Raper, 1998). Notably, furin proteases are widely present in tissue, which provides a built-in regulatory mechanism for Semaphorin 3A. Yet, these proteases may also lead to a short-lived activity of Semaphorin 3A.

The binding of Semaphorin 3A to Nrp1 is responsible for Semaphorin 3A-induced entry of macrophages into avascular cancer areas fostering cancer progression (Casazza et al., 2013). Therefore, the high affinity interaction of wild type Semaphorin 3A with Nrp1 limits its exploitability as an effective anti-cancer drug and even potentially favors cancer progression.

The technical problem underlying the present invention is the provision of means and methods for an improved therapy of angiogenic disorders, tumorous diseases and/or cancer.

The technical problem is solved by provision of the embodiments provided herein below and as characterized in the appended claims.

Summary

Disclosed herein is a mutated Semaphorin 3 or a functional fragment thereof wherein said mutated Semaphorin 3 is selected from the group consisting of Semaphorin 3A, Semaphorin 3B, Semaphorin 3C, and Semaphorin 3D and wherein said mutated Semaphorin 3 or said functional fragment thereof comprises an amino acid sequence $CX_1X_2A_3GKD$, wherein X_1 is an amino acid, which is K or N, X_2 is an amino acid selected from the group of W, M and L, and wherein the alanine (A_3) is replaced by a hydrophilic amino acid, wherein the mutated Semaphorin 3 or the functional fragment thereof inhibits angiogenesis and/or functions as vascular normalizing agent.

Also disclosed herein is a fusion protein comprising the mutated Semaphorin 3 or the functional fragment thereof as defined herein that functions as an inhibitor of angiogenesis and/or as vascular normalizing agent.

Also disclosed herein is an antibody specifically binding to the mutated Semaphorin 3 or the functional fragment thereof as defined herein or to the fusion protein as defined herein, wherein said antibody specifically binds to an epitope comprising a hydrophilic amino acid in

place of the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2; a hydrophilic amino acid in place of the alanine corresponding to position 105 of the wild type Semaphorin 3B as shown in SEQ ID NO: 6; a hydrophilic amino acid in place of the alanine corresponding to position 104 of the wild type Semaphorin 3C as shown in SEQ ID NO: 10; or a hydrophilic amino acid in place of the alanine corresponding to position 120 of the wild type Semaphorin 3D as shown in SEQ ID NO: 14.

Also disclosed herein is a nucleic acid molecule encoding the mutated Semaphorin 3 or the functional fragment thereof as defined herein or the fusion protein as defined herein.

Also disclosed here is a vector comprising the nucleic acid molecule as defined herein.

Disclosed herein is a host transformed with a vector as defined herein or comprising the nucleic acid molecule as defined herein.

Disclosed herein is a method for producing the mutated Semaphorin 3, the functional fragment thereof or the fusion protein encoded by the nucleic acid molecule as defined herein, wherein the method comprises culturing/raising the host as defined herein and optionally isolating the produced polypeptide.

Disclosed herein is a pharmaceutical composition comprising (a) the mutated Semaphorin 3 or the functional fragment thereof as defined herein; (b) the fusion protein as defined herein; and/or (c) the nucleic acid molecule as defined herein; optionally comprising a pharmaceutical excipient.

The mutated Semaphorin 3 or the functional fragment thereof as defined herein, the fusion protein as defined herein, the nucleic acid molecule as defined herein, the pharmaceutical composition as defined herein, and/or the polypeptide as defined herein for use as a medicament; or for use in the treatment of a cancer, tumor, tumorous disease, angiogenic disorder, vascular retinopathy, blood-brain barrier permeability alterations, neuroinflammatory disorder, osteoporosis, obesity, mycobacterial infection, and/or granuloma, preferably wherein said tumor, cancer, and/or tumorous disease is a solid tumor, more preferably wherein the cancer is selected from the group consisting of pancreatic

cancer, cervical cancer, breast cancer, colon cancer, melanoma, prostate cancer, bladder cancer and tongue cancer, and most preferably wherein said tumor, cancer, or tumorous disease is a pancreatic tumor/cancer.

