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FUNDACION CENTRO NACIONAL DE INVESTIGACIONES ONCOLOGICAS (CNIO), ES

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(54) Titre : ANTICORPS DIRIGE CONTRE L'EPHRINE B2 ET UTILISATION CORRESPONDANTE
(54) Title: ANTI-EPHRIN B2 ANTIBODY AND ITS USE

(57) Abrégé/Abstract:
The invention relates to a novel anti-ephrin-B2 antibody and to the use thereof for the detection of this protein and as a drug for inhibiting angiogenesis and lymphangiogenesis in the treatment of diseases involving said processes, such as cancer.

ABSTRACT

The present invention relates to a novel antibody against ephrin B2 and its use to detect the protein and as a medicament for inhibiting angiogenesis and lymphangiogenesis in the treatment of diseases in which these processes are implicated, for example, cancer.

ANTI-EPHRIN B2 ANTIBODY AND ITS USE

The present invention belongs to the field of Biomedicine and Biotechnology and relates to a new ephrin B2 specific antibody capable of blocking the formation of blood vessels (angiogenesis) and lymphatic vessels (Lymphangiogenesis). Furthermore, the present invention relates to the use of the mentioned antibody, for example, for the preparation of a medicament.

STATE OF THE ART

Angiogenesis, or new blood vessel formation from pre-existing ones plays a key role in numerous physiological processes during embryonic development and postnatal life: reproduction, scarring and inflammation. Although the molecular mechanisms responsible for the transition of an endothelial cell to an angiogenic phenotype are not completely known, it is a complex process that involves the proliferation, migration and assembly of endothelial cells, followed by the recruitment of other perivascular cells such as pericytes or muscle cells and the remodelling of the extracellular matrix (Risau, W. Nature 1997, 386:671-674). The uncontrolled growth of blood vessels is an underlying disorder in numerous

pathologies such as rheumatoid arthritis or diabetic retinopathy, and especially neoplastic processes. Tumour growth will depend on the constant supply of oxygen and nutrients through the formation of a network of new blood vessels, such that in the absence of adequate vascularisation, cells undergo a process of necrosis and / or apoptosis that inhibits or moderates the increase of tumour volume.

In addition to blood vessels, the vertebrate circulatory system is comprised of lymphatic vessels that also play a critical role during the organism development and pathological processes. The lymphatic system drains the interstitial fluid of the tissues and drives it to the blood system, also absorbs lipids from digestive system, is part of the individual immune defense transporting cells of the immune system, for example in inflammation, and in various pathological conditions induces types of lymphedema, inflammatory diseases and is involved

CLAIMS

What is claimed is:

1. An isolated polypeptide characterized by:

(a) comprising an amino acid sequence at least 76% sequence identity with SEQ ID NO: 1 and

(b) specifically recognizes and binds to ephrin B2.

2. The polypeptide according to the previous claim wherein the amino acid sequence is SEQ ID NO: 1.

3. The polypeptide according to any of the preceding claims wherein said polypeptide is an antibody.

4. The polypeptide according to the preceding claim wherein said antibody is human.

5. The polypeptide according to the preceding claim wherein the human antibody isotype is IgG1, IgG2, IgG3, IgG4, or IgA.

6. The polypeptide according to any preceding claim characterized by further comprising a signal peptide

7. The polypeptide according to the preceding claim wherein the signal peptide is SEQ ID NO: 6.

8. The polypeptide according to any preceding claim characterized by further comprising at least one marker.

9. The polypeptide according to the preceding claim wherein the label is

selected from the list comprising: c-myc, FLAG, HA, histidine chain, GST, biotin, VSV-G, HSVtk, V5, biotin, avidin, streptavidin, maltose-binding protein and a fluorescent protein.

10. The polypeptide according to the preceding claim wherein the label is a chain of histidines, c-myc or both.

11. The polypeptide according to the previous claim wherein the amino acid sequence of said polypeptide is SEQ ID NO: 7.

12. An antibody against ephrin B2 whose amino acid sequence comprises the polypeptide according to any of the preceding claims.

