[0004]

Under such circumstances, selection of cells suitable for transplantation using marker genes for dopaminergic neural cells or dopaminergic neuron progenitor cells has been proposed (Patent Documents 1 to 4). However, these methods still need improvement in the process of selection of markers. Moreover, whether administration of cells immediately after the selection is preferred, or administration of cells induced from these intermediate cells is preferred, has not been discussed in these documents.

[0005]

The methods for producing dopaminergic neural cells may still need to be improved also from the viewpoint of reducing the influence of lot-to-lot variability due to biological components contained therein, and suppressing increases in the prices.

PRIOR ART DOCUMENTS

[Patent Documents]

[0006]

Patent Document 1: WO 2005/052190

Patent Document 2: WO 2006/009241

Patent Document 3: WO 2007/119759

Patent Document 4: WO 2013/015457

[Non-patent Document]

[0007]

Non-patent Document 1: Wernig M, et al., Proc Natl Acad Sci U S A. 2008, 105: 5856-5861

SUMMARY

[0008]

An object of the present disclosure is to produce dopaminergic neuron

progenitor cells which are preferred as a therapeutic agent for Parkinson's disease. Thus, the present disclosure aims to provide a production process for dopaminergic neuron progenitor cells, or a kit necessary for the production.

[0009]

In order to solve the above-described problems, the present inventors focused attention on cell surface membrane proteins Corin and Lrtm1, which are thought to be marker genes for dopaminergic neuron progenitor cells. The present inventors discovered that, by extracting cells using Corin and/or Lrtm1 as an index/indices and culturing the cells followed by their transplantation, the dopamine-producing cells can survive after the transplantation. The present inventors thus found that dopaminergic neuron progenitor cells as a therapeutic agent for Parkinson's disease can be obtained by this production process, thereby completed the present disclosure.

[0009a]

In one aspect, there is provided a method for producing dopaminergic neuron progenitor cells from pluripotent stem cells, said method comprising the steps of:

(i) performing adherent culture of pluripotent stem cells on an extracellular matrix in a medium containing a reagent(s) selected from the group consisting of BMP inhibitor, TGFβ inhibitor, SHH signal-stimulating agent, FGF8, and GSK3β inhibitor;

(ii) collecting Corin- and/or Lrtm1-positive cells from the cells obtained in Step (i) using a substance which binds to Corin and/or a substance which binds to Lrtm1; and

(iii) performing suspension culture of the cells obtained in Step (ii) in a medium containing a neurotrophic factor.

[0010]

The present disclosure relates to the followings:

[1] A method for producing dopaminergic neuron progenitor cells from pluripotent stem cells, said method comprising the steps of:

(i) performing adherent culture of pluripotent stem cells on an extracellular matrix in a medium containing a reagent(s) selected from the group consisting of BMP inhibitor, TGFβ inhibitor, SHH signal-stimulating agent, FGF8, and GSK3β inhibitor;

(ii) collecting Corin- and/or Lrtm1-positive cells from the cells obtained in Step (i); and

(iii) performing suspension culture of the cells obtained in Step (ii) in a medium containing a neurotrophic factor.

[2] The method according to [1], wherein said extracellular matrix is laminin 511 or a fragment thereof.

[3] The method according to [2], wherein said laminin 511 is laminin 511E8.

CLAIMS

1. A method for producing dopaminergic neuron progenitor cells from pluripotent stem cells, said method comprising the steps of:

(i) performing adherent culture of pluripotent stem cells on an extracellular matrix in a medium containing a reagent(s) selected from the group consisting of BMP inhibitor, TGFβ inhibitor, SHH signal-stimulating agent, FGF8, and GSK3β inhibitor;

(ii) collecting Corin- and/or Lrtm1-positive cells from the cells obtained in Step (i) using a substance which binds to Corin and/or a substance which binds to Lrtm1; and

(iii) performing suspension culture of the cells obtained in Step (ii) in a medium containing a neurotrophic factor.

2. The method according to claim 1, wherein Step (i) comprises the steps of:

(a) performing adherent culture of pluripotent stem cells on an extracellular matrix in a medium containing BMP inhibitor and TGFβ inhibitor;

(b) performing adherent culture of the cells obtained in Step (a) on an extracellular matrix in a medium containing BMP inhibitor, TGFβ inhibitor, SHH signal-stimulating agent, and FGF8;

(c) performing adherent culture of the cells obtained in Step (b) on an extracellular matrix in a medium containing

BMP inhibitor, TGFβ inhibitor, SHH signal-stimulating agent, FGF8, and GSK3β inhibitor; and

(d) performing adherent culture of the cells obtained in Step (c) on an extracellular matrix in a medium containing BMP inhibitor and GSK3β inhibitor.

3. The method according to claim 1 or 2, wherein said neurotrophic factor is BDNF and GDNF.

4. The method according to any one of claims 1 to 3, wherein the medium in Step (iii) further comprises B27 supplement, ascorbic acid, and dibutyryl cyclic AMP.

5. The method according to any one of claims 1 to 4, wherein said Step (i) is carried out for at least 10 days.

6. The method according to any one of claims 1 to 5, wherein said Step (i) is carried out for 12 days to 21 days.

7. The method according to any one of claims 1 to 6, wherein said Step (iii) is carried out for at least 7 days.

8. The method according to any one of claims 1 to 7, wherein said Step (iii) is carried out for 14 days to 30 days.

9. The method according to any one of claims 1 to 8, wherein said extracellular matrix is laminin or a fragment(s) thereof.

10. The method according to any one of claims 1 to 9, wherein said extracellular matrix is laminin 511E8.

11. The method according to any one of claims 1 to 10, wherein said substance which binds to Corin or said substance

which binds to Lrtm1 is an antibody or an aptamer which binds to Corin or Lrtm1.