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, 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 inhibitor comprises a TGFβ receptor inhibitor and a ROCK inhibitor and the highly proliferating cells are cells

having 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.

[0003b]

In another aspect, the present disclosure refers to highly proliferating cells produced by the method as disclosed herein.

[0003c]

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

having 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 and the small-molecule signaling pathway inhibitor comprises a TGFβ receptor inhibitor and a ROCK inhibitor.

[0003e]

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: a cell-secreted product produced by the method as disclosed herein.

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, 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 inhibitor comprises a TGFβ receptor inhibitor and a ROCK inhibitor and

the highly proliferating cells are cells having 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.

2. The method according to claim 1, wherein the ectodermal cells which serve as the raw material comprise cells of the central nervous system.

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

4. The method according to any one of claims 1 to 3, wherein the inhibitor comprises at least one compound selected from the group consisting of Y-27632 and A-83-01.

5. The method according to claim 1, wherein the concentration of the TGFβ receptor inhibitor is in a range of 0.001 μM to 100 μM.

6. The method according to claim 1, wherein the concentration of the ROCK inhibitor is in a range of 0.001 μM to 100 μM.

7. The method according to any one of claims 1 to 6, 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.

8. The method according to any one of claims 1 to 7,

wherein the highly proliferating cells are negative for at least one selected from the group consisting of Musashi1, Notch1, Nestin, and SOX2.

9. Highly proliferating cells produced by the method according to any one of claims 1 to 8.

10. The cells according to claim 9, wherein the highly proliferating cells comprise cells negative for at least one selected from the group consisting of Musashi1, Notch1, Nestin, and SOX2.

11. The cells according to claim 9 or 10, 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.

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

(a) producing highly proliferating cells by the method according to any one of claims 1 to 8 and obtaining a culture containing a cell-secreted product secreted from the highly proliferating cells, or (b) producing highly proliferating cells according to any one of claims 9 to 11; and

separating the cell-secreted product from the culture.

13. The method according to claim 12, wherein the cell-secreted product is an exosome.

14. 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 having 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 and

the small-molecule signaling pathway inhibitor comprises a TGFβ receptor inhibitor and a ROCK inhibitor.

15. The method according to claim 14, further comprising:

isolating the highly proliferating cells from the culture.

16. The method according to claim 12 or 13, wherein the cell-secreted product comprises 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).

17. The method according to claim 16, wherein the cell-secreted product is an exosome.

18. 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:

a cell-secreted product produced by the method according to claim 12 or 13.

19. The use according to claim 18,

wherein the pharmaceutical composition is for inhibiting sympathetic nervous system.