

hemorrhage or lead to scarring that may end in retinal detachment. Current treatments for proliferative retinopathies seek to destroy the existing pathological vessels but do not address the underlying ischemia that drives their growth. Currently, standard treatment for proliferative retinopathy involves destruction of a portion of the retina with a laser in an attempt to stop new vessel growth and preserve central vision. These treatments are however to some extent inefficient. While some patients may maintain a stable vision for many years, a high percentage of patients suffering from retinopathy eventually suffers from total visual loss.

[0004] Retinopathies may also be characterized by an increased retinal vascular leakage resulting in macular edema. Currently, patients suffering from diabetic macular edema are treated with compounds targeting vascular endothelial growth factor A (VEGF-A), a growth factor that is driving both angiogenesis and vascular permeability. This therapeutic strategy may prove insufficient for treating patients who suffers from both macular ischemia and macular edema.

[0005] There is thus still a need for a therapeutic approach to treat patients who could benefit from the pro-angiogenic properties of VEGF-A, in particular in patients suffering from both macular ischemia and macular edema. Consequently, there is still an unfulfilled need for new therapeutic approaches for efficiently treating eye or retinal diseases.

SUMMARY OF THE INVENTION

[0006] Neuropilins (NRPs) are transmembrane glycoprotein receptors that play an important role in the development of the neuronal and vascular systems as receptors for members of the class-3 semaphorin family (SEMA3) of axonal guidance factors and members of the vascular endothelial growth factor (VEGF) family of angiogenesis factors. Two neuropilin proteins, neuropilin-1 (Nrp-1) and neuropilin-2 (Nrp-2) have been identified. Their extracellular region contains three domains: two CUB homology domains (A-domain of Nrp1, also referred herein as "Nrp1A") as Sema3 ligand-binding domain, two coagulation factor V/VIII homology domains (B-domain of Nrp1, also

referred herein as “Nrp1B”) as VEGF binding domain, and a MAM domain (c) involved in Nrp-1 dimerization. Nrp-1 can bind VEGF-A165, VEGF-B, VEGF-E, PlGF, Sema3A, Sema3B and Sema3C, whereas Nrp2 binds VEGF-A165, VEGF-A145, VEGF-C, VEGF-D, SEMA3B, Sema3C, Sema3F and Sema3G. The binding site for VEGF ligands has been localized on the B-domain of Nrp1 whereas the binding of semaphorins has been localized on the A-domain of Nrp1.

[0007] The sequence of human Nrp1 is available online, the Nrp1 isoform A precursor is depicted in SEQ ID NO: 26 and is available under the Reference protein sequence NP_003864. Nrp1 has been studied in tumor angiogenesis and metastasis for years, but its effects on retinal revascularization and neovascularization are not fully understood. The inventors have shown here that targeting Nrp1, especially the A-domain of Nrp1, is a highly effective strategy for treating eye and retinal diseases.

[0008] In a **first-one aspect**, the present **inventiondisclosure** provides an anti-Nrp1A antibody or an antigen-binding fragment thereof comprising:

- a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 1 (H-CDR1); the amino acid sequence of SEQ ID NO: 2 (H-CDR2); and the amino acid sequence of SEQ ID NO: 3 (H-CDR3); and
- a light chain variable region comprising the amino acid sequence of SEQ ID NO: 4 (L-CDR1); the amino acid sequence of SEQ ID NO: 5 (L-CDR2); and the amino acid sequence of SEQ ID NO: 6 (L-CDR3).

[0009] In one **embodimentexample**, the present **inventiondisclosure** provides an anti-Nrp1A antibody or an antigen-binding fragment thereof comprising:

- a heavy chain variable region comprising an amino acid sequence at least 80%, at least 90%, at least 95%, at least 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16 or SEQ ID NO: 17; and

- a light chain variable region comprising an amino acid sequence at least 80%, at least 90%, at least 95%, at least 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO: 11.

[0010] In one embodimentexample, the present inventiondisclosure provides an anti-Nrp1A antibody or an antigen-binding fragment thereof comprising:

- a heavy chain variable region comprising an amino acid sequence at least 80%, at least 90%, at least 95%, at least 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16 or SEQ ID NO: 17; and
- a light chain variable region comprising an amino acid sequence at least 80%, at least 90%, at least 95%, at least 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO: 11;

wherein:

- the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 1 (H-CDR1), the amino acid sequence of SEQ ID NO: 2 (H-CDR2), and the amino acid sequence of SEQ ID NO: 3 (H-CDR3); and
- the light chain variable region comprising the amino acid sequence of SEQ ID NO: 4 (L-CDR1), the amino acid sequence of SEQ ID NO: 5 (L-CDR2), and the amino acid sequence of SEQ ID NO: 6 (L-CDR3).