Detailed Description

The present invention relates to a non-naturally occurring/genetically modified/mutated Semaphorin of class 3, particularly of a non-naturally occurring/genetically modified/mutated Semaphorin selected from the group consisting of Semaphorin 3A, Semaphorin 3B, Semaphorin 3C and Semaphorin 3D, most preferably of a non-naturally occurring/genetically modified/mutated Semaphorin 3A.

Accordingly, the present invention relates to a mutated Semaphorin 3 (or a functional fragment thereof functioning as an inhibitor of angiogenesis or a fusion protein/polypeptide comprising said mutated Semaphorin or said functional fragment) that

(a) comprises a hydrophilic amino acid in place of the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2; or

(b) comprises a hydrophilic amino acid in place of the alanine at the position which corresponds in other Semaphorin 3 proteins by comparison of homology to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2; and

(c) wherein said Semaphorin 3 is selected from the group of Semaphorin 3A, Semaphorin 3B, Semaphorin 3C and Semaphorin 3D, wherein preferably said Semaphorin 3 is Semaphorin 3A.

Claims

1. A mutated Semaphorin 3 or a functional fragment thereof wherein said mutated Semaphorin 3 is selected from the group consisting of Semaphorin 3A, Semaphorin 3B, Semaphorin 3C, and Semaphorin 3D and wherein said mutated Semaphorin 3 or said functional fragment thereof comprises an amino acid sequence $CX_1X_2A_3GKD$, wherein

   X_1 is an amino acid, which is K or N,

   X_2 is an amino acid selected from the group of W, M and L,

   and wherein the alanine (A_3) is replaced by a hydrophilic amino acid,

   wherein the mutated Semaphorin 3 or the functional fragment thereof inhibits angiogenesis and/or functions as vascular normalizing agent.

2. The mutated Semaphorin 3 or the functional fragment thereof of claim 1, wherein said mutated Semaphorin 3 or said functional fragment thereof comprises

   said hydrophilic amino acid in place of the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2;

   said hydrophilic amino acid in place of the alanine corresponding to position 105 of the wild type Semaphorin 3B as shown in SEQ ID NO: 6;

   said hydrophilic amino acid in place of the alanine corresponding to position 104 of the wild type Semaphorin 3C as shown in SEQ ID NO: 10; or

   said hydrophilic amino acid in place of the alanine corresponding to position 120 of the wild type Semaphorin 3D as shown in SEQ ID NO: 14.

3. The mutated Semaphorin 3 or the functional fragment thereof of claim 1 or 2, wherein said Semaphorin 3 is selected from the group of:

   (a) a polypeptide that is encoded by a nucleic acid sequence selected from the group of SEQ ID NO: 1, SEQ ID NO: 5, SEQ ID NO: 9 and SEQ ID NO: 13,

   wherein the nucleotides GCT at position 631 to 633 of SEQ ID NO: 1 are replaced by nucleotides encoding said hydrophilic amino acid,

   wherein the nucleotides GCA at position 559 to 561 of SEQ ID NO: 5 are replaced by nucleotides encoding said hydrophilic amino acid,

wherein the nucleotides GCT at position 872 to 874 of SEQ ID NO: 9 are replaced by nucleotides encoding said hydrophilic amino acid, and
wherein the nucleotides GCC at position 398 to 400 of SEQ ID NO: 13 are replaced by nucleotides encoding said hydrophilic amino acid;

(b) a polypeptide having the amino acid sequence selected from the group of SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 10 and SEQ ID NO: 14, wherein the alanine residue at position 106 of SEQ ID NO: 2, at position 105 of SEQ ID NO: 6, at position 104 of SEQ ID NO: 10 or at position 120 of SEQ ID NO: 14 is replaced by said hydrophilic amino acid;

(c) a polypeptide that is encoded by a nucleic acid sequence that hybridizes under stringent conditions to the complementary strand of a nucleic acid molecule encoding a polypeptide as defined in (a) or (b);

(d) a polypeptide that has at least 55% identity to any one of the polypeptides referred to in (b).

