticle, a dendrimer, a polymer, liposomes, or a cationic delivery system. Positively charged cationic delivery systems facilitate binding of an RNA interference molecule (negatively charged) and also enhance interactions at the negatively charged cell membrane to permit efficient uptake of an siRNA by the cell. Cationic lipids, dendrimers, or polymers can either be bound to an RNA interference molecule, or induced to form a vesicle or micelle (see e.g., Kim SH., et al (2008) *Journal of Controlled Release* 129(2):107-116) that encases an RNAi molecule. The formation of vesicles or micelles further prevents degradation of the RNAi molecule when administered systemically. Methods for making and administering cationic-RNAi complexes are well within the abilities of one skilled in the art (see e.g., Sorensen, DR., et al (2003) *J. Mol. Biol* 327:761-766; Verma, UN., et al (2003) *Clin. Cancer Res.* 9:1291-1300; Arnold, AS et al (2007) *J. Hypertens.* 25:197-205, which are incorporated herein by reference in their entirety).

[0078] Some non-limiting examples of drug delivery systems useful for systemic administration of RNAi include DOTAP (Sorensen, DR., et al (2003), *supra*; Verma, UN., et al (2003), *supra*), Oligofectamine, "solid nucleic acid lipid particles" (Zimmermann, TS., et al (2006) *Nature* 441:111-114), cardiolipin (Chien, PY., et al (2005) *Cancer Gene Ther.* 12:321-328; Pal, A., et al (2005) *Int J. Oncol.* 26:1087-1091), polyethyleneimine (Bonnet ME., et al

(2008) Pharm. Res. Aug 16 Epub ahead of print; Aigner, A. (2006) J. Biomed. Biotechnol. 71659), Arg-Gly-Asp (RGD) peptides (Liu, S. (2006) Mol. Pharm. 3:472-487), and polyamidoamines (Tomalia, DA., et al (2007) Biochem. Soc. Trans. 35:61-67; Yoo, H., et al (1999) Pharm. Res. 16:1799-1804). In some embodiments, an RNAi molecule forms a complex with cyclodextrin for systemic administration. Methods for administration and pharmaceutical compositions of RNAi molecules and cyclodextrins can be found in U.S. Patent No. 7, 427, 605, which is herein incorporated by reference in its entirety. Specific methods for administering an RNAi molecule for the inhibition of angiogenesis can be found in e.g., U.S. Patent Application No. 20080152654, which is herein incorporated by reference in its entirety.

Fibromodulin Polypeptides

[0079] A fibromodulin polypeptide or a portion thereof functional to promote angiogenesis can be administered to an individual in need thereof. In one approach, a soluble fibromodulin polypeptide, produced, for example, in cultured cells bearing a recombinant fibromodulin expression vector can be administered to the individual. The fibromodulin polypeptide or portion thereof will generally be administered intravenously. This approach rapidly delivers the protein throughout the system and maximizes the chance that the protein is intact when delivered. Alternatively, other routes of therapeutic protein administration are contemplated, such as by inhalation. Technologies for the administration of agents, including protein agents, as aerosols are well known and continue to advance. Alternatively, the polypeptide agent can be formulated for topical delivery, including, for example, preparation in liposomes. Further contemplated are, for example, transdermal administration, and rectal or vaginal administration. Further options for the delivery of fibromodulin polypeptides as described herein are discussed in the section "Pharmaceutical Compositions" herein below.

[0080] Vectors for transduction of a fibromodulin-encoding sequence are well known in the art. While overexpression using a strong non-specific promoter, such as a CMV promoter, can be used, it can be helpful to include a tissue- or cell-type-specific promoter on the expression construct – for example, the use of a skeletal muscle-specific promoter or other cell-type-specific promoter can be advantageous, depending upon what cell type is used as a host. Further, treatment can include the administration of viral vectors that drive the expression of fibromodulin polypeptides in infected host cells. Viral vectors are well known to those skilled in the art.

[0081] These vectors are readily adapted for use in the methods of the present invention. By the appropriate manipulation using recombinant DNA/molecular biology techniques to insert an operatively linked fibromodulin encoding nucleic acid segment into the selected expression/delivery vector, many equivalent vectors for the practice of the methods described herein can be generated. It will be appreciated by those of skill in the art that cloned genes readily can be manipulated to alter the amino acid sequence of a protein.

[0082] The cloned gene for fibromodulin can be manipulated by a variety of well known techniques for in vitro mutagenesis, among others, to produce variants of the naturally occurring human protein, herein referred to as muteins or variants or mutants of fibromodulin, which may be used in accordance with the methods and compositions described herein. The variation in primary structure of muteins of fibromodulin useful in the invention, for instance, may include deletions, additions and substitutions. The substitutions may be conservative or non-conservative. The differences between the natural protein and the mutein generally conserve desired properties, mitigate or eliminate undesired properties and add desired or new properties. The fibromodulin polypeptide can also be a fusion polypeptide, fused, for example, to a polypeptide that targets the product to a desired location, or, for example, a tag that facilitates its purification, if so desired. Fusion to a polypeptide sequence that increases the stability of the fibromodulin polypeptide is also contemplated. For example, fusion to a serum protein, e.g., serum albumin, can increase the circulating half-life of a fibromodulin polypeptide. Tags and fusion partners can be designed to be cleavable, if so desired. Another modification specifically contemplated is attachment, e.g., covalent attachment, to a polymer. In one aspect, polymers such as polyethylene glycol (PEG) or methoxypolyethylene glycol (mPEG) can increase the in vivo half-life of proteins to which they are conjugated. Methods of PEGylation of polypeptide agents are well known to those skilled in the art, as are considerations of, for example, how large a PEG polymer to use. In another aspect, biodegradable or absorbable polymers can provide extended, often localized, release of polypeptide agents. Such synthetic bioabsorbable, biocompatible polymers, which may release proteins over several weeks or months can include, for example, poly- α -hydroxy acids (e.g. polylactides, polyglycolides and their copolymers), polyanhydrides, polyorthoesters, segmented block copolymers of polyethylene glycol and polybutylene terephthalate (PolyactiveTM), tyrosine derivative polymers or poly(ester-amides). Suitable bioabsorbable polymers to be used in manufacturing of drug delivery materials and implants are discussed e.g. in U.S. Pat. Nos. 4,968,317; 5,618,563, among others, and in "Biomedical

Polymers" edited by S. W. Shalaby, Carl Hanser Verlag, Munich, Vienna, New York, 1994 and in many references cited in the above publications. The particular bioabsorbable polymer that should be selected will depend upon the particular patient that is being treated.

Diseases

[0083] Essentially any angiogenesis-associated disease can be treated with the methods and compositions described herein. In one embodiment, methods of treatment described herein include the step of diagnosing an individual with an angiogenesis-associated or angiogenesis-related disease or disorder. While it is anticipated that fibromodulin inhibitors would target angiogenesis in tissues with elevated levels of fibromodulin, such elevated levels are not necessarily required for anti-fibromodulin therapy to be effective.

[0084] One example of a disease mediated by angiogenesis is ocular neovascular disease. This disease is characterized by invasion of new blood vessels into the structures of the eye, such as the retina or cornea. It is the most common cause of blindness and is involved in approximately twenty eye diseases. In age-associated macular degeneration, the associated visual problems are caused by an ingrowth of choroidal capillaries through defects in Bruch's membrane with proliferation of fibrovascular tissue beneath the retinal pigment epithelium.

[0085] Angiogenic damage is also associated with diabetic retinopathy, retinopathy of prematurity/ retrolental fibroplasia, corneal graft rejection and neovascular glaucoma. Other diseases or conditions associated with corneal neovascularization include, but are not limited to, epidemic keratoconjunctivitis, Vitamin A deficiency, trachoma, contact lens overwear, atopic keratitis, superior limbic keratitis, pterygium keratitis sicca, Sjogrens disease, acne rosacea, phlyctenulosis, syphilis, Mycobacteria infections, lipid degeneration, chemical burns, bacterial ulcers, fungal ulcers, Herpes simplex infection, Herpes simplex keratitis, Herpes zoster infections, protozoan infections, Kaposi's sarcoma, Mooren's ulcer, Terrien's marginal degeneration, marginal keratolysis, rheumatoid arthritis, systemic lupus, polyarteritis, trauma, Wegener's sarcoidosis, scleritis, Stevens-Johnson's disease, pemphigoid, trachoma and radial keratotomy.

