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(54) Title: HUMAN CORD BLOOD AS A SOURCE OF NEURAL TISSUE FOR REPAIR OF THE BRAIN AND SPINAL CORD

(57) Abstract: The present invention relates to the use of umbilical cord blood cells from a donor or patient to provide neural cells which may be used in transplantation. The isolated cells according to the present invention may be used to effect autologous and allogeneic transplantation and repair of neural tissue, in particular, tissue of the brain and spinal cord and to treat neurodegenerative diseases of the brain and spinal cord.
Claims:

1. Neural cells obtained by exposing pluripotent stem or progenitor cells obtained from umbilical cord blood to an amount of a differentiation agent effective for changing the phenotype of said stem or progenitor cells to a neural phenotype.

2. The cells of claim 1 wherein said differentiation agent is selected from the group consisting of retinoic acid, fetal or mature neuronal cells, BDNF, GDNF, NGF, FGF, TGF, CNTF, BMP, LIF, GGF, TNF, IGF, CSF, KIT-SCF, interferon, triiodothyronine, thyroxine, erythropoietin, thrombopoietin, silencers, SHC, neuroproteins, proteoglycans, glycoproteins, neural adhesion molecules, cell signalling molecules and mixtures, thereof.

3. The cells of claim 1 or 2 wherein said differentiation agent is a mixture of retinoic acid and NGF.

4. A method of producing neural cells from umbilical cord blood comprising:
   a. obtaining a sample of mononuclear cells from said umbilical cord blood; and
   b. growing said mononuclear cells from step a in a culture medium containing an effective amount of a differentiation agent for a period sufficient to change the phenotype of said stem or progenitor cells to neural.

5. The method according to claim 4 wherein said differentiation agent is selected from the group consisting of retinoic acid, fetal or mature neuronal cells, BDNF, GDNF, NGF, FGF, TGF, CNTF, BMP, LIF, GGF, TNF, IGF, CSF, KIT, interferon, triiodothyronine, thyroxine, erythropoietin, thrombopoietin, silencers, SHC, neuroproteins, proteoglycans, glycoproteins, neural adhesion molecules, cell signalling molecules and mixtures, thereof.

6. The method according to claim 4 or 5 wherein said differentiation agent is a mixture of retinoic acid and NGF.

7. The method according to any of claims 4-6 wherein said neuronal cells are selected from
the group consisting of mesencephalic cells and striatal cells.

8. A method of producing neural cells from umbilical cord blood comprising:
   a. obtaining a sample of mononuclear cells from said umbilical cord blood;
   b. selecting for and isolating a sample of pluripotent stem or progenitor cells within
      said sample; and
   c. growing said stem or progenitor cells from step b in a culture medium containing an
      effective amount of a differentiation agent for a period sufficient to change the
      phenotype of said stem or progenitor cells to neural.

9. The method according to claim 8 wherein said selecting and isolating step b is carried out
   using a magnetic cell separator to separate out cells containing a CD marker.

10. The method according to claim 8 or 9 wherein said differentiation agent is selected from
    the group consisting of retinoic acid, fetal or mature neuronal cells, BDNF, GDNF, NGF,
    FGF, TGF, CNTF, BMP, LIF, GGF, TNF, IGF, CSF, KIT, interferon, triiodothyronine,
    thyroxine, erythropoietin, thrombopoietin, silencers, SHC, neuroproteins, proteoglycans,
    glycoproteins, neural adhesion molecules, cell signalling molecules and mixtures,
    thereof.

11. A method of producing neural cells from umbilical cord blood comprising:
    a. obtaining a sample of mononuclear cells from said umbilical cord blood;
    b. growing said mononuclear cells from step b in a culture medium containing an
       effective amount of a differentiation agent for a period sufficient to change the
       phenotype of pluripotent stem or progenitor cells within said mononuclear cells to
       neural; and
    c. selecting for and isolating said neural cells from said sample of pluripotent stem or
       progenitor cells within said sample by essentially eliminating from said sample
       mononuclear cells having a CD marker.

