as a marker of dopaminergic neuron cells, or dopaminergic neuron progenitor cells has been proposed (Patent Literature 1). However, further improvement is required to reduce the influence of lot-to-lot variation due to inclusion of biological components, and to enhance production efficiency.

Citation List

Patent Literature

[0005]

[Patent Literature 1] WO 2015/34012

Non Patent Literature

[0006]

[Non Patent Literature 1] Wernig M, et al., Proc Natl Acad Sci USA, 2008, 105: 5856-5861

Summary

[0006a]

In a first aspect of the present disclosure, there is provided a method for producing a cell population comprising Corin positive and/or Lrtm1 positive cells comprising the following steps (1) and (2):

(1) a step of performing adhesion culture of pluripotent stem cells in a medium for maintaining undifferentiated state containing a Sonic hedgehog (SHH) signal stimulant, and an undifferentiated state-maintaining factor in the absence of feeder cells but in the presence of an extracellular matrix, and

(2) a step of culturing the cells obtained in Step (1) in a medium containing one or more differentiation-inducing factors.

[0006b]

In a second aspect of the present disclosure, there is provided a method for producing dopaminergic neuron progenitor cells comprising the following steps:

(3) a step of collecting Corin positive and/or Lrtm1 positive cells from the cell population obtained by the producing method according to any one of claims 1 to 14 using a substance that binds to Corin and/or a substance that binds to Lrtm1, and

(4) a step of performing suspension culture of the Corin positive and/or Lrtm1 positive cells obtained in Step (3) in a medium containing one or more neurotrophic factors.

[0006c]

In a third aspect of the present disclosure, there is provided a cell population obtained by the producing method of the first aspect, having the content of Corin- positive and/or Lrtm1 positive cells of 10% or more.

[0006d]

In a fourth aspect of the present disclosure, there is provided a dopaminergic neuron progenitor cell obtained by the producing method according to the second aspect.

[0006e]

In a fifth aspect of the present disclosure, there is provided a therapeutic agent for use in treating Parkinson's disease comprising a dopaminergic neuron progenitor cell obtained by the producing method according to the second aspect.

[0006f]

In a sixth aspect of the present disclosure, there is provided a use of a therapeutic agent comprising a dopaminergic neuron progenitor cell obtained by the producing method according to the second aspect, in the manufacture of a medicament for treating Parkinson's disease.

Technical Problem

[0007]

An object of the present invention is to produce dopaminergic neuron progenitor cells suitable for a therapeutic agent for Parkinson's disease. Therefore, one aspect of the present invention is to provide a method for producing dopaminergic neuron progenitor cells, and the like.

Solution to Problem

[0008]

The present inventors made investigations to achieve the above object focusing attention on Corin and/or Lrtm1 that are cell surface membrane proteins and found a method for producing efficiently a cell population comprising cells that express such proteins. Further, the inventors also found that dopaminergic neuron progenitor cells can be obtained by extracting cells using Corin and/or Lrtm1 as an index from the cell population, and culturing the obtained cells, thereby completed the present invention.

[0009]

The present invention includes the followings.

[1] A method for producing a cell population comprising Corin positive and/or Lrtm1 positive cells comprising the steps (1) and (2):

(1) a step of performing adhesion culture of pluripotent stem cells in a medium

24 hours before the start of differentiation induction by exchange with a differentiation medium, the medium was exchanged with StemFit AK03N (Ajinomoto Healthy Supply Co., Inc.), which was a medium for maintaining undifferentiated state containing SAG (Enzo Life Sciences, Inc., 300 nM), an SHH signal stimulant, to obtain a cell population comprising iPS cells.

The cell population comprising iPS cells after culturing for 24 hours in the presence of SAG as described above was dissociated using TrypLE CTS (Life Technologies), and the cells were inoculated on a 6-well plate coated with Laminin-511 E8 (iMatrix-511, Nippi, Inc.) prepared separately, in an amount of 5×10^6 cells per well. The medium was replaced with a differentiation medium [a basal medium A [GMEM (Invitrogen) supplemented with 8% KSR (Invitrogen), 1 mM Sodium pyruvate (Invitrogen), 0.1 mM MEM nonessential amino acid (Invitrogen), and 0.1 mM 2-Mercaptoethanol (WAKO)] containing 10 μM Y-27632 (WAKO), 0.1 μM LDN 193189 (Stemgent, Inc.), and 0.5 μM A83-01 (WAKO)]. On the next day (Day 1), the medium was replaced with Basal Medium A supplemented with 0.1 μM LDN193189, 0.5 μM A83-01, 2 μM Purmorphamine (WAKO), and 100 ng/mL FGF8 (WAKO). Two days later (Day 3), the medium was replaced with Basal Medium A supplemented with 0.1 μM LDN193189, 0.5 μM A83-01, 2 μM Purmorphamine, 100 ng/mL FGF8, and 3 μM CHIR99021 (WAKO). Four days later (Day 7), the medium was replaced with Basal Medium A supplemented with 0.1 μM LDN193189 and 3 μM CHIR99021. During the above period, the medium was replaced once a day. A flow cytometry analysis using an anti-Corin antibody was performed on the 12th day of the start of differentiation induction (Day 12) (Fig. 1).

An anti-Corin antibody was prepared by the following method. First, from the Corin gene of Macaca fascicularis, a gene sequence encoding a part of the extracellular domain (79th-453rd amino acids) was introduced into 293E cells, and the cells were then allowed to express the extracellular-domain fragment of Corin protein, followed by collecting the protein. Mice were immunized with the recovered protein, and lymphocytes were then extracted and fused with myeloma cells. From the cell population after the fusion, a clone having reactivity with Corin was selected. The culture supernatant of the clone was used after attaching a fluorescent label as the

16. The producing method according to claim 15, wherein the substance that binds to Corin or the substance that binds to Lrtm1 is an antibody or aptamer that binds to Corin or Lrtm1.

17. The producing method according to claim 15 or 16, wherein the neurotrophic factors are BDNF and GDNF.

18. The producing method according to any one of claims 15 to 17, wherein the medium containing a neurotrophic factor further contains B27 Supplement, ascorbic acid, and a cAMP analog.

19. The producing method according to claim 18, wherein the cAMP analogue is Dibutyryl cyclic AMP.

20. The producing method according to any one of claims 15 to 19, wherein the suspension culture in a medium containing a neurotrophic factor is performed for at least 7 days.

21. A cell population obtained by the producing method according to any one of claims 1 to 14, having the content of Corin- positive and/or Lrtm1 positive cells of 10% or more.

22. A dopaminergic neuron progenitor cell obtained by the producing method according to any one of claims 15 to 20.

23. A therapeutic agent for use in treating Parkinson's disease comprising a dopaminergic neuron progenitor cell obtained by the producing method according to any one of claims 15 to 20.

24. Use of a therapeutic agent comprising a dopaminergic neuron progenitor cell obtained by the producing method according to any one of claims 15 to 20, in the manufacture of a medicament for treating Parkinson's disease.