[0086] Diseases associated with retinal/choroidal neovascularization include, but are not limited to, diabetic retinopathy, macular degeneration, sickle cell anemia, sarcoidosis, syphilis, pseudoxanthoma elasticum, Paget's disease, vein occlusion, artery occlusion, carotid obstructive disease, chronic uveitis/vitritis, Mycobacteria infections, Lyme disease, systemic lupus erythematosus, retinopathy of prematurity, Eales' disease, Behcet's disease, infections causing retinitis or choroiditis, presumed ocular histoplasmosis, Best's disease, myopia, optic

pits, Stargardt's disease, pars planitis, chronic retinal detachment, hyperviscosity syndromes, toxoplasmosis, histoplasmosis, trauma and post-laser complications. Other eye-associated diseases that can involve inappropriate angiogenesis include, but are not limited to, diseases associated with rubeosis (neovascularization of the angle) and diseases caused by the abnormal proliferation of fibrovascular or fibrous tissue, including all forms of proliferative vitreoretinopathy.

[0087] Another angiogenesis associated disease is rheumatoid arthritis. The blood vessels in the synovial lining of the joints undergo angiogenesis. In addition to forming new vascular networks, the endothelial cells release factors and reactive oxygen species that lead to pannus growth and cartilage destruction. The factors involved in angiogenesis may actively contribute to, and help maintain, the chronically inflamed state of rheumatoid arthritis.

[0088] Angiogenesis may also play a role in osteoarthritis and gout. The activation of the chondrocytes by angiogenic-associated factors contributes to the destruction of the joint. At a later stage, the angiogenic factors promote new bone growth. Therapeutic intervention that prevents the bone destruction could halt the progress of the disease and provide relief for persons suffering with arthritis.

[0089] Angiogenesis has been associated with a number of different types of cancer, including solid tumors and blood-borne tumors. Solid tumors with which angiogenesis has been associated include, but are not limited to cancer of the prostate, lung, breast, brain, ovarian, stomach, pancreas, larynx, esophagus, testes, liver, parotid, biliary tract, colon, rectum, cervix, uterus, endometrium, kidney, bladder and thyroid; as well as rhabdomyosarcomas, retinoblastoma, Ewing's sarcoma, neuroblastoma, and osteosarcoma, to name but a few. Tumors in which angiogenesis is important include benign tumors such as acoustic neuroma, neurofibroma, trachoma, and pyogenic granulomas. Prevention of angiogenesis could halt the growth of these tumors and the resultant damage to the animal due to the presence of the tumor.

[0090] Angiogenesis is also associated with blood-borne tumors, such as leukemias, any of various acute or chronic neoplastic diseases of the bone marrow in which unrestrained proliferation of white blood cells occurs, usually accompanied by anemia, impaired blood clotting, and enlargement of the lymph nodes, liver and spleen. It is believed that angiogenesis plays a role in the abnormalities in the bone marrow and lymph nodes that give rise to lymphoma, myelodysplastic syndrome and multiple myeloma.

[0091] One of the most frequent angiogenic diseases of childhood is the hemangioma. A hemangioma is a tumor composed of newly-formed blood vessels. In most cases the tumors

are benign and regress without intervention. In more severe cases, the tumors progress to large cavernous and infiltrative forms and create clinical complications. Systemic forms of hemangiomas, hemangiomatoses, have a high mortality rate. Therapy-resistant hemangiomas exist that cannot be treated with therapeutics currently in use.

[0092] Angiogenesis is prominent in solid tumor formation and metastasis. Angiogenic factors have been found associated with several solid tumors such as rhabdomyosarcomas, retinoblastoma, Ewing's sarcoma, neuroblastoma, and osteosarcoma. A tumor cannot expand without a blood supply to provide nutrients and remove cellular wastes. Tumors in which angiogenesis is important include solid tumors, and benign tumors such as acoustic neuroma, neurofibroma, trachoma and pyogenic granulomas. Prevention of angiogenesis could halt the growth of these tumors and the resultant damage to the animal due to the presence of the tumor.

[0093] Angiogenesis is important in two stages of tumor metastasis. The first stage where angiogenesis stimulation is important is in the vascularization of the tumor which allows tumor cells to enter the blood stream and to circulate throughout the body. After the tumor cells have left the primary site, and have settled into the secondary, metastasis site, angiogenesis must occur before the new tumor can grow and expand. Therefore, prevention of angiogenesis could lead to the prevention of metastasis of tumors and possibly contain the neoplastic growth at the primary site.

[0094] Knowledge of the role of angiogenesis in the maintenance and metastasis of tumors has led to a prognostic indicator for breast cancer. The amount of neovascularization found in the primary tumor was determined by counting the microvessel density in the area of the most intense neovascularization in invasive breast carcinoma. A high level of microvessel density was found to correlate with tumor recurrence. Control of angiogenesis by therapeutic means can lead to cessation of the recurrence of the tumors.

[0095] Angiogenesis is also responsible for damage found in hereditary diseases such as Osler-Weber-Rendu disease, or hereditary hemorrhagic telangiectasia. This is an inherited disease characterized by multiple small angiomas, tumors of blood or lymph vessels. The angiomas are found in the skin and mucous membranes, often accompanied by epistaxis (nose bleeds) or gastrointestinal bleeding and sometimes with pulmonary or hepatic arteriovenous fistula.

[0096] Angiogenesis is also involved in normal physiological processes, such as reproduction and wound healing. Angiogenesis is an important step in ovulation and also in implantation of the blastula after fertilization. Prevention of angiogenesis could be used to

induce amenorrhea, to block ovulation, or to prevent implantation by the blastula. In wound healing, excessive repair or fibroplasia can be a detrimental side effect of surgical procedures and may be caused or exacerbated by angiogenesis. Adhesions are a frequent complication of surgery and lead to problems such as small bowel obstruction. Other angiogenesis dependent diseases of the reproductive system include endometriosis, ectopic pregnancy and uterine fibroids.

[0097] Diseases associated with chronic inflammation are accompanied by angiogenesis and can be treated by the compositions and methods of the present invention. Diseases with symptoms of chronic inflammation include obesity, inflammatory bowel diseases such as Crohn's disease and ulcerative colitis, psoriasis, sarcoidosis, atherosclerosis including plaque rupture, Sjogrens disease, acne rosacea, syphilis, chemical burns, bacterial ulcers, fungal ulcers, Behcet's syndrome, Stevens-Johnson's disease, Mycobacteria infections, Herpes simplex infections, Herpes zoster infections, protozoan infections, Mooren's ulcer, leprosy, Wegener's sarcoidosis, pemphigoid, lupus, systemic lupus erythematosus, polyarteritis, lyme's disease, Bartonellosis, tuberculosis, histoplasmosis and toxoplasmosis. Angiogenesis is a key element that these chronic inflammatory diseases have in common. The chronic inflammation depends on continuous formation of capillary sprouts to maintain an influx of inflammatory cells. The influx and presence of the inflammatory cells sometimes produce granulomas to help maintain the chronic inflammatory state. Inhibition of angiogenesis by the compositions and methods of the present invention would prevent the formation of the granulomas and alleviate the disease.

[0098] The inflammatory bowel diseases also show extraintestinal manifestations such as skin lesions. Such lesions are characterized by inflammation and angiogenesis and can occur at many sites other than the gastrointestinal tract. The compositions and methods of the present invention are also capable of treating these lesions by preventing the angiogenesis, thus, reducing the influx of inflammatory cells and the lesion formation.

[0099] Sarcoidosis is another chronic inflammatory disease that is characterized as a multisystem granulomatous disorder. The granulomas of this disease may form anywhere in the body, and, thus, the symptoms depend on the site of the granulomas and whether the disease active. The granulomas are created by the angiogenic capillary sprouts providing a constant supply of inflammatory cells.

[00100] Lastly, tissue and organ growth is regulated by the available vascular supply and new supply from angiogenesis. Thus obesity has been found to be inhibited by angiogenesis inhibitors since fat pad growth requires angiogenesis. Inhibitors of angiogenesis, as described

herein, are contemplated for use in the treatment of obesity, for weight loss, for weight control, or for maintenance of a weight following e.g., surgery or dietary intervention (see e.g., U.S. Patent No. 6,306,819, which is herein incorporated by reference in its entirety). Alternatively, activators of angiogenesis can be used to promote weight gain in e.g., anorexic or malnourished individuals.