[0011] In yet another embodimentexample, the present inventiondisclosure provides an anti-Nrp1A antibody or an antigen-binding fragment thereof comprising:

- a heavy chain variable region comprising the amino acid sequences of SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16 or SEQ ID NO: 17; and

- a light chain variable region comprising the amino acid sequence of SEQ ID NO: 11.

[0012] In another embodimentexample, the present inventiondisclosure provides an anti-Nrp1A antibody or an antigen-binding fragment thereof comprising:

- a. a variable heavy chain and a variable light chain comprising the amino acid sequences of SEQ ID NO: 10 and SEQ ID NO: 11, respectively;
- b. a variable heavy chain and a variable light chain comprising the amino acid sequences of SEQ ID NO: 12 and SEQ ID NO: 11, respectively;
- c. a variable heavy chain and a variable light chain comprising the amino acid sequences of SEQ ID NO: 13 and SEQ ID NO: 11, respectively;
- d. a variable heavy chain and a variable light chain comprising the amino acid sequences of SEQ ID NO: 14 and SEQ ID NO: 11, respectively;
- e. a variable heavy chain and a variable light chain comprising the amino acid sequences of SEQ ID NO: 15 and SEQ ID NO: 11, respectively;
- f. a variable heavy chain and a variable light chain comprising the amino acid sequences of SEQ ID NO: 16 and SEQ ID NO: 11, respectively; or
- g. a variable heavy chain and a variable light chain comprising the amino acid sequences of SEQ ID NO: 17 and SEQ ID NO: 11, respectively.

[0013] In yet another embodimentexample, the present inventiondisclosure provides an anti-Nrp1A antibody or an antigen-binding fragment thereof comprising:

- a heavy chain comprising, preferably consisting of, the amino acid sequence of SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24 or SEQ ID NO: 25; and

- a light chain comprising, preferably consisting of, the amino acid sequence of SEQ ID NO: 19.

[0014] In a particular ~~embodiment~~example, the ~~invention~~disclosure relates to an anti-Nrp1A antibody or an antigen-binding fragment thereof comprising:

- a. a heavy chain comprising the amino acid sequence of SEQ ID NO: 18 and a light chain comprising the amino acid sequence of SEQ ID NO: 19;
- b. a heavy chain comprising the amino acid sequence of SEQ ID NO: 20 and a light chain comprising the amino acid sequence of SEQ ID NO: 19;
- c. a heavy chain comprising the amino acid sequence of SEQ ID NO: 21 and a light chain comprising the amino acid sequence of SEQ ID NO: 19;
- d. a heavy chain comprising the amino acid sequence of SEQ ID NO: 22 and a light chain comprising the amino acid sequence of SEQ ID NO: 19;
- e. a heavy chain comprising the amino acid sequence of SEQ ID NO: 23 and a light chain comprising the amino acid sequence of SEQ ID NO: 19;
- f. a heavy chain comprising the amino acid sequence of SEQ ID NO: 24 and a light chain comprising the amino acid sequence of SEQ ID NO: 19; or
- g. a heavy chain comprising the amino acid sequence of SEQ ID NO: 25 and a light chain comprising the amino acid sequence of SEQ ID NO: 19.

[0015] In a particular preferred ~~embodiment~~example, the anti-Nrp1A antibody is a humanized anti-Nrp1A antibody.

[0016] **In a ~~second~~another aspect**, the present ~~invention~~disclosure provides an anti-Nrp1A antibody or an antigen-binding fragment thereof that binds to at least one amino acid residue within amino acid regions 68-77 of the human Nrp1 as ~~depicted~~set forth in SEQ ID NO: 26.

[0017] In one embodimentexample, the present inventiondisclosure provides an anti-Nrp1A antibody or an antigen-binding fragment thereof that binds to at least one amino acid residue within amino acid regions as set forth in SEQ ID NO: 26. In a preferred embodimentexample, the present inventiondisclosure provides an anti-Nrp1A antibody or an antigen-binding fragment thereof that binds the amino acid regions as set forth in SEQ ID NO: 27. In another aspect, the present disclosure provides an anti-Nrp1A antibody or an antigen-binding fragment thereof that binds to the sequence set forth in SEQ ID NO: 27.

[0018] ~~In a third aspect, the~~The present inventiondisclosure provides an anti-Nrp1A antibody or an antigen-binding fragment thereof for use as a medicament. In another aspect, the present disclosure provides an antibody or an antigen-binding fragment as described herein for use as a medicament.

[0019] In one embodimentexample, the present inventiondisclosure provides an anti-Nrp1A or an antigen-binding fragment for inhibiting the vasorepulsive effect of Sema3A, and/or for improving revascularisation of the retina. In a further embodimentexample, the present inventiondisclosure provides an anti-Nrp1A or an antigen-binding fragment for inhibiting the permeability of the blood retinal barrier (BRB) induced by Sema3A and for inhibiting the permeability of the blood retinal barrier induced by VEGF, preferably VEGF-A.