12. The method according to claim 11 wherein said selecting and isolating step c is carried
    out using a magnetic cell separator to separate out cells containing a CD marker.
13. The method according to claim 11 or 12 wherein said differentiation agent is selected from the group consisting of retinoic acid, fetal or mature neuronal cells, BDNF, GDNF, NGF, FGF, TGF, CNTF, BMP, LIF, GGF, TNF, IGF, CSF, KIT, interferon, triiodothyronine, thyroxine, erythropoietin, thrombopoietin, silencers, SHC, neuroproteins, proteoglycans, glycoproteins, neural adhesion molecules, cell signalling molecules and mixtures, thereof.

14. The method according to any of claims 11-13 wherein said neuronal cells are selected from the group consisting of mesencephalic cells and striatal cells.
15. A method of producing a sample of enriched neural cells from a sample of mononuclear cells obtained from umbilical cord blood comprising:
   a. subjecting the mononuclear cells to an amount of an anti-proliferating agent effective to eliminate essentially all proliferating cells from said mononuclear cell sample;
   b. exposing the remaining non-proliferating cells from step a to a mitogen to provide a population of differentiated cells and quiescent cells comprising a population of pluripotent stem or progenitor cells;
   c. growing said population of said differentiated cells and quiescent cells from step b to selectively grow said quiescent cells to the essential exclusion of differentiated cells.

16. The method according to claim 15 comprising the further step of incubating a cell population obtained from step c to a differentiation agent effective to induce a neural phenotype in said pluripotent stem or progenitor cells.

17. The method according to claim 15 or 16 wherein said anti-proliferating agent is Ara-C.

18. The method according to any of claims 15-17 wherein said mitogen is selected from the group consisting of epidermal growth factor and pokeweed mitogen.

19. The method according to any of claims 15-18 wherein said differentiation agent is selected from the group consisting of retinoic acid, fetal or mature neuronal cells, BDNF, GDNF, NGF, FGF, TGF, CNTF, BMP, LIF, GGF, TNF, IGF, CSF, KIT, interferon, triiodothyronine, thyroxine, erythropoietin, thrombopoietin, silencers, SHC, neuroproteins, proteoglycans, glycoproteins, neural adhesion molecules, cell signalling molecules and mixtures, thereof.

20. The method according to claim 19 wherein said retinoic acid is selected from 9-cis retinoic acid, all transretinoic acid and mixtures, thereof.

21. The method according to any of claims 1-20 wherein said neural cells are used in allogeneic transplantation.

22. The method according to any of claims 1-20 wherein said neural cells are used in
allogeneic transplantation.

23. A method of treating a damaged brain or spinal cord comprising transplanting into said brain or spinal cord an effective number of neural cells according to any of claims 1-20.

24. A method of treating a patient with a neurodegenerative disease comprising administering an effective number of neural cells according to any of claims 1-20 to said patient.

25. The method according to claim 24 wherein said neurodegenerative disease is selected from the group consisting of Parkinson's disease, Alzheimer's disease, Huntington's disease, amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), Tay Sach's disease, Rett Syndrome, lysosomal storage disease, ischemia, spinal cord damage, traumatic brain injury, ataxia, alcoholism, amyotrophic lateral sclerosis, schizophrenia and autism.

26. The method according to claim 25 wherein said ischemia is caused by a stroke or heart attack in said patient.

27. A method of treating a patient with a neurodegenerative disease comprising administering an effective number of neural cells in umbilical cord blood or a mononuclear cell fraction thereof to said patient.

28. The method according to claim 27 wherein said neurodegenerative disease is selected from the group consisting of Parkinson's disease, Huntington's disease, Alzheimer's disease, amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), Tay Sach's disease, Rett Syndrome, lysosomal storage disease, ischemia, spinal cord damage, traumatic brain injury, ataxia, alcoholism, amyotrophic lateral sclerosis, schizophrenia and autism.

29. A method of treating a patient with a neurodegenerative disease other than amyotrophic lateral sclerosis comprising administering an effective number of neural cells to said patient.

30. The method according to claim 29 wherein said neurodegenerative disease is selected
from the group consisting of Parkinson's disease, Huntington's disease, Alzheimer's disease, amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), Tay Sach's disease (beta hexosaminidase deficiency), Rett Syndrome, lysosomal storage disease ischemia, spinal cord damage, traumatic brain injury, ataxia, alcoholism, schizophrenia and autism.