Dosage and Administration

[00101] In one aspect, the methods described herein provide a method for treating an angiogenesis-associated disease in a subject. In one embodiment, the subject can be a mammal. In another embodiment, the mammal can be a human, although the approach is effective with respect to all mammals. The method comprises administering to the subject an effective amount of a pharmaceutical composition comprising an agent that inhibits fibromodulin activity, in a pharmaceutically acceptable carrier.

[00102] The dosage range for the agent depends upon the potency, and include amounts large enough to produce the desired effect, e.g., a reduction in invasion of new blood vessels in the eye or elsewhere. The dosage should not be so large as to cause unacceptable adverse side effects. Generally, the dosage will vary with the type of fibromodulin activity inhibitor (e.g., an antibody or fragment, small molecule, siRNA, etc.), and with the age, condition, and sex of the patient. The dosage can be determined by one of skill in the art and can also be adjusted by the individual physician in the event of any complication. Typically, the dosage ranges from 0.001mg/kg body weight to 5 g/kg body weight. In some embodiments, the dosage range is from 0.001 mg/kg body weight to 1g/kg body weight, from 0.001 mg/kg body weight to 0.5 g/kg body weight, from 0.001 mg/kg body weight to 0.1 g/kg body weight, from 0.001 mg/kg body weight to 50 mg/kg body weight, from 0.001 mg/kg body weight to 25 mg/kg body weight, from 0.001 mg/kg body weight to 10 mg/kg body weight, from 0.001 mg/kg body weight to 5 mg/kg body weight, from 0.001 mg/kg body weight to 1 mg/kg body weight, from 0.001 mg/kg body weight to 0.1 mg/kg body weight, from 0.001 mg/kg body weight to 0.005 mg/kg body weight. Alternatively, in some embodiments the dosage range is from 0.1 g/kg body weight to 5 g/kg body weight, from 0.5 g/kg body weight to 5 g/kg body weight, from 1 g/kg body weight to 5 g/kg body weight, from 1.5 g/kg body weight to 5 g/kg body weight, from 2 g/kg body weight to 5 g/kg body weight, from 2.5 g/kg body weight to 5 g/kg body weight, from 3 g/kg body weight to 5 g/kg body weight, from 3.5 g/kg body weight to 5 g/kg body weight, from 4 g/kg body weight to 5 g/kg body weight, from 4.5 g/kg body weight to 5 g/kg body weight, from 4.8 g/kg body weight to 5 g/kg body weight. In one

embodiment, the dose range is from 5µg/kg body weight to 30µg/kg body weight.

Alternatively, the dose range will be titrated to maintain serum levels between 5µg/mL and 30µg/mL.

[00103] Administration of the doses recited above can be repeated for a limited period of time. In some embodiments, the doses are given once a day, or multiple times a day, for example but not limited to three times a day. In a preferred embodiment, the doses recited above are administered daily for several weeks or months. The duration of treatment depends upon the subject's clinical progress and responsiveness to therapy. Continuous, relatively low maintenance doses are contemplated after an initial higher therapeutic dose.

[00104] A therapeutically effective amount is an amount of an agent that is sufficient to produce a statistically significant, measurable change in neovascular formation, number of blood vessels etc. (see "Efficacy Measurement" below). Such effective amounts can be gauged in clinical trials as well as animal studies for a given fibromodulin activity inhibitor.

[00105] Agents useful in the methods and compositions described herein can be administered topically, intravenously (by bolus or continuous infusion), orally, by inhalation, intraperitoneally, intramuscularly, subcutaneously, intracavity, and can be delivered by peristaltic means, if desired, or by other means known by those skilled in the art. It is preferred that the agents for the methods described herein are administered topically to the eye. For the treatment of tumors, the agent can be administered systemically, or alternatively, can be administered directly to the tumor e.g., by intratumor injection or by injection into the tumor's primary blood supply.

[00106] Therapeutic compositions containing at least one agent can be conventionally administered in a unit dose. The term "unit dose" when used in reference to a therapeutic composition refers to physically discrete units suitable as unitary dosage for the subject, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required physiologically acceptable diluent, i.e., carrier, or vehicle.

[00107] The compositions are administered in a manner compatible with the dosage formulation, and in a therapeutically effective amount. The quantity to be administered and timing depends on the subject to be treated, capacity of the subject's system to utilize the active ingredient, and degree of therapeutic effect desired. An agent can be targeted by means of a targeting moiety, such as e.g., an antibody or targeted liposome technology. In some embodiments, a fibromodulin activity inhibitor can be targeted to tissue- or tumor-specific

targets by using bispecific antibodies, for example produced by chemical linkage of an anti-ligand antibody (Ab) and an Ab directed toward a specific target. To avoid the limitations of chemical conjugates, molecular conjugates of antibodies can be used for production of recombinant bispecific single-chain Abs directing ligands and/or chimeric inhibitors at cell surface molecules. The addition of an antibody to a fibromodulin activity inhibitor permits the agent attached to accumulate additively at the desired target site. Antibody-based or non-antibody-based targeting moieties can be employed to deliver a ligand or the inhibitor to a target site. Preferably, a natural binding agent for an unregulated or disease associated antigen is used for this purpose.

[00108] Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner and are particular to each individual. However, suitable dosage ranges for systemic application are disclosed herein and depend on the route of administration. Suitable regimes for administration are also variable, but are typified by an initial administration followed by repeated doses at one or more intervals by a subsequent injection or other administration. Alternatively, continuous intravenous infusion sufficient to maintain concentrations in the blood in the ranges specified for in vivo therapies are contemplated.

[00109] An agent may be adapted for catheter-based delivery systems including coated balloons, slow-release drug-eluting stents or other drug-eluting formats, microencapsulated PEG liposomes, or nanobeads for delivery using direct mechanical intervention with or without adjunctive techniques such as ultrasound.

[00110] In some embodiments, an inhibitor may be combined with one or more agents such as chemotherapeutic or anti-angiogenic agents, for the treatment of an angiogenesis associated disease.

Pharmaceutical Compositions

[00111] The present invention involves therapeutic compositions useful for practicing the therapeutic methods described herein. Therapeutic compositions contain a physiologically tolerable carrier together with an active agent as described herein, dissolved or dispersed therein as an active ingredient. In a preferred embodiment, the therapeutic composition is not immunogenic when administered to a mammal or human patient for therapeutic purposes. As used herein, the terms "pharmaceutically acceptable", "physiologically tolerable" and grammatical variations thereof, as they refer to compositions, carriers, diluents and reagents, are used interchangeably and represent that the materials are capable of administration to or

upon a mammal without the production of undesirable physiological effects such as nausea, dizziness, gastric upset and the like. A pharmaceutically acceptable carrier will not promote the raising of an immune response to an agent with which it is admixed, unless so desired. The preparation of a pharmacological composition that contains active ingredients dissolved or dispersed therein is well understood in the art and need not be limited based on formulation. Typically such compositions are prepared as injectable either as liquid solutions or suspensions, however, solid forms suitable for solution, or suspensions, in liquid prior to use can also be prepared. The preparation can also be emulsified or presented as a liposome composition. The active ingredient can be mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient and in amounts suitable for use in the therapeutic methods described herein. Specifically contemplated pharmaceutical compositions are active RNAi ingredients in a preparation for delivery as described herein above, or in references cited and incorporated herein in that section. Suitable excipients include, for example, water, saline, dextrose, glycerol, ethanol or the like and combinations thereof. In addition, if desired, the composition can contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents and the like which enhance the effectiveness of the active ingredient. The therapeutic composition of the present invention can include pharmaceutically acceptable salts of the components therein. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the polypeptide) that are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, tartaric, mandelic and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine and the like. Physiologically tolerable carriers are well known in the art. Exemplary liquid carriers are sterile aqueous solutions that contain no materials in addition to the active ingredients and water, or contain a buffer such as sodium phosphate at physiological pH value, physiological saline or both, such as phosphate-buffered saline. Still further, aqueous carriers can contain more than one buffer salt, as well as salts such as sodium and potassium chlorides, dextrose, polyethylene glycol and other solutes. Liquid compositions can also contain liquid phases in addition to and to the exclusion of water. Exemplary of such additional liquid phases are glycerin, vegetable oils such as cottonseed oil, and water-oil emulsions. The amount of an active agent used in the methods described herein that will be

effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques.