4. The mutated Semaphorin 3 or the functional fragment thereof of any one of claims 1 to 3, wherein said Semaphorin 3 or said functional fragment thereof comprises a hydrophilic amino acid

(a) at position 106 of SEQ ID NO: 2;

(b) at position 105 of SEQ ID NO: 6;

(c) at position 104 of SEQ ID NO: 10; or

(d) at position 120 of SEQ ID NO: 14.

5. The mutated Semaphorin 3 or the functional fragment thereof of any one of claims 1 to 4, wherein said Semaphorin 3 or said functional fragment thereof comprises a functional sema domain and comprises at least one additional mutation selected from the group consisting of amino acid substitution(s), addition(s), deletions(s) and duplication(s).

6. The mutated Semaphorin 3 or the functional fragment thereof of any one of claims 1 to 5, wherein said hydrophilic amino acid is selected from the group of K, R, N, Q, S,T, E, D, and H, preferably wherein said hydrophilic amino acid is K or R, and most preferably wherein said hydrophilic amino acid replacing said alanine is K.

7. The mutated Semaphorin 3 or the functional fragment thereof of any one of claims 1 to 6, wherein said Semaphorin 3 or said functional fragment thereof comprises said functional sema domain, wherein said sema domain is selected from an amino acid sequence as shown in:
   (a) SEQ ID NO: 21, wherein the alanine residue corresponding to position 106 of SEQ ID NO: 2 is replaced by a hydrophilic amino acid;
   (b) SEQ ID NO: 22, wherein the alanine residue corresponding to position 105 of SEQ ID NO: 6 is replaced by a hydrophilic amino acid;
   (c) SEQ ID NO: 23, wherein the alanine residue corresponding to position 104 of SEQ ID NO: 10 is replaced by a hydrophilic amino acid; or
   (d) SEQ ID NO: 24, wherein the alanine residue corresponding to position 120 of SEQ ID NO: 14 is replaced by a hydrophilic amino acid.

8. A fusion protein comprising the mutated Semaphorin 3 or the functional fragment thereof as defined in any one of claims 1 to 7 that functions as an inhibitor of angiogenesis and/or as vascular normalizing agent.

9. The fusion protein of claim 8, wherein said fusion protein comprises said mutated Semaphorin 3 or said functional fragment thereof as defined in any one of claims 1 to 7, wherein said fusion protein further comprises:
   (a) a stabilizer domain; and/or
   (b) a dimerization domain.

10. The fusion protein of claim 9, wherein
   (a) said stabilizer domain is a Plexin Semaphorin Integrin (PSI) domain, wherein said PSI domain comprises one or more of the following sequences SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47 or SEQ ID NO: 48; and/or
   (b) said dimerization domain has a dissociation constant K_D in the range of 10^{-5} M to 10^{-6} M with another such dimerization domain and/or wherein said dimerization domain is selected from the group of a C-terminal IgG constant domain, DARPin and leucine zipper, and/or wherein the IgG constant domain is IgG1 or IgG3.