13. A nucleic acid encoding the polypeptide according to any of claims 1 to 11 or the antibody according to claim 12.

14. A vector comprising the nucleic acid according to the preceding claim.

15. The vector according to the preceding claim wherein the vector is an expression vector.

16. A cell comprising the vector according to any of claims 14 or 15.

17. The cell according to the preceding claim wherein said cell is prokaryotic.

18. The cell according to claim 16 wherein said cell is eukaryotic.

19. The cell according to the preceding claim wherein said cell is a mammalian cell.

20. A method of obtaining a polypeptide according to any of claims 1 to 11 or the antibody according to claim 12 comprising the steps (a) expressing the vector described according to claim 15 in a cell and (b) purifying the polypeptide expressed in the step (a).

21. The method according to the preceding claim wherein the cell is prokaryotic.

22. The method according to claim 20 wherein the cell is eukaryotic.

23. A method of detection and/or quantification of the ephrin B2 comprising the steps (a) contacting an isolated biological sample with the polypeptide according to any of claims 1 to 11 or with the antibody according to claim 12 and (b) detecting and/or quantifying the complex formed by the ephrin B2 and said polypeptide or said antibody in the sample used in (a).

24. A method of diagnosing a disease associated with expression of ephrin B2 comprising the steps (a) contacting a isolated biological sample with the polypeptide according to any of claims 1 to 11 or with the antibody according to claim 12, (b) detecting and/or quantifying the complex formed by the ephrin B2 and said polypeptide or said antibody in the sample used in (a), (c) compare ephrin B2 levels detected with control levels and (d) associating the result of this comparison to the presence or absence of disease.

25. Use of the polypeptide according to any of claims 1 to 11 or the antibody according to claim 12 for preparing a medicament.

26. Use according to the preceding claim for inhibiting angiogenesis.

27. Use according to any of the two preceding claims for the prophylactic or therapeutic treatment of a pathological condition associated with angiogenesis.

28. Use according to any of the three preceding claims for the prophylactic or therapeutic treatment of a tumour or cancer.

29. Use according to the preceding claim wherein the tumor or cancer is solid.

30. Use according to the preceding claim wherein the cancer is pancreatic, colon or lung.

31. A composition comprising the polypeptide according to any of claims 1 to 11, the antibody according to claim 12, the nucleic acid according to claim 13, the vector according to claims 14 or 15 or the cell according to any of claims 17 to 19.

32. The composition according to the preceding claim, wherein said composition is a pharmaceutical composition.

33. The composition according to the preceding claim characterized by further comprising a pharmaceutically acceptable excipient.

34. The composition according to any of claims 31 or 32 characterized by further comprising an antiangiogenic agent.

35. The composition according to any of claims 31 or 32 characterized by further comprising a chemotherapeutic agent.

36. Use of the composition according to any of the five preceding claims for preparing a medicament.

37. Use according to the preceding claim for inhibiting angiogenesis.

38. Use according to any of the two preceding claims for the prophylactic or therapeutic treatment of a pathological condition associated with angiogenesis.