Efficacy measurement

[00112] The efficacy of a given treatment for an angiogenesis-associated disease can be determined by the skilled clinician. However, a treatment is considered “effective treatment,” as the term is used herein, if any one or all of the signs or symptoms of, as but one example, ocular neovascular disease are altered in a beneficial manner, other clinically accepted symptoms or markers of disease are improved, or even ameliorated, e.g., by at least 10% following treatment with a fibromodulin inhibitor. Efficacy can also be measured by a failure of an individual to worsen as assessed by hospitalization or need for medical interventions (i.e., progression of the disease is halted or at least slowed). Methods of measuring these indicators are known to those of skill in the art and/or described herein. Treatment includes any treatment of a disease in an individual or an animal (some non-limiting examples include a human, or a mammal) and includes: (1) inhibiting the disease, e.g., arresting, or slowing the pathogenic growth of new blood vessels; or (2) relieving the disease, e.g., causing regression of symptoms, reducing the number of new blood vessels in a tissue exhibiting pathology involving angiogenesis (eg., the eye); and (3) preventing or reducing the likelihood of the development of a neovascular disease, e.g., an ocular neovascular disease).

[00113] An effective amount for the treatment of a disease means that amount which, when administered to a mammal in need thereof, is sufficient to result in effective treatment as that term is defined herein, for that disease. Efficacy of an agent can be determined by assessing physical indicators of, for example ocular neovascular disease, such as e.g., visual problems, new blood vessel invasion, rate of vessel growth, angiogenesis, etc.

[00114] For treatment of a subject with a fibromodulin polypeptide (or other fibromodulin activity activator), the in vivo efficacy of an agent can be measured by e.g., rate of wound healing, size of scar, increased fertility, as well as by assessing an increase in various markers of angiogenesis as described herein.

[00115] It is understood that the foregoing detailed description and the following examples are illustrative only and are not to be taken as limitations upon the scope of the invention. Various changes and modifications to the disclosed embodiments, which will be apparent to those of skill in the art, may be made without departing from the spirit and scope of the present invention. Further, all patents, patent applications, and publications identified are

expressly incorporated herein by reference for the purpose of describing and disclosing, for example, the methodologies described in such publications that might be used in connection with the present invention. These publications are provided solely for their disclosure prior to the filing date of the present application. Nothing in this regard should be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior invention or for any other reason. All statements as to the date or representation as to the contents of these documents are based on the information available to the applicants and do not constitute any admission as to the correctness of the dates or contents of these documents.

The present invention may be as defined in any one of the following numbered paragraphs.

1. A fibromodulin activity inhibitor for use in the treatment of an angiogenesis-related disease.
2. The use of paragraph 1, wherein the fibromodulin activity inhibitor is selected from the group consisting of an antibody, an RNA interference molecule, a small molecule, a peptide and an aptamer.
3. The use of paragraph 2, wherein the fibromodulin activity inhibitor comprises an antibody.
4. The use of paragraph 1, wherein the angiogenesis-related disease is age-related macular degeneration.
5. Use of a fibromodulin activity inhibitor in the manufacture of a medicament for the treatment of an angiogenesis-related disease.
6. The use of paragraph 5, wherein the fibromodulin activity inhibitor is selected from the group consisting of an antibody, an RNA interference molecule, a small molecule, a peptide and an aptamer.
7. The use of paragraph 6, wherein the fibromodulin activity inhibitor comprises an antibody.
8. The use of paragraph 5, wherein the angiogenesis-related disease is age-related macular degeneration.
9. Use of a fibromodulin polypeptide or fragment thereof in the manufacture of a medicament for the treatment of a disorder of impaired angiogenesis.
10. The use of paragraph 9, wherein the disorder is impaired response to wound healing.
11. The use of paragraph 9, wherein the disorder is impaired fertility.

12. Use of a fibromodulin polypeptide or fragment thereof in the manufacture of a medicament for the treatment of a wound.
13. A method for inhibiting endothelial cell proliferation and/or migration, the method comprising contacting an endothelial cell with a fibromodulin activity inhibitor.
14. The method of paragraph 13, wherein the fibromodulin activity inhibitor is selected from the group consisting of an antibody, an RNA interference molecule, a small molecule, a peptide and an aptamer.
15. The method of paragraph 13, wherein the fibromodulin activity inhibitor comprises an antibody.
16. The method of paragraph 13, wherein the cell is selected from the group consisting of a primary cell or a cell of a cell line.
17. The method of paragraph 13, wherein the cell is human.
18. A method of treating an angiogenesis-related disease, the method comprising administering a therapeutically effective amount of a fibromodulin activity inhibitor to a mammal having an angiogenesis-related disease.
19. The method of paragraph 18, wherein the angiogenesis-related disease is age-related macular degeneration.
20. The method of paragraph 18, wherein the fibromodulin activity inhibitor is selected from the group consisting of an antibody, an RNA interference molecule, a small molecule, a peptide and an aptamer.
21. The method of paragraph 18, wherein the fibromodulin activity inhibitor comprises an antibody.
22. The method of paragraph 18, wherein the mammal is a human.
23. A method for inhibiting angiogenesis, the method comprising administering a therapeutically effective amount of a fibromodulin activity inhibitor to a mammal in need thereof.
24. The method of paragraph 23, wherein the fibromodulin activity inhibitor is selected from the group consisting of an antibody, an RNA interference molecule, a small molecule, a peptide and an aptamer.
25. The method of paragraph 23, wherein the fibromodulin activity inhibitor comprises an antibody.
26. The method of paragraph 23, wherein the mammal is a human.

27. A method for inhibiting fibromodulin activity in a mammal, the method comprising administering a therapeutically effective amount of a fibromodulin activity inhibitor to a mammal in need thereof.
28. The method of paragraph 27, wherein the fibromodulin activity inhibitor is selected from the group consisting of an antibody, an RNA interference molecule, a small molecule, a peptide and an aptamer.
29. The method of paragraph 28, wherein the fibromodulin activity inhibitor comprises an antibody.
30. The method of paragraph 27, wherein the mammal is a human.
31. A method for promoting endothelial cell proliferation and/or migration, the method comprising: contacting an endothelial cell with an agent that activates fibromodulin activity.
32. The method of paragraph 31, wherein the agent is a fibromodulin polypeptide, or a fragment thereof.
33. The method of paragraph 31, wherein the cell is selected from the group consisting of a primary cell or a cell of a cell line.
34. The method of paragraph 31, wherein the cell is human.
35. A method for promoting angiogenesis in a subject, the method comprising: administering a therapeutically effective amount of an agent that activates fibromodulin activity to a subject in need thereof.
36. The method of paragraph 35, wherein the agent is a fibromodulin polypeptide, or a fragment thereof.
37. The method of paragraph 35, wherein the subject is a mammal.
38. The method of paragraph 37, wherein the mammal is a human.
39. A method for promoting wound healing in a subject, the method comprising: administering an agent that activates fibromodulin activity.
40. The method of paragraph 39, wherein the agent is a fibromodulin polypeptide, or a fragment thereof.
41. The method of paragraph 39, wherein the subject is a mammal.
42. The method of paragraph 41, wherein the mammal is a human.

EXAMPLES

[00116] Fibromodulin is a member of a family of small interstitial proteoglycans with leucine-rich repeats. The inventors have discovered a novel property of fibromodulin that

relates to the proliferation and migration of vascular endothelial cells. Promoting vascular growth is useful for the treatment of disorders with insufficient angiogenesis such as wound healing. Further the inventors have found that inhibitors of fibromodulin, including antibodies to fibromodulin can significantly inhibit endothelial cell proliferation and can thus serve as treatments to inhibit angiogenesis dependent diseases such as macular degeneration and cancer, among others.

[00117] The term "pathological angiogenesis" refers to the excessive formation and growth of blood vessels during the maintenance and the progression of several disease states. Examples where pathological angiogenesis can occur are found in ocular disorders, such as age related macular degeneration and diabetic retinopathy, as well as in many other disorders such as cancer and arthritis.

[00118] The role of FMOD in vasculogenesis in the developing embryo and postnatal angiogenesis has not yet been identified in literature. Through detailed in vitro and in vivo studies of fibromodulin, the inventors have found that FMOD plays a key role in pathological angiogenesis and that inhibitors of FMOD can be used to prevent blood vessel formation.

EXAMPLE 1: PRO-ANGIOGENIC EFFECTS OF FIBROMODULIN

FMOD affects migration of endothelial cells.