[0020] In yet a further embodimentexample, the present inventiondisclosure provides an anti-Nrp1A or an antigen-binding fragment for:

- redirecting angiogenesis towards ischemic regions, in order to improve revascularisation of the retina;
- preventing pathological neovascularization of the vitreous region;
- preventing the blood retinal barrier breakdown induced by Sema3A; and
- preventing the blood retinal barrier breakdown induced by VEGF-A.

[0021] In one ~~embodiment~~example, the present ~~invention~~disclosure provides an anti-Nrp1A antibody or an antigen-binding fragment thereof for use in the treatment or prevention of a retinal or eye disease. In another aspect, the present disclosure provides an antibody or an antigen-binding fragment as described herein for use in treating or preventing an eye or a retinal disease. In another aspect, the present disclosure provides use of an antibody or an antigen-binding fragment as described herein in the manufacture of a medicament for treating or preventing an eye or a retinal disease.

[0022] In another ~~embodiment~~example, the present ~~invention~~disclosure relates to a method for treating one or more retinal or eye diseases, comprising administering a

pharmaceutically effective amount of an antibody or an antigen-binding fragment according to a patient in need thereof.

[0023] ~~In a fourth aspect, the~~The present ~~invention~~disclosure provides an anti-Nrp1A antibody or an antigen-binding fragment thereof for use in the treatment or prevention of a disease selected from the group consisting of retinopathy, proliferative retinopathy (PR) such as retinopathy of prematurity, ischemic retinopathy, diabetic retinopathy (DR) including proliferative diabetic retinopathy (PDR) and non-proliferative diabetic retinopathy, diabetic macular edema (DME), diabetic macular ischemia (DMI), age-related macular degeneration, retinitis pigmentosa, inherited retinal dystrophy, myopic degeneration, retinal vein occlusions, retinal artery occlusions, endophthalmitis, uveitis, cystoid macular edema, choroidal neovascular membrane secondary to any retinal diseases, optic neuropathies, glaucoma, retinal detachment, toxic retinopathy, radiation retinopathy, traumatic retinopathy, drug-induced retinal vasculopathy, retinal neovascularisation, polypoidal choroidal vasculopathy, retinal vasculitis, retinal microaneurysm, Fuch's dystrophy, macular telangiectasia, usher syndrome, and Stargardt disease.

[0024] In another ~~embodiment~~example, the present ~~invention~~disclosure provides an anti-Nrp1A antibody or an antigen-binding fragment thereof for use in the treatment or prevention of a disease selected from the group consisting of diabetic retinopathy including proliferative diabetic retinopathy and non-proliferative diabetic retinopathy, ischemic retinopathy, diabetic macular edema, diabetic macular ischemia, age-related macular edema, retinal neovascularization, glaucoma and choroidal neovascularization. Preferably, said disease is diabetic macular edema and/or diabetic macular ischemia.

[0025] In a preferred ~~embodiment~~example, the present ~~invention~~disclosure provides an anti-Nrp1A antibody or an antigen-binding fragment thereof for use in the treatment of diabetic macular ischemia, by promoting vascular regeneration within the ischemic retina (revascularization) and reducing pathological neovascularization of the vitreous region of the eye. In one ~~embodiment~~example, the antibody according to the ~~invention~~disclosure does not inhibit the angiogenesis induced by VEGF, preferably VEGF-A.

[0026] In another preferred ~~embodiment~~example, the present ~~invention~~disclosure provides an anti-Nrp1A antibody or an antigen-binding fragment thereof for use in the treatment of diabetic macular edema, by reducing, preferably preventing, the permeability of blood retinal barrier induced by Sema3A and by reducing, preferably preventing, the permeability of blood retinal barrier induced by VEGF-A.

[0027] ~~In a fifth aspect, the~~The present ~~invention~~disclosure provides a pharmaceutical composition comprising an anti-Nrp1A antibody or an antigen-binding fragment thereof and a pharmaceutically acceptable carrier. In another aspect, the present disclosure provides a pharmaceutical composition comprising an antibody or an antigen-binding fragment as described herein and a pharmaceutically acceptable carrier.

[0028] In one ~~embodiment~~example, the present ~~invention~~disclosure provides an anti-Nrp1A antibody or an antigen-binding fragment thereof or a pharmaceutical composition comprising an anti-Nrp1A antibody or an antigen-binding fragment thereof, wherein said antibody or an antigen-binding fragment thereof is administered by a parenteral route, intravenous route, intravitreal route or subcutaneous route of administration, preferably by intravitreal route.

