METHOD FOR PRODUCING HIGHLY PROLIFERATIVE CELL, AND HIGHLY PROLIFERATIVE CELL AND USE THEREOF

Technical Field

[0001]

The present invention relates to a production method for highly proliferating cells and highly proliferating cells and use thereof.

Background Art

[0002]

In the field of regenerative medicine, various cells in the living body, cells at various differentiation stages, or cells that have been induced to differentiate in a specific direction are used. Considering that these cells are to be used in living bodies, it is desirable that no genetic modification takes place in these cells. Up until now, it has been reported that, by bringing mature endodermal cells into contact with a specific low-molecular compound, the mature cells can be reprogrammed into stem cells or progenitor cells without genetic modification (WO 2017/119512 A and WO 2020/080550 A).

Summary

[0003]

Meanwhile, many of the cells that are obtained

without performing genetic modification, especially cells that are comparatively close to mature cells, have limited proliferative ability and tend to be incapable of being maintained in vivo and in vitro over a long period of time. For this reason, the need for highly proliferating cells that are effective in long-term usage of the function of the cells or mass production of a useful substance generated by the cells still remains. In particular, ectodermal cells, including cells of the nervous system, are not being fully utilized, unlike endodermal cells.

[0003a]

In one aspect, the present disclosure refers to a method for producing highly proliferating cells, the method comprising: (i) preparing ectodermal cells which serve as a raw material; (ii) culturing the ectodermal cells which serve as the raw material in a medium containing a small-molecule signaling pathway inhibitor by bringing the inhibitor into contact with the ectodermal cells; and (iii) after the contact, performing additional culturing in a medium containing the inhibitor to obtain a culture containing highly proliferating cells that have higher cell proliferative ability than the ectodermal cells which serve as the raw material.

[0003b]

In another aspect, the present disclosure refers to

highly proliferating cells having characteristics of ectodermal cells and proliferative ability that allows the number of the highly proliferating cells after more than 28 days of a culturing period during which the cells are brought into contact with a small-molecule signaling pathway inhibitor to be greater than 1.0 times the number of ectodermal cells which are cultured under the same culturing conditions, except that the cells are not brought into contact with the inhibitor, for the same culturing period.

[0003c]

In another aspect, the present disclosure refers to a method for producing a cell-secreted product, the method comprising: obtaining a culture containing a cell-secreted product secreted from the highly proliferating cells obtaining by the production method as disclosed herein or the highly proliferating cells as disclosed herein; and separating the cell-secreted product from the culture.

[0003d]

In another aspect, the present disclosure refers to a method for producing ectodermal progenitor cells, the method comprising: (i) preparing ectodermal cells which serve as a raw material; (ii) culturing, for a period of more than 28 days, the ectodermal cells which serve as the raw material in a medium containing a small-molecule

signaling pathway inhibitor by bringing the inhibitor into contact with the ectodermal cells; and (iii) after the contact, performing additional culturing in a medium containing the inhibitor to obtain a culture containing highly proliferating cells that have higher cell proliferative ability than the ectodermal cells which serve as the raw material, wherein the ectodermal progenitor cells are the highly proliferating cells.

[0003e]

In another aspect, the present disclosure refers to a cell-secreted product comprising: a protein and/or a miRNA, wherein the protein comprises a combination of vinculin (P18206), integrin β-1, CD29 (P05556), pyruvate kinase M1/2 (P14618), and ephrin type-A receptor 2 (P29317), and wherein the miRNA comprises a combination of hsa-miR-382-5p (MIMAT0000737), hsa-miR-155-5p (MIMAT0000646), hsa-miR-379-5p (MIMAT0000733), hsa-miR-16-5p (MIMAT0000069), hsa-miR-382-5p (MIMAT0000737), hsa-miR-16-5p (MIMAT0000069), hsa-miR-382-5p (MIMAT0000737), hsa-miR-21-5p (MIMAT0000076), hsa-let-7a-5p (MIMAT0000062), hsa-miR-16-5p (MIMAT0000069), hsa-miR-409-3p (MIMAT0001639), hsa-let-7a-5p (MIMAT0000062), and hsa-let-7f-5p (MIMAT0000067).

[0003f]

In another aspect, the present disclosure refers to

a use of a pharmaceutical composition in the manufacture of a medicament for preventing or treating a disorder associated with peripheral nerve cells or central nerve cells, the pharmaceutical composition comprising any one of: a cell-secreted product secreted from the highly proliferating cells obtained by the production method as disclosed herein or from the highly proliferating cells as disclosed herein, and the cell-secreted product as disclosed herein.

[0003g]

In another aspect, the present disclosure refers to a use of any one of: a cell-secreted product secreted from the highly proliferating cells obtained by the production method as disclosed herein or from the highly proliferating cells as disclosed herein, and the cell-secreted product as disclosed herein in the manufacture of a medicament for inhibiting sympathetic nervous system.

Technical Problem

[0004]

An object of the present disclosure is to provide a method for producing highly proliferating cells and highly proliferating cells and use thereof.

Solution to Problem

[0005]

As a result of intensive research, the present inventors found that highly proliferating cells are obtained by bringing a small-molecule signaling pathway inhibitor into contact with ectodermal cells.