[00119] Cell migration is a fundamental function of normal cellular processes, including embryonic development, angiogenesis and wound healing. A standard migration assay was used to measure the migration of cells through a membrane. The cells which migrate through the membrane are dissociated from the membrane and counted using the CyQuant GR dye (molecular probe). This fluorescent dye binds to nucleic acids and gives an increasing signal with increasing cell number.

FMOD affects endothelial cell proliferation.

[00120] Cell proliferation was assessed by staining proliferating cells with an assay based on the cleavage of the tetrazolium salt WST-1 to formazan by cellular mitochondrial dehydrogenases. Expansion in the number of viable cells results in an increase in the overall activity of the mitochondrial dehydrogenases in the sample. As shown in Figure 1, human microvessel endothelial cells, dermal (HMVEC-d) cell proliferation is significantly increased by incubation with recombinant FMOD ($p < 0.001$).

Fibromodulin increases endothelial cell migration *in vivo* as tested in the Matrigel assay.

[00121] Angiogenesis was studied *in vivo* in a matrigel assay performed as described. Two groups of 8-week-old C57BL/6 mice were injected subcutaneously with matrigel containing 6.5pmol FMOD, or 6.5pmol GST as control. On day 6, animals were sacrificed and fluorescence-activated cell sorting (FACS) analysis was used for determination of the matrigel liberated cells. In order to distinguish the endothelial from hematopoietic cells the inventors used the antibodies CD31-PE, and CD45-APC respectively.

Model of Dermatological Wound Healing.

[00122] Vascular remodeling was studied in a model of skin wound healing, since it is dependent on neovascularization. The size of the wounds was standardized through the use of a biopsy punch which creates a circular wound measuring 1 mm. A full-thickness skin incision was made on the dorsal aspect of the mouse ear. Wound healing was observed and imaging analyzed throughout the entire healing process by daily measuring the width and the length of the wound. New blood vessel formation was analyzed *in vivo* and on skin sections harvested five days after wounding.

[00123] The ears of mice were treated daily with either matrigel, or matrigel containing GST or FMOD (8nM) respectively, over the course of five days. In order to evaluate the wound neovascularization, mice were anesthetized and injected with dextran-FITC to label specifically the endothelial cells. The ear/wound was observed and as shown in Figure 3, the angiogenesis around the circular wound among the treated mice was visualized with intravenous dextran-FITC.

Fibromodulin injected into the vitreous dramatically increases *in vivo* angiogenesis in a mouse model of Choroidal neovascularization (CNV).

[00124] To evaluate how Fibromodulin modulates the angiogenic process in the eye, a choroidal neovascularization assay was performed. CNV is an abnormal vessel growth from the choriocapillaris through Bruch's membrane resulting in hemorrhage, scarring, exudation, and/or retinal detachment, with the ultimate consequence of a severe loss of high-acuity central vision. It is the leading cause of blindness seen in age-related macular degeneration. Briefly, for the assay, 6-8 week-old C57BL/6J mice were anesthetized followed by 1% Tropicamide for pupillary dilation. Three burns of 532-nm diode laser photocoagulation (50- μ m spot size, 0.1-s duration, 200 mW) were performed. Experiments were performed to investigate the effect of intravitreal injections of GST (5.25nM) and FMOD (5.25nM) on

laser-induced CNV. Animals were injected immediately after laser injury into the vitreous. One week later, mice were anesthetized and perfused with fluorescein-labeled dextran (1×10^6 average molecular weight) (Sigma–Aldrich, St. Louis, MO). Eyes were enucleated and labeled with anti Lectin and or anti-CD31PE (invitrogen). Choroidal flatmounts were prepared, and the CNV area was measured as described previously.

[00125] Five mice were used for each group with three burns in each eye ($n = 15\text{--}18$ successful burns in each group). For quantitative analysis of lesion intensity and size, CNV images were batch-processed by using imageJ. Perimeter, area, and mean diameter (pixels) were calculated for each region of interest and exported to Excel (Microsoft, Redmond, WA). Analyses were performed by a blind observer to eliminate user bias.

[00126] These data indicate that a significant effect of fibromodulin on CNV was observed (Figure 4).

EXAMPLE 2

siRNA-FMOD reduces migration of endothelial cells.

[00127] A standard migration assay, which measures the migration of cells through a membrane, was used to determine the effect of fibromodulin inhibition in cells. The cells which migrate through the membrane are dissociated from the membrane and counted using the CyQuant GR dye (molecular probe). This fluorescent dye binds to nucleic acids and gives an increasing signal with increasing cell number. Figure 5 shows that siRNA-fibromodulin reduces migration of human microvessel endothelial cell migration.

Anti-Fibromodulin decrease s endothelial cell migration in vivo as tested in the Matrigel assay

[00128] Angiogenesis was studied in vivo in a matrigel assay performed as described. Three groups of 8-week-old C57BL/6 mice were injected subcutaneously with non pigment melanocytes cell mixed with matrigel containing anti FMOD antibody, or IgG-Goat or only cells. On day 6, animals were sacrificed and fluorescence-activated cell sorting (FACS) analysis was used to determine the number of matrigel liberated cells. In order to distinguish the endothelial from hematopoietic cells the following antibodies were used: CD31-PE (endothelial cell marker), and CD45-APC (hematopoietic cell marker). As shown in Figure 6, 2.25 times more endothelial cells (0.09% versus 0.04%) (upper left panel) infiltrated the matrigel plugs compared to when only cells or cells with IgG were included. This observation

confirms the hypothesis that FMOD is a potent angiogenic factor which has a synergistic effect on endothelial cell migration.

EXAMPLE 3

Corneal micropocket assay

[00129] To further evaluate the role of fibromodulin as an angiogenic regulator, a corneal micropocket assay was performed. Results are shown in Figure 7 and indicate that fibromodulin promotes angiogenesis in the cornea.

Retinal neovascularization

[00130] Retinal neovascularization was measured in 4-day old pups, injected with either recombinant fibromodulin or a GST control. The peripheral avascular area was expressed as a percentage of the total retinal area. Results are shown in Figure 8, indicating that pups treated with fibromodulin have a lower percent avascular area and an increased percent neovascularization compared to pups not treated with fibromodulin. These results indicate that fibromodulin promotes angiogenesis and formation of new blood vessels in the retina.