[0029] ~~In a sixth~~another aspect, the present ~~invention~~disclosure provides an isolated polynucleotide or polynucleotides comprising:

- a sequence encoding a heavy chain as shown in SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24 or SEQ ID NO: 25 or a heavy chain variable region as shown in SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16 or SEQ ID NO: 17; and
- a sequence encoding a light chain as shown in SEQ ID NO: 19 or a light chain variable region as shown in SEQ ID NO: 11.

[0030] In one embodimentexample, the present inventiondisclosure provides an expression vector comprising an isolated polynucleotide or polynucleotides comprising a sequence encoding a heavy chain as shown in SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24 or SEQ ID NO: 25 or a heavy chain

variable region as shown in SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16 or SEQ ID NO: 17; and a sequence encoding a light chain as shown in SEQ ID NO: 19 or a light chain variable region as shown in SEQ ID NO: 11. In another aspect, the present disclosure provides an expression vector comprising the isolated polynucleotide or polynucleotides as described herein.

[0031] In one ~~embodiment~~example, the present ~~invention~~disclosure provides a viral vector comprising an isolated polynucleotide or polynucleotides comprising a sequence encoding a heavy chain as shown in SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24 or SEQ ID NO: 25 or a heavy chain variable region as shown in SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16 or SEQ ID NO: 17; and a sequence encoding a light chain as shown in SEQ ID NO: 19 or a light chain variable region as shown in SEQ ID NO: 11.

[0032] In one ~~embodiment~~example, the present ~~invention~~disclosure provides a host cell comprising an expression vector or an isolated polynucleotide or polynucleotides comprising a sequence encoding a heavy chain as shown in SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24 or SEQ ID NO: 25 or a heavy chain variable region as shown in SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16 or SEQ ID NO: 17; and a sequence encoding a light chain as shown in SEQ ID NO: 19 or a light chain variable region as shown in SEQ ID NO: 11. In another aspect, the present disclosure provides a host cell comprising an isolated polynucleotide or polynucleotides as described herein or an expression vector as described herein.

[0033] In one ~~embodiment~~example, the present ~~invention~~disclosure provides a method for producing an anti-Nrp1A antibody or an antigen-binding fragment thereof comprising obtaining a host cell comprising an expression vector or an isolated

polynucleotide or polynucleotides comprising a sequence encoding a heavy chain as shown in SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24 or SEQ ID NO: 25 or a heavy chain variable region as shown in SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16 or SEQ ID NO: 17; and a sequence encoding a light chain as shown in SEQ ID NO: 19 or a light chain variable region as shown in SEQ ID NO: 11; and cultivating the host cell. In another aspect, the present disclosure provides a method for producing an anti-Nrp1A antibody or an antigen-binding fragment thereof comprising:

- a. obtaining a host cell as described herein; and
- b. cultivating the host cell.

[0034] In one embodiment example, the method for producing an anti-Nrp1A antibody or an antigen-binding fragment thereof further comprises recovering and purifying the anti-Nrp1A antibody or an antigen-binding fragment thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

[0035] **FIGURE 1: Localization of Sema3A in human eyes**

[0036] This figure shows the localization of Sema3A in human eyes in prespecified retinal samples from human donors with a history of Diabetic Retinopathy or primary open angle glaucoma (POAG) in comparison to age matched controls (Age ctrl) and subjects with Diabetes, but no ocular pathology (DM ctrl). Sema3A was found in the vasculature wall of retinal blood vessels. Additionally, unidentified but distinctive Sema3A fluorescent objects were observed in the retinal ganglion cell layer.

[0037] **FIGURE 2: Inhibition of VEGF-mediated effects - VEGF-A induced permeability in the retina of Brown Norway rats**

[0038] Retinal Evans Blue fluorescence is used as a read-out for permeability to assess vascular leakage. Intravitreal injection of VEGF-A induces hyperpermeability in the retina. The antibody of the invention binding to the A-domain of Nrp1 inhibits VEGF-A-induced retinal permeability, similar to the VEGF trap (aflibercept). The antibody directed against the Nrp1 ligand semaphorin 3A (Sema3A antibody) does not inhibit VEGF-A-induced permeability in the retina.

[0039] **FIGURE 3: Effect of the antibody of the invention and anti-VEGF treatment on tip cell density, avascular area and pre-retinal tufts in mouse OIR model**

[0040] Tip cell density, avascular area and pre-retinal neovascularization (tufts) were investigated in a model of oxygen-induced retinopathy in mouse pups. Animals were exposed 75% oxygen from P7 to P12 and received a single intravitreal injection of antibody after returning to normoxia on P12. A control antibody is directed against trinitrophenol. On P17, retinal flatmounts were prepared, stained with isolectin B4 and