11. The fusion protein of any one of claims 8 to 10, wherein said fusion protein comprises an amino acid sequence:

spanning from amino acid residues 1 to 548 of SEQ ID NO: 2 and an amino acid sequence as shown in SEQ ID NO: 41, wherein the alanine residue at position 106 of SEQ ID NO: 2 is replaced by a hydrophilic amino acid;

spanning from amino acid residues 1 to 547 of SEQ ID NO: 6 and an amino acid sequence as shown in SEQ ID NO: 41, wherein the alanine residue at position 105 of SEQ ID NO: 6 is replaced by a hydrophilic amino acid;

spanning from amino acid residues 1 to 565 of SEQ ID NO: 10 and an amino acid sequence as shown in SEQ ID NO: 41, wherein the alanine residue at position 104 of SEQ ID NO: 10 is replaced by a hydrophilic amino acid; or

spanning from amino acid residues 1 to 545 of SEQ ID NO: 14 and an amino acid sequence as shown in SEQ ID NO: 41, wherein the alanine residue at position 120 of SEQ ID NO: 14 is replaced by said hydrophilic amino acid; or

wherein the fusion protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 18, SEQ ID NO: 76, SEQ ID NO: 78 and SEQ ID NO: 79.

12. An antibody specifically binding to the mutated Semaphorin 3 or the functional fragment thereof as defined in any one of claims 1 to 7 or to the fusion protein as defined in any one of claims 8 to 11, wherein said antibody specifically binds to an epitope comprising a hydrophilic amino acid in place of the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2; a hydrophilic amino acid in place of the alanine corresponding to position 105 of the wild type Semaphorin 3B as shown in SEQ ID NO: 6; a hydrophilic amino acid in place of the alanine corresponding to position 104 of the wild type Semaphorin 3C as shown in SEQ ID NO: 10; or a hydrophilic amino acid in place of the alanine corresponding to position 120 of the wild type Semaphorin 3D as shown in SEQ ID NO: 14.

13. A nucleic acid molecule encoding the mutated Semaphorin 3 or the functional fragment thereof of any one of claims 1 to 7 or the fusion protein of any one of claims 8 to 11.

14. A vector comprising the nucleic acid molecule of claim 13.

15. A host transformed with a vector of any one of claim 14 or comprising the nucleic acid molecule of claim 13.

16. A method for producing the mutated Semaphorin 3, the functional fragment thereof or the fusion protein encoded by the nucleic acid molecule of claim 13, wherein the method comprises culturing/raising the host of any one of claim 15 and optionally isolating the produced polypeptide.

17. A pharmaceutical composition comprising
    (a) the mutated Semaphorin 3 or the functional fragment thereof according to any one of claims 1 to 7;
    (b) the fusion protein of any one of claims 8 to 11; and/or
    (c) the nucleic acid molecule of claim 13;
    optionally comprising a pharmaceutical excipient.

18. The mutated Semaphorin 3 or the functional fragment thereof of any one of claims 1 to 7, the fusion protein of any one of claims 8 to 11, the nucleic acid molecule of claim 13, the pharmaceutical composition of claim 17, and/or the polypeptide as produced by claim 16 for use as a medicament; or for use in the treatment of a cancer, tumor, tumorous disease, angiogenic disorder, vascular retinopathy, blood-brain barrier permeability alterations, neuroinflammatory disorder, osteoporosis, obesity, mycobacterial infection, and/or granuloma, preferably wherein said tumor, cancer, and/or tumorous disease is a solid tumor, more preferably wherein the cancer is selected from the group consisting of pancreatic cancer, cervical cancer, breast cancer, colon cancer, melanoma, prostate cancer, bladder cancer and tongue cancer, and most preferably wherein said tumor, cancer, or tumorous disease is a pancreatic tumor/cancer.

19. The mutated Semaphorin 3 or the functional fragment thereof of any one of claims 1 to 7 for use according to claim 18, the fusion protein of any one of claims 8 to 11 for

use according to claim 18 , the nucleic acid molecule of claim 13 for use according to claim 18, the pharmaceutical composition of claim 17 for use according to claim 18, and/or the polypeptide as produced by claim 16 for use according to claim 18, wherein the mutated Semaphorin 3 or the functional fragment thereof, the fusion protein, the nucleic acid molecule, the pharmaceutical composition and/or the polypeptide is to be administered in combination with an anti-proliferative drug, an anticancer drug, a cytostatic drug, a cytotoxic drug and/or radiotherapy.