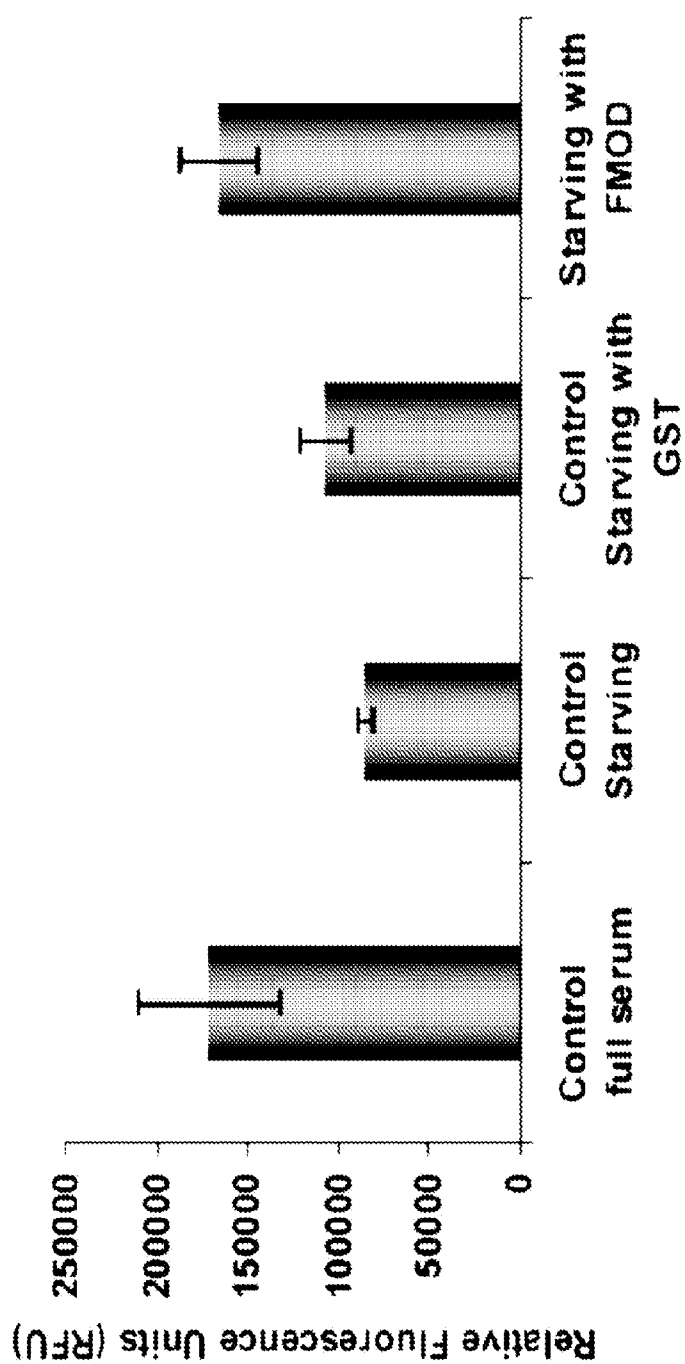
CLAIMS

1. A fibromodulin activity inhibitor for use in the treatment of an angiogenesis-related disease.
2. The use of claim 1, wherein said fibromodulin activity inhibitor is selected from the group consisting of an antibody, an RNA interference molecule, a small molecule, a peptide and an aptamer.
3. The use of claim 2, wherein said fibromodulin activity inhibitor comprises an antibody.
4. The use of claim 1, wherein said angiogenesis-related disease is age-related macular degeneration.
5. Use of a fibromodulin activity inhibitor in the manufacture of a medicament for the treatment of an angiogenesis-related disease.
6. The use of claim 5, wherein said fibromodulin activity inhibitor is selected from the group consisting of an antibody, an RNA interference molecule, a small molecule, a peptide and an aptamer.
7. The use of claim 6, wherein said fibromodulin activity inhibitor comprises an antibody.
8. The use of claim 5, wherein said angiogenesis-related disease is age-related macular degeneration.
9. Use of a fibromodulin polypeptide or fragment thereof in the manufacture of a medicament for the treatment of a disorder of impaired angiogenesis.
10. The use of claim 9, wherein said disorder is impaired response to wound healing.
11. The use of claim 9, wherein said disorder is impaired fertility.
12. Use of a fibromodulin polypeptide or fragment thereof in the manufacture of a medicament for the treatment of a wound.
13. A method for inhibiting endothelial cell proliferation and/or migration, the method comprising contacting an endothelial cell with a fibromodulin activity inhibitor.
14. The method of claim 13, wherein said fibromodulin activity inhibitor is selected from the group consisting of an antibody, an RNA interference molecule, a small molecule, a peptide and an aptamer.
15. The method of claim 13, wherein said fibromodulin activity inhibitor comprises an antibody.

16. The method of claim 13, wherein said cell is selected from the group consisting of a primary cell or a cell of a cell line.
17. The method of claim 13, wherein said cell is human.
18. A method of treating an angiogenesis-related disease, the method comprising administering a therapeutically effective amount of a fibromodulin activity inhibitor to a mammal having an angiogenesis-related disease.
19. The method of claim 18, wherein said angiogenesis-related disease is age-related macular degeneration.
20. The method of claim 18, wherein said fibromodulin activity inhibitor is selected from the group consisting of an antibody, an RNA interference molecule, a small molecule, a peptide and an aptamer.
21. The method of claim 18, wherein said fibromodulin activity inhibitor comprises an antibody.
22. The method of claim 18, wherein said mammal is a human.
23. A method for inhibiting angiogenesis, the method comprising administering a therapeutically effective amount of a fibromodulin activity inhibitor to a mammal in need thereof.
24. The method of claim 23, wherein said fibromodulin activity inhibitor is selected from the group consisting of an antibody, an RNA interference molecule, a small molecule, a peptide and an aptamer.
25. The method of claim 23, wherein said fibromodulin activity inhibitor comprises an antibody.
26. The method of claim 23, wherein said mammal is a human.
27. A method for inhibiting fibromodulin activity in a mammal, the method comprising administering a therapeutically effective amount of a fibromodulin activity inhibitor to a mammal in need thereof.
28. The method of claim 27, wherein said fibromodulin activity inhibitor is selected from the group consisting of an antibody, an RNA interference molecule, a small molecule, a peptide and an aptamer.
29. The method of claim 28, wherein said fibromodulin activity inhibitor comprises an antibody.
30. The method of claim 27, wherein said mammal is a human.
31. A method for promoting endothelial cell proliferation and/or migration, the method comprising: contacting an endothelial cell with an agent that activates fibromodulin activity.

32. The method of claim 31, wherein said agent is a fibromodulin polypeptide, or a fragment thereof.
33. The method of claim 31, wherein said cell is selected from the group consisting of a primary cell or a cell of a cell line.
34. The method of claim 31, wherein said cell is human.
35. A method for promoting angiogenesis in a subject, the method comprising:
administering a therapeutically effective amount of an agent that activates fibromodulin activity to a subject in need thereof.
36. The method of claim 35, wherein said agent is a fibromodulin polypeptide, or a fragment thereof.
37. The method of claim 35, wherein said subject is a mammal.
38. The method of claim 37, wherein said mammal is a human.
39. A method for promoting wound healing in a subject, the method comprising:
administering an agent that activates fibromodulin activity.
40. The method of claim 39, wherein said agent is a fibromodulin polypeptide, or a fragment thereof.
41. The method of claim 39, wherein said subject is a mammal.
42. The method of claim 41, wherein said mammal is a human.