31. A composition comprising umbilical cord blood or a mononuclear cell fraction, thereof, in combination with an effective amount of at least one neural differentiation agent.

32. The composition according to claim 31 further comprising a cell medium to which said differentiation agent is added.

33. The composition according to claim 31 or 32 wherein said differentiation agent is selected from the group consisting of retinoic acid, fetal or mature mesencephalic or striatal cells brain derived neurotrophic factor (BDNF), glial derived neurotrophic factor (GDNF), glial growth factor (GFF), nerve growth factor (NGF), fibroblast growth factor (FGF), transforming growth factors (TGF), ciliary neurotrophic factor (CNTF), bone-morphogenetic proteins (BMP), leukemia inhibitory factor (LIF), glial growth factor (GGF), tumor necrosis factors (TNF), interferon, insulin-like growth factors (IGF), colony stimulating factors (CSF), KIT receptor stem cell factor (KIT-SCF), interferon, triiodothyronine, thyroxine, erythropoietin, thrombopoietin, glial-cell missing silenter factor, neuron restrictive silencer factor, SRC-homology-2-domain-containing transforming protein, neuroproteins, proteoglycans, glycoproteins and neural adhesion molecules.

34. The composition according to any of claims 31-33 wherein said differentiation agent is selected from the group consisting of retinoic acid, fetal or mature mesencephalic or striatal cells, brain derived neurotrophic factor (BDNF), glial derived neurotrophic factor (GDNF), glial growth factor (GFF), nerve growth factor (NGF) and mixtures, thereof.

35. The composition according to any of claims 31-34 wherein said differentiation agent is selected from the group consisting of mixtures of retinoic acid, brain derived neurotrophic factor (BDNF), glial derived neurotrophic factor (GDNF), glial growth factor (GFF) and
nerve growth factor (NGF).

36. The composition according to any of claims 31-35 wherein said differentiation agent is a mixture of retinoic acid and nerve growth factor.

37. A method of producing a pharmaceutical composition comprising a sample of mononuclear cells being enriched with cells having a neural phenotype marker, said method comprising:
   a. obtaining a sample of mononuclear cells from said umbilical cord blood; and
   b. growing said mononuclear cells from step a in a culture medium containing an effective amount of a differentiation agent for a period sufficient to change the phenotype of said stem or progenitor cells to neural; and
   c. combining said cells obtained from step b with a pharmaceutically acceptable carrier, additive or excipient.

38. The method according to claim 37 wherein said differentiation agent is selected from the group consisting of retinoic acid, fetal or mature neuronal cells, BDNF, GDNF, NGF, FGF, TGF, CNTF, BMP, LIF, GGF, TNF, IGF, CSF, KIT, interferon, triiodothyronine, thyroxine, erythropoietin, thrombopoietin, silencers, SHC, neuroproteins, proteoglycans, glycoproteins, neural adhesion molecules, cell signalling molecules and mixtures, thereof.

39. The method according to claim 37 or 38 wherein said differentiation agent is a mixture of retinoic acid and NGF.

40. The method according to any of claims 37-39 wherein said neural cells are selected from the group consisting of mesencephalic cells and striatal cells.

41. A method of producing a pharmaceutical composition comprising neural cells obtained from umbilical cord blood comprising:
   a. obtaining a sample of mononuclear cells from said umbilical cord blood;
   b. selecting for and isolating a sample of pluripotent stem or progenitor cells within said sample;
c. growing said stem or progenitor cells from step b in a culture medium containing an effective amount of a differentiation agent for a period sufficient to change the phenotype of said stem or progenitor cells to neural; and

d. combining said cells obtained from step b with a pharmaceutically acceptable carrier, additive or excipient.

42. The method according to claim 41 wherein said selecting and isolating step b is carried out using a magnetic cell separator to separate out cells containing a CD marker.

43. The method according to claim 41 or 42 wherein said differentiation agent is selected from the group consisting of retinoic acid, fetal or mature neuronal cells, BDNF, GDNF, NGF, FGF, TGF, CNTF, BMP, LIF, GGF, TNF, IGF, CSF, KIT, interferon, triiodothyronine, thyroxine, erythropoietin, thrombopoietin, silencers, SHC, neuroproteins, proteoglycans, glycoproteins, neural adhesion molecules, cell signalling molecules and mixtures thereof.