39. Use according to the preceding claim for prophylactic or therapeutic treatment of a tumour or cancer.

40. Use according to the preceding claim wherein the tumor or cancer is solid.

41. Use according to the preceding claim wherein the cancer is pancreatic, colon or lung.

Application number / Numéro de demande: ES 201107065 5

Figures: 1-10, 12A

Pages: 10 pages

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FIG. 11A

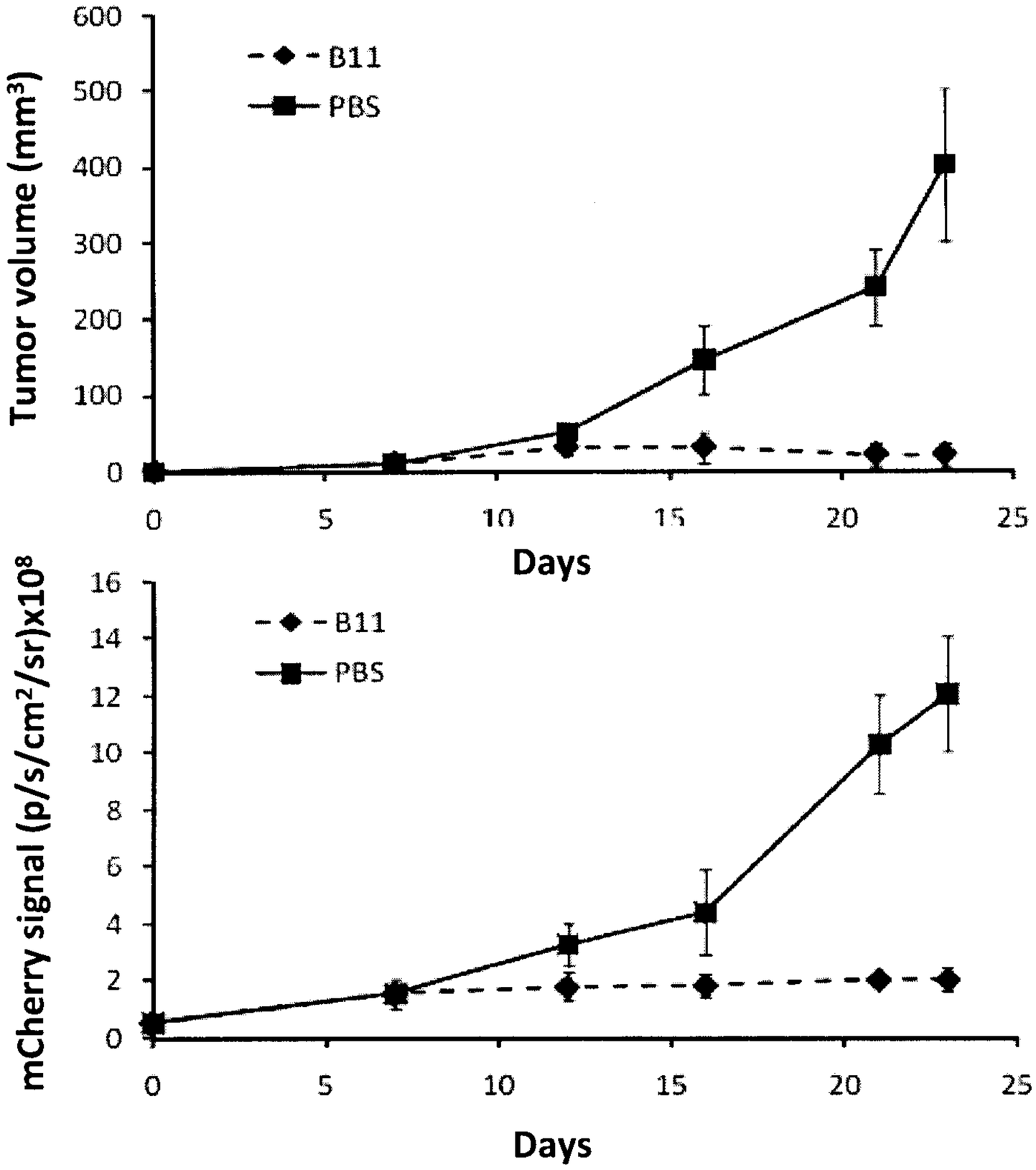