1/10

**FIG. 1**

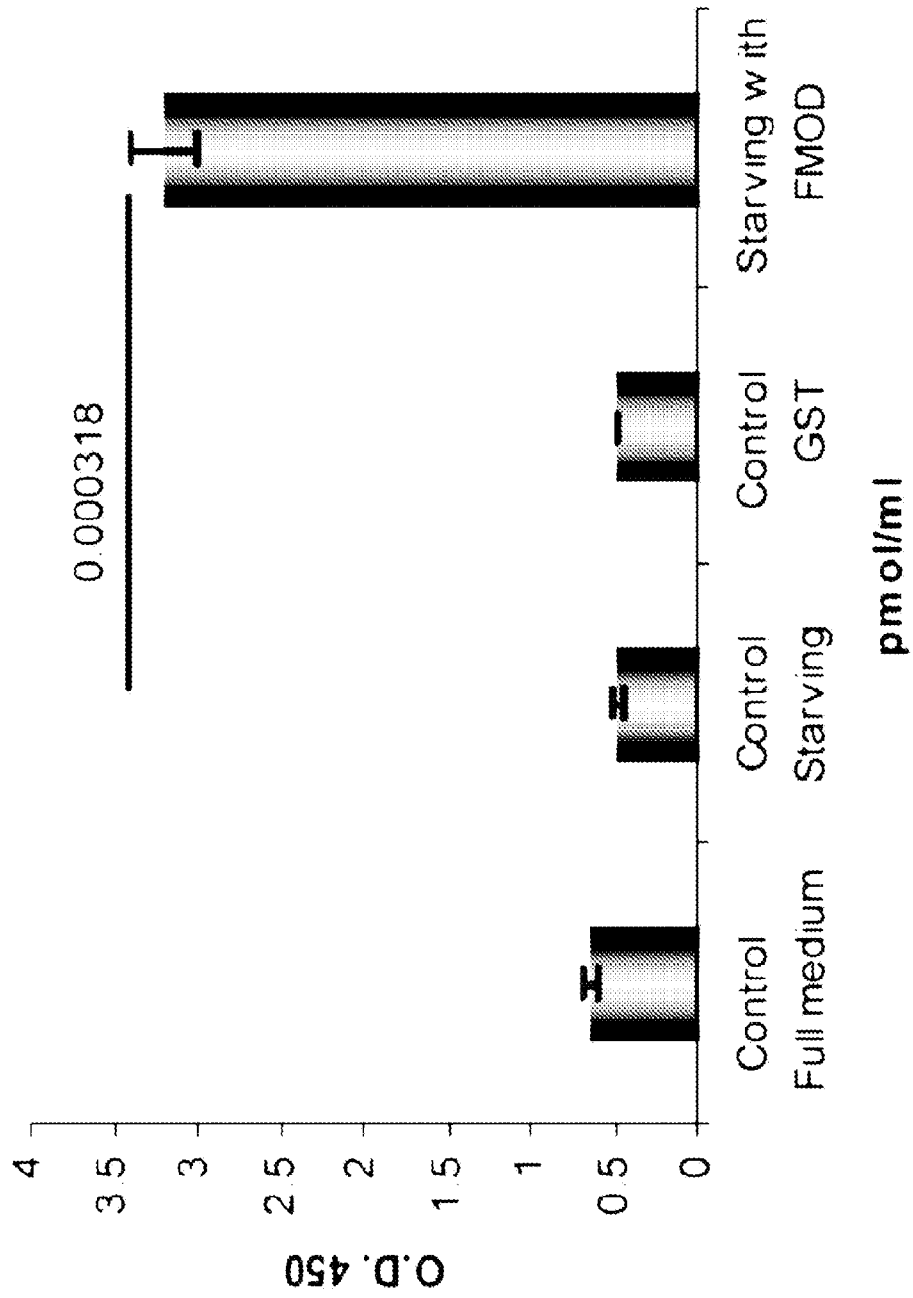


FIG. 2

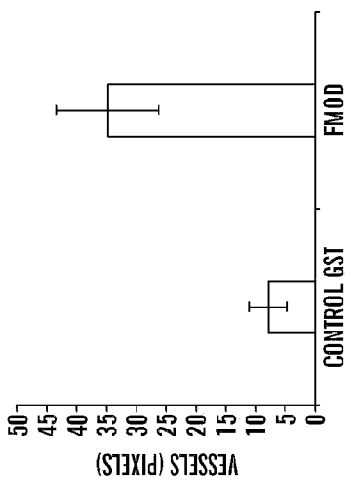


FIG. 3B

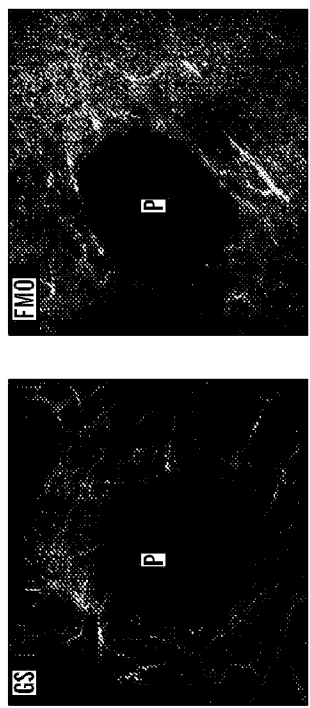


FIG. 3A

4/10

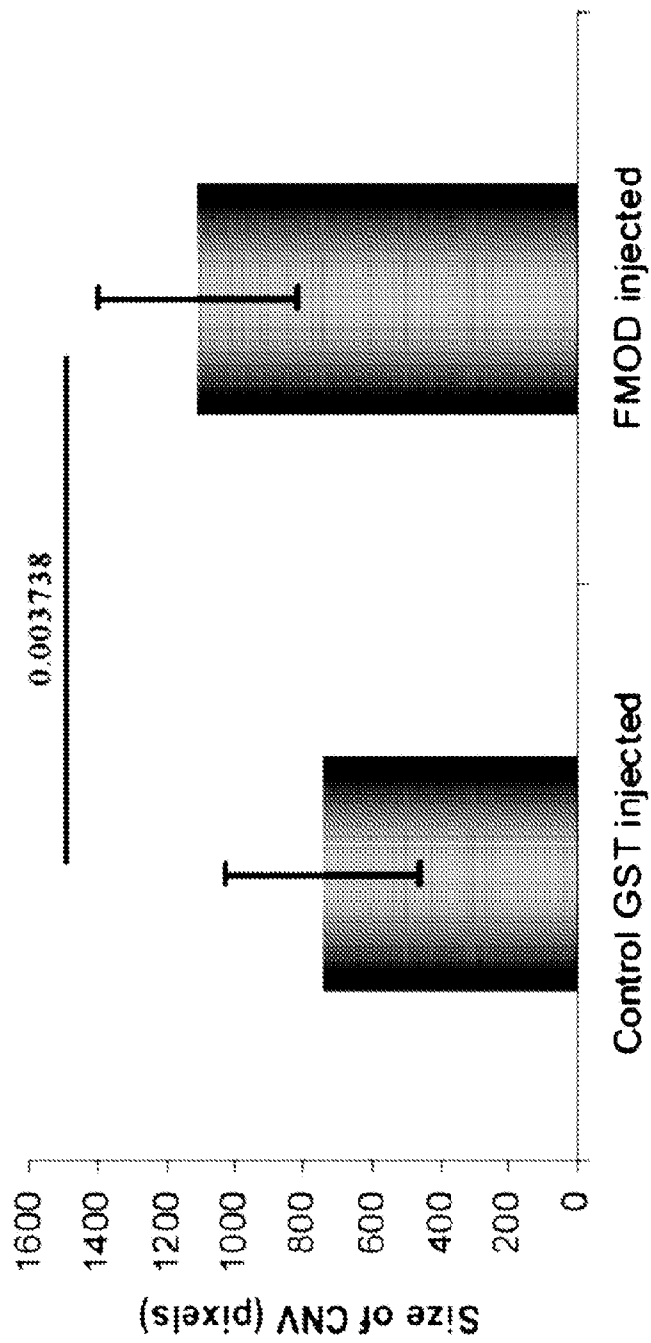


FIG. 4

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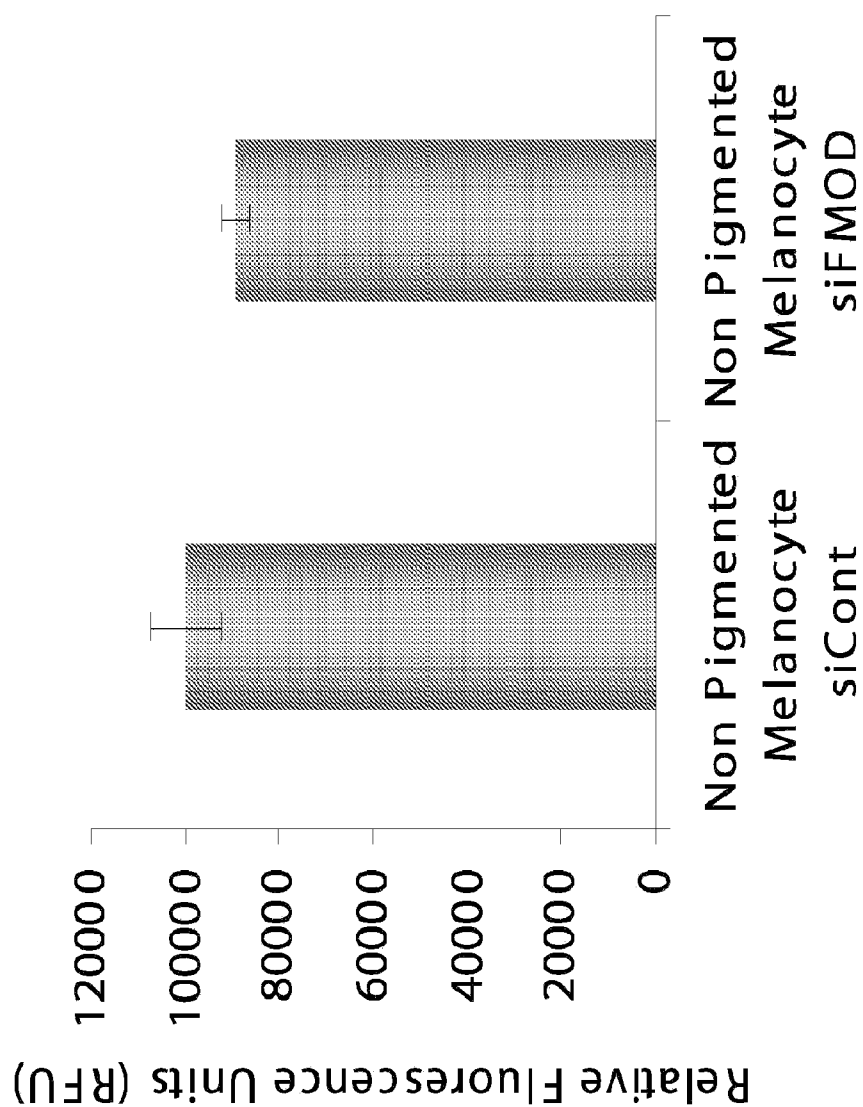