44. A method of producing a pharmaceutical composition comprising neural cells obtained from umbilical cord blood comprising:

a. obtaining a sample of mononuclear cells from said umbilical cord blood;

b. growing said mononuclear cells from step b in a culture medium containing an effective amount of a differentiation agent for a period sufficient to change the phenotype of pluripotent stem or progenitor cells within said mononuclear cells to neural; and

c. selecting for and isolating said neural cells from said sample of pluripotent stem or progenitor cells within said sample by essentially eliminating from said sample mononuclear cells having a CD marker; and

d. combining said neural cells isolated from step c with a pharmaceutically acceptable carrier, additive or excipient.

45. The method according to claim 44 wherein said selecting and isolating step c is carried out using a magnetic cell separator to separate out cells containing a CD marker.

46. The method according to claim 44 or 45 wherein said differentiation agent is selected
from the group consisting of retinoic acid, fetal or mature neuronal cells, BDNF, GDNF, NGF, FGF, TGF, CNTF, BMP, LIF, GGF, TNF, IGF, CSF, KIT, interferon, triiodothyronine, thyroxine, erythropoietin, thrombopoietin, silencers, SHC, neuroproteins, proteoglycans, glycoproteins, neural adhesion molecules, cell signalling molecules and mixtures, thereof.

47. The method according to any of claims 44-46 wherein said differentiation agent is a mixture of retinoic acid and nerve growth factor.

48. The method according to any of claims 44-47 wherein said neural cells are selected from the group consisting of mesencephalic cells and striatal cells.

49. A method of treating a patient for a neurodegenerative disease selected from the group consisting of multiple sclerosis (MS), Tay Sach’s disease (beta hexosaminidase deficiency), Rett Syndrome, and lysosomal storage disease said method comprising administering to said patient an effective amount of human umbilical cord blood or a mononuclear cell fraction thereof to said patient.

50. The method according to claim 49 wherein said human umbilical cord blood or said mononuclear cell fraction thereof is administered via a parenteral route of administration.

51. A method of treating a patient in need thereof for a neurodegenerative disease other than amyotrophic lateral sclerosis, said method comprising administering an effective amount of human umbilical cord blood or a mononuclear cell fraction thereof to said patient.

52. The method according to claim 51 wherein said neurodegenerative disease is selected from the group consisting of Parkinson’s disease, Huntington’s disease, Alzheimer’s disease, multiple sclerosis (MS), Tay Sach’s disease, Rett Syndrome, lysosomal storage disease, ischemia, spinal cord damage, traumatic brain injury, ataxia, alcoholism, schizophrenia and autism.

53. A method of treating a patient in need thereof for a neurodegenerative disease
comprising administering an effective amount of neural cells to said patient in the absence of a radiation step or chemotherapeutic step which is used to impair bone marrow production of hematopoietic cells.

54. The method according to claim 53 wherein neural cells are administered to said patient via a route of administration selected from the group consisting of intrathecal, intraventricular, intraparenchymal, intracisternal, intracranial, intrastriatal, and intranigral.

55. The method according to claim 53 or 54 wherein said neurodegenerative disorder is selected from the group consisting of Parkinson's disease, Huntington's disease, Alzheimer's disease, multiple sclerosis, Tay Sach's disease, Rett Syndrome, lysosomal storage disease, spinal cord damage, traumatic brain injury, ataxia, schizophrenia and autism.

56. A method of treating amyotrophic lateral sclerosis in a patient in need thereof, said method comprising administering an effective amount of human umbilical cord blood or a mononuclear cell fraction thereof to said patient in the absence of a radiation step or chemotherapeutic step which is used to impair bone marrow production of hematopoietic cells.
**INTERNATIONAL SEARCH REPORT**

**A. CLASSIFICATION OF SUBJECT MATTER**

<table>
<thead>
<tr>
<th>IPC(7)</th>
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<td>C12N 5/00, 5/02, 5/06, 5/08</td>
<td>435/2, 325, 368, 372, 405</td>
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</table>

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)


Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please see Continuation Sheet

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
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Further documents are listed in the continuation of Box C. See patent family annex.

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