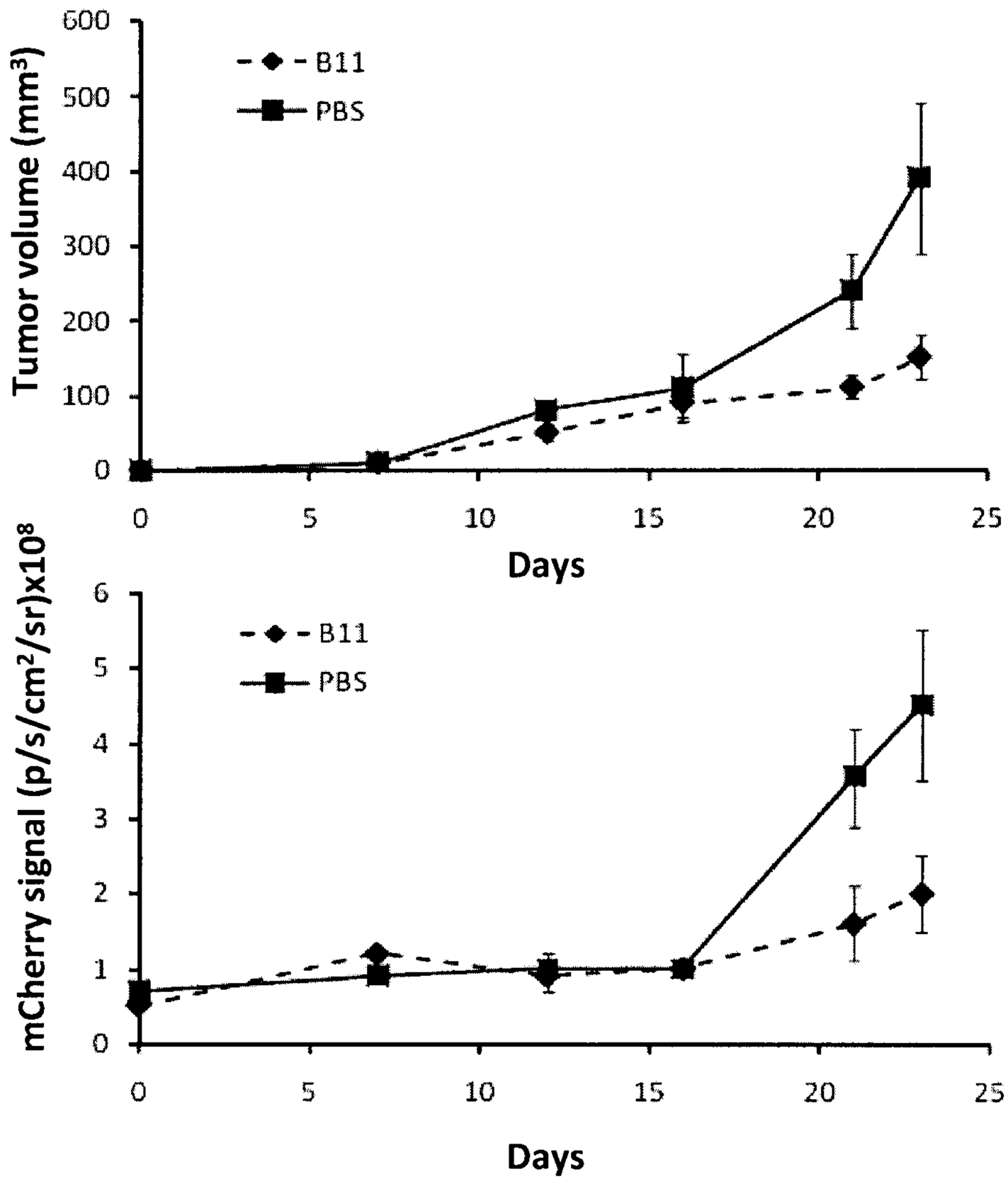

600
500
400
300
200
100
0
Tumor volume (mm³)
B11
PBS
0
5
10
15
20
25
Days
16
14
12
10
8
6
4
2
0
mCherry signal (p/s/cm²/sr)x10⁸
B11
PBS
0
5
10
15
20
25
Days

FIG. 11B


600
500
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Tumor volume (mm³)
B11
PBS
0
5
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15
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25
Days
6
5
4
3
2
1
0
mCherry signal (p/s/cm²/sr)x10⁸
B11
PBS
0
5
10
15
20
25
Days

FIG. 12B