FIG. 5

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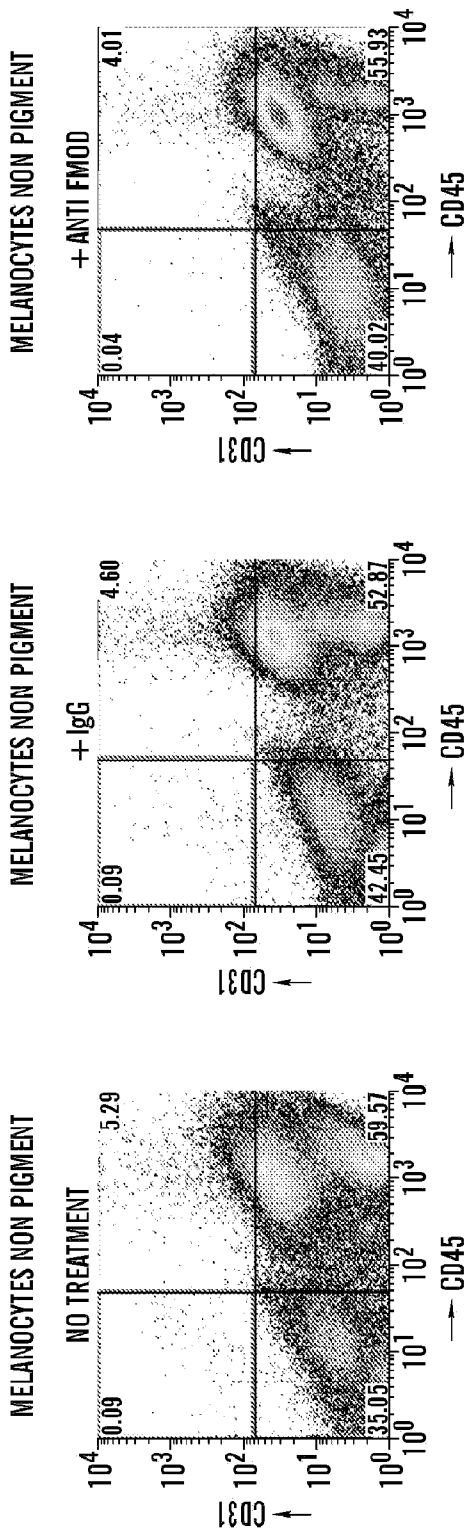


FIG. 6C

FIG. 6B

FIG. 6A

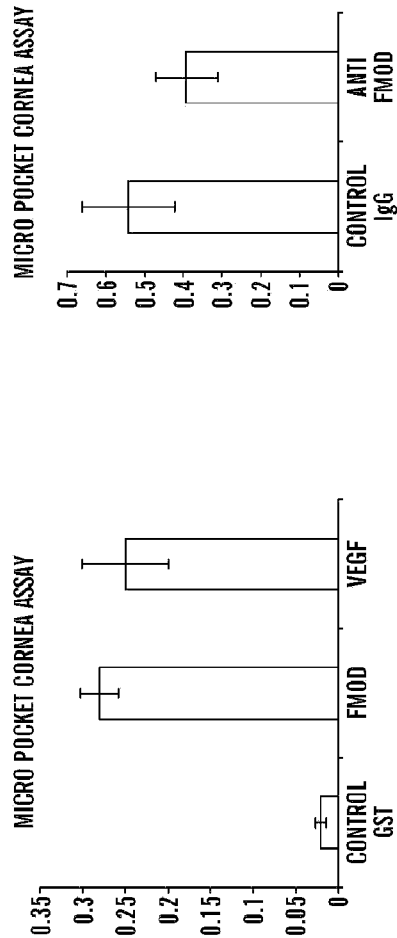
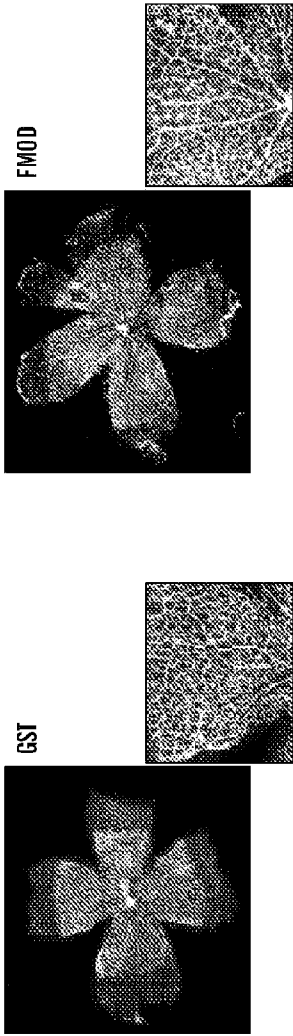


FIG. 7B

FIG. 7A

8/10



RETINA PUPS 5-7 DAY

FIG. 8A

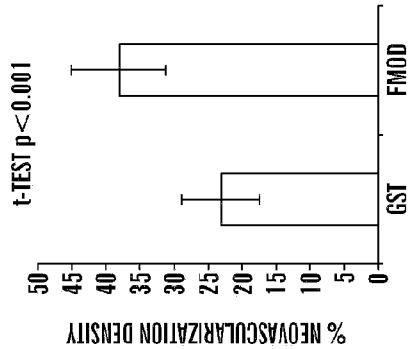


FIG. 8C

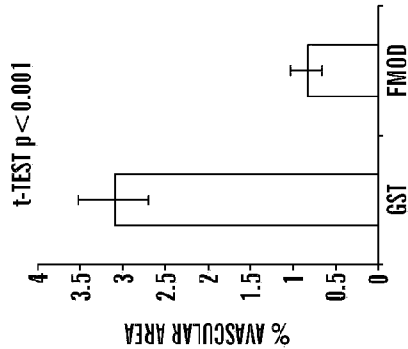


FIG. 8B

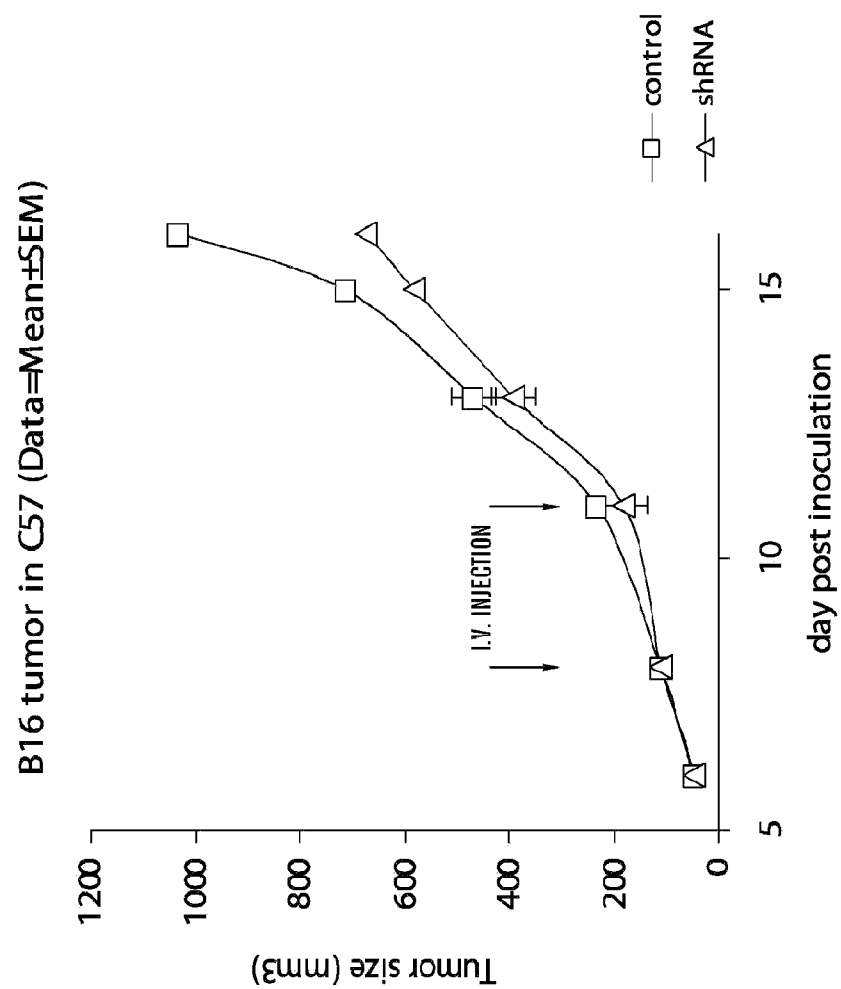


FIG. 9