Cancer Inst 101 : 237-247 ; Delloye-Bourgeois, 2009, JEM 206 : 833-847). WO2012025618 discloses DCC-fusion proteins having improved DCC decoy.

EP 1 989 546 (WO2007099133) discloses monoclonal or polyclonal antibodies directed specifically against netrin-1 or netrin-1 receptors, particularly directed to the extracellular domain of the netrin-1 receptors or to the netrin-1 fragment able to interact with the extracellular domain of said netrin-1 receptors, as a medicament.

Summary

In one aspect, the present invention refers to an isolated or purified polypeptide consisting of sequence SEQ ID NO: 3 comprising SEQ ID NO: 35, or encoded by the cDNA of sequence SEQ ID NO: 4 or SEQ ID NO: 36, or a variant by virtue of the degeneracy of the genetic code. In another aspect, the present invention refers to use of a polypeptide as disclosed herein for the preparation of a monoclonal antibody. In yet another aspect, the present invention refers to a netrin-1 binding antibody which comprises a CDR1-H of sequence SEQ ID NO: 5, a CDR2-H of sequence SEQ ID NO: 6, a CDR3-H of sequence SEQ ID NO: 7, and a CDR1-L of sequence SEQ ID NO: 8, a CDR2-L of sequence YAS and a CDR3-L of sequence SEQ ID NO: 9, wherein the antibody has the property of binding to netrin-1 and inducing cell death or apoptosis of a tumor cell via an UNC5 receptor or DCC receptor. In a further aspect, the present invention refers to a netrin-1 binding antibody which comprises a CDR1-H of sequence SEQ ID NO: 28, a CDR2-H of sequence SEQ ID NO: 29, a CDR3-H of sequence SEQ ID NO: 30, and a CDR1-L of sequence SEQ ID NO: 31, a CDR2-L of sequence SEQ ID NO: 32 and a CDR3-L of sequence SEQ ID NO: 9, wherein the antibody has the property of binding to netrin-1 and inducing cell death or apoptosis of a tumor cell via an UNC5 receptor or DCC receptor. In another aspect, the present invention refers to a pharmaceutical composition comprising an antibody as disclosed herein, and a pharmaceutically acceptable vehicle, carrier or diluent. In yet another aspect, the present invention refers to use of a pharmaceutical composition as disclosed herein. in the manufacture of a medicament for treating cancer, wherein the cancer has tumoral cells expressing a netrin-1 receptor, in the presence of netrin-1. In a further aspect, the present invention refers to use of a pharmaceutical composition as disclosed herein in the manufacture of a medicament for treating cancer, wherein the cancer has tumoral cells expressing a netrin-1 receptor and netrin-1 or a tumoral cells expressing netrin-1 within stroma cells expressing netrin-1. In another aspect, the present invention refers to use of a pharmaceutical composition as disclosed herein in the manufacture of a medicament for treating cancer. In a further aspect, the present invention refers to use of the netrin-1 binding antibody as disclosed herein in the manufacture of a medicament for treating cancer.

The Applicant has now determined a linear or substantially linear epitope on netrin-1 that likely corresponds to the specific binding region of netrin-1 to receptors, in particular of the UNC5 class, especially UNC5B and UNC5A, or alternatively corresponds to a region nearby the specific binding region of netrin-1 to receptor that when bound to an antibody prevents netrin-1/receptor interaction. This determination of a linear epitope allows the Applicant to produce antibodies binding to netrin-1 and interfering with netrin-1/receptors interaction, thereby inducing apoptosis or cell death of tumour cells expressing or overexpressing netrin-1 and at least one netrin-1 receptor, owing the fact that this interaction inhibits netrin-1 binding to a receptor and the multimerization of the receptor. The Applicant also produced a murine monoclonal antibody directed against this epitope and various humanized forms thereof.

The full-length amino acid sequence of netrin-1 is given as SEQ ID NO: 1 and a cDNA coding therefore is given at SEQ ID NO: 2. A linear epitope has been characterized in the second EGF-like domain of netrin-1 and is depicted on SEQ ID NO: 3 and more particularly on SEQ ID NO:35. A cDNA encoding this epitope is depicted on SEQ ID NO: 4, respectively 36.