[0006]

Specifically, the present disclosure includes the

WHAT IS CLAIMED IS:

1. A method for producing highly proliferating cells, the method comprising:

(i) preparing ectodermal cells which serve as a raw material;

(ii) culturing the ectodermal cells which serve as the raw material in a medium containing a small-molecule signaling pathway inhibitor by bringing the inhibitor into contact with the ectodermal cells; and

(iii) after the contact, performing additional culturing in a medium containing the inhibitor to obtain a culture containing highly proliferating cells that have higher cell proliferative ability than the ectodermal cells which serve as the raw material.

2. The production method according to claim 1, wherein the highly proliferating cells have proliferative ability that allows the number of the highly proliferating cells after more than 28 days of a culturing period during which the cells are brought into contact with the inhibitor to be greater than 1.0 times the number of ectodermal cells which are cultured under the same culturing conditions, except that the cells are not brought into contact with the inhibitor, for the same culturing period.

3. The production method according to claim 1 or 2, wherein the ectodermal cells which serve as the raw

material comprise cells of the central nervous system.

4. The production method according to any one of claims 1 to 3, wherein the ectodermal cells which serve as the raw material comprise astrocytes.

5. The production method according to any one of claims 1 to 4, wherein the inhibitor comprises at least one compound selected from the group consisting of a TGFβ receptor inhibitor and a ROCK inhibitor.

6. The production method according to claim 5, wherein the concentration of the TGFβ receptor inhibitor is in a range of 0.001 μM to 100 μM.

7. The production method according to claim 5 or 6, wherein the concentration of the ROCK inhibitor is in a range of 0.001 μM to 100 μM.

8. The production method according to any one of claims 1 to 7, wherein the highly proliferating cells express at least one gene specific to mature cells at the same level as or at a higher level than the ectodermal cells which serve as the raw material, and express at least one gene specific to progenitor cells at the same level as or at a higher level than the ectodermal cells which serve as the raw material.

9. The production method according to any one of claims 1 to 8, wherein the highly proliferating cells are negative for at least one selected from the group consisting of

Musashi1, Notch1, Nestin, and SOX2.

10. Highly proliferating cells having characteristics of ectodermal cells and proliferative ability that allows the number of the highly proliferating cells after more than 28 days of a culturing period during which the cells are brought into contact with a small-molecule signaling pathway inhibitor to be greater than 1.0 times the number of ectodermal cells which are cultured under the same culturing conditions, except that the cells are not brought into contact with the inhibitor, for the same culturing period.

11. The cells according to claim 10, wherein the highly proliferating cells comprise cells negative for at least one selected from the group consisting of Musashi1, Notch1, Nestin, and SOX2.

12. The cells according to claim 10 or 11, wherein the NG2 expression level is higher than the NG2 expression level in the ectodermal cells that are not brought into contact with the inhibitor.

13. A method for producing a cell-secreted product, the method comprising:

obtaining a culture containing a cell-secreted product secreted from the highly proliferating cells obtaining by the production method according to any one of claims 1 to 9 or the highly proliferating cells according

to any one of claims 10 to 12; and

separating the cell-secreted product from the culture.

14. The method according to claim 13, wherein the cell-secreted product is an exosome.

15. A method for producing ectodermal progenitor cells, the method comprising:

(i) preparing ectodermal cells which serve as a raw material;

(ii) culturing, for a period of more than 28 days, the ectodermal cells which serve as the raw material in a medium containing a small-molecule signaling pathway inhibitor by bringing the inhibitor into contact with the ectodermal cells; and

(iii) after the contact, performing additional culturing in a medium containing the inhibitor to obtain a culture containing highly proliferating cells that have higher cell proliferative ability than the ectodermal cells which serve as the raw material,

wherein the ectodermal progenitor cells are the highly proliferating cells.

16. The production method according to claim 15, further comprising:

isolating the highly proliferating cells from the culture.

17. A cell-secreted product comprising:

a protein and/or a miRNA,

wherein the protein comprises a combination of vinculin (P18206), integrin β-1, CD29 (P05556), pyruvate kinase M1/2 (P14618), and ephrin type-A receptor 2 (P29317), and

wherein the miRNA comprises a combination of hsa-miR-382-5p (MIMAT0000737), hsa-miR-155-5p (MIMAT0000646), hsa-miR-379-5p (MIMAT0000733), hsa-miR-16-5p (MIMAT0000069), hsa-miR-382-5p (MIMAT0000737), hsa-miR-16-5p (MIMAT0000069), hsa-miR-382-5p (MIMAT0000737), hsa-miR-21-5p (MIMAT0000076), hsa-let-7a-5p (MIMAT0000062), hsa-miR-16-5p (MIMAT0000069), hsa-miR-409-3p (MIMAT0001639), hsa-let-7a-5p (MIMAT0000062), and hsa-let-7f-5p (MIMAT0000067).

18. The cell-secreted product according to claim 17, which is an exosome.

19. Use of a pharmaceutical composition in the manufacture of a medicament for preventing or treating a disorder associated with peripheral nerve cells or central nerve cells, the pharmaceutical composition comprising any one of:

a cell-secreted product secreted from the highly proliferating cells obtained by the production method according to claim 1 or 2 or from the highly proliferating

cells according to any one of claims 10 to 12, and

the cell-secreted product according to claim 17 or 18.

20. Use of any one of:

a cell-secreted product secreted from the highly proliferating cells obtained by the production method according to claim 1 or 2 or from the highly proliferating cells according to any one of claims 10 to 12, and

the cell-secreted product according to claim 17 or 18 in the manufacture of a medicament for inhibiting sympathetic nervous system.