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(54) Title: EXPANSION AND DIFFERENTIATION OF NEURAL STEM CELLS UNDER LOW OXYGEN CONDITIONS

(57) Abstract: The present invention encompasses methods and compositions for enhancing the growth of neural stem cells (NSCs). The invention relates to the benefit of culturing NSCs under lowered oxygen conditions as compared to environmental oxygen conditions traditionally employed in cell culture techniques.



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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US07/00567

A. CLASSIFICATION OF SUBJECT MATTER

IPC: C12N 5/00(2006.01),5/02(2006.01),5/06(2006.01),5/08(2006.01)

USPC: 435/325,363,366,368,375,377

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)

U.S. : 435/325,363,366,368,375,377

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)
WEST, STN, MEDLINE, CAPLUS, BIOSIS, SCISEARCH, LIFESCI

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6,759,242 B1 (CSETE et al) 6 July 2004 (06.07.2004), column 2, lines 16-22, column 5, lines 15-22.	1,2,4,6
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Y		3
Y	US 6,184,035 B1 (CSETE et al) 6 February 2001 (06.02.2001), column 7, lines 32-52.	9
Y	ZHU et al., Effects of Hypoxia on the Proliferation and Differentiation of NSCs, Molecular Neurobiology, 2005, Vol. 31, pages 231-242, especially page 234.	8
Y	MORRISON et al., Culture in Reduced Levels of Oxygen Promotes Clonogenic Sympathoadrenal Differentiation by Isolated Neural Crest Stem Cells, The Journal of Neuroscience, October 1, 2000, Vol. 20, No. 19, pages 7370-7376, especially page 7371, left column.	5



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T"

later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X"

document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y"

document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&"

document member of the same patent family

Date of the actual completion of the international search

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US07/00567

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of any additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-6,8 and 9

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

BOX III. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claim(s) 1-6, 8 and 9, drawn to a method of increasing cellular differentiation of an isolated neural stem cell (NSC) comprising differentiating said NSC in low oxygen conditions ranging from 2.5% to 5% oxygen, wherein said low oxygen conditions increase the cellular differentiation of said NSC, and wherein said cellular differentiation could be in the presence of BDNF or IGF-1.

Group II, claim(s) 7, drawn to a method of increasing cellular differentiation of an isolated neural stem cell (NSC) comprising differentiating said NSC in low oxygen conditions ranging from 2.5% to 5% oxygen, wherein said low oxygen conditions increase the cellular differentiation of said NSC, and wherein exogenous genetic material has been introduced into said NSC.

Group III, claim(s) 10-13, 15 and 16, drawn to an isolated differentiated neural stem cell (NSC) prepared by differentiating said NSC in low oxygen conditions ranging from 2.5% to 5% oxygen, wherein said cellular differentiation could be in the presence of BDNF or IGF-1.

Group IV, claim(s) 14, drawn to an isolated differentiated neural stem cell (NSC) prepared by differentiating said NSC in low oxygen conditions ranging from 2.5% to 5% oxygen, wherein exogenous genetic material has been introduced into said NSC.

Group V, claim(s) 17-26 and 28, drawn to a method of treating a mammal having a CNS disease, disorder or condition comprising obtaining an isolated NSC from a donor, differentiating said NSC in low oxygen condition ranging from 2.5% to 5% oxygen, and administering said differentiated NSC to the CNS of said mammal.

Group VI, claim(s) 27, drawn to a method of treating a mammal having a CNS disease, disorder or condition comprising obtaining an isolated NSC from a donor, differentiating said NSC in low oxygen conditions ranging from 2.5% to 5% oxygen, and administering said differentiated NSC to the CNS of said mammal, wherein said NSC is genetically modified before administration.

Group VII, claim(s) 29-34, drawn to a method of in vitro expansion and maintenance of the multipotentiality of a neural stem cell (NSC) comprising culturing said NSC in low oxygen conditions ranging from 2.5% to 5% oxygen, wherein said low oxygen conditions increase the cellular proliferation of said NSC.

Group VIII, claim(s) 35-37, drawn to an isolated neural stem cell (NSC) prepared by a method of culturing said NSC in low oxygen conditions ranging from 2.5% to 5% oxygen, wherein said low oxygen conditions increase the cellular proliferation of said NSC.

Group IX, claim(s) 38, drawn to an isolated neural stem cell (NSC) prepared by a method of culturing said NSC in low oxygen conditions ranging from 2.5% to 5% oxygen, wherein said low oxygen conditions increase the cellular proliferation of said NSC, and wherein exogenous genetic material has been introduced into said NSC.

Group X, claim(s) 39-46, drawn to a method of treating a mammal having a CNS disease, disorder or condition comprising obtaining an isolated NSC from a donor, culturing said NSC in low oxygen condition ranging from 2.5% to 5% oxygen, and administering said cultured NSC to the CNS of said mammal.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US07/00567

The inventions listed as Groups I-X do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The putative special technical feature common to groups I-X is differentiation or proliferation of neural stem cell (NSC) in low oxygen conditions ranging from 2.5% to 5% oxygen. Csete et al., 2004 (US Patent No. 6,759,242 B1) discloses a method of culturing at least one neural crest stem cell (NCSC) to enhance survival, proliferation or differentiation of the NCSC in low ambient oxygen conditions, wherein said survival, proliferation or differentiation is enhanced compared to a control NCSC cultured in ambient oxygen conditions (e.g. column 2, lines 16-22). The low oxygen condition could be at 0.5%, 1%, 1.5%, 2%, 2.5%, 3%, 3.5%, 4%, 4.5%, 5% ... etc., or another oxygen condition between any of these figure (e.g. column 5, lines 15-22). Csete et al., 2001 (US Patent No. 6,184,035 B1) discloses a method of isolating, maintaining, and/or enriching for stem or progenitor cells derived from diverse organ or tissue sources (e.g. abstract). The medium for the proliferation of stem/progenitor cells and the medium for differentiation of those cells could be the same, and the medium can be used in conjunction with low oxygen level culturing. The medium can be supplemented with a variety of growth factors, such as bFGF, VEGF, and IGF (e.g. column 7, lines 32-52). In view of the reasons set forth above, there is no common special technical feature contributed by the instant invention over the prior art. Thus, groups I-X do not relate to a single general inventive concept under PCT Rule 13.1.