A first object of the invention is thus a polypeptide representing a linear epitope of netrin-1 or a fragment or variant of said linear epitope. More specifically, the invention relates to:

- an isolated or purified polypeptide of sequence SEQ ID NO: 3,
- a variant polypeptide having at least 85, 90, 95, 96, 97, 98 or 99 % of identity to SEQ ID NO: 3,
- a variant polypeptide of at most 200, 150, 100, 90, 80, 70, 60, 50 amino acids and comprising SEQ ID NO: 3 or variant sequence as described above, such as a variant polypeptide consisting of the 22 consecutive amino acids of SEQ ID NO: 3 from position 9 (A) to position 30 (C), including these amino acids, which variant is presented as SEQ ID NO: 35,
- a variant polypeptide comprising at least 20, 25 or 30 consecutive amino acids of SEQ ID NO: 3 such as a variant polypeptide of sequence SEQ ID NO: 35, or variant sequence as described above,
- a polypeptide of sequence SEQ ID NO: 35, or variant sequence as described above,

CLAIMS:

1. An isolated or purified polypeptide:

- consisting of sequence SEQ ID NO: 3 or SEQ ID NO: 35 or a variant consisting of a fragment of sequence SEQ ID NO: 3 comprising SEQ ID NO: 35, or

- encoded by the cDNA of sequence SEQ ID NO: 4 or SEQ ID NO: 36, or a variant thereof by virtue of the degeneracy of the genetic code.

2. Use of a polypeptide according to claim 1 for the preparation of a monoclonal antibody.

3. A netrin-1 binding antibody which comprises a CDR1-H of sequence SEQ ID NO: 5, a CDR2-H of sequence SEQ ID NO: 6, a CDR3-H of sequence SEQ ID NO: 7, and a CDR1-L of sequence SEQ ID NO: 8, a CDR2-L of sequence YAS and a CDR3-L of sequence SEQ ID NO: 9, wherein the antibody has the property of binding to netrin-1 and inducing cell death or apoptosis of a tumor cell via an UNC5 receptor or DCC receptor.

4. A netrin-1 binding antibody which comprises a CDR1-H of sequence SEQ ID NO: 28, a CDR2-H of sequence SEQ ID NO: 29, a CDR3-H of sequence SEQ ID NO: 30, and a CDR1-L of sequence SEQ ID NO: 31, a CDR2-L of sequence SEQ ID NO: 32 and a CDR3-L of sequence SEQ ID NO: 9, wherein the antibody has the property of binding to netrin-1 and inducing cell death or apoptosis of a tumor cell via an UNC5 receptor or DCC receptor.

5. The netrin-1 binding antibody of claim 3 or 4, which is an epitope binding fragment thereof.

6. The netrin-1 binding antibody of any one of claims 3 to 5, which comprises an amino acid sequence SEQ ID NO: 10, 11, 12 or 13, preferably both sequences SEQ ID NO: 10 and 11, or SEQ ID NO: 12 and 13.

7. The netrin-1 binding antibody of any one of claims 3 to 6, which comprises an amino acid sequence selected from the group of SEQ ID NO:14 to 19 and/or from the group of SEQ ID NO: 20 to 27, preferably it comprises an amino acid sequence selected from the group of SEQ ID NO: 14 to 19 and an amino acid sequence selected from the group of SEQ ID NO: 20 to 27.

8. The netrin-1 binding antibody of claim 7, which comprises a pair of amino acid sequences selected from the pairs SEQ ID NO: 14 and 20; 15 and 21; 16 and 22; 17 and 23; 17 and 24; 16 and 25; 17 and 26; 17 and 22; 18 and 25; and 16 and 21.

9. A pharmaceutical composition comprising an antibody according to any one of claims 3 to 8, and a pharmaceutically acceptable vehicle, carrier or diluent.

10. Use of a pharmaceutical composition of claim 9 in the manufacture of a medicament for treating cancer, wherein the cancer has tumoral cells expressing a netrin-1 receptor, in the presence of netrin-1.

11. Use of a pharmaceutical composition of claim 9 in the manufacture of a medicament for treating cancer, wherein the cancer has tumoral cells expressing a netrin-1 receptor and netrin-1 or a tumoral cells expressing netrin-1 within stroma cells expressing netrin-1.

12. Use of a pharmaceutical composition according to claim 9 in the manufacture of a medicament for treating cancer.

13. Use of the netrin-1 binding antibody of any one of claims 3 to 8 in the manufacture of a medicament for treating cancer.