Claims

[Claim 1]

A method for producing neural crest cells, comprising the steps of:

(1) obtaining neural crest cells; and

(2) suspension-culturing the neural crest cells for a period of 35 days or longer in a medium comprising a GSK3β inhibitor and a basic fibroblast growth factor, wherein the medium comprises

(a) the GSK3β inhibitor with a concentration that exhibits an effect equivalent to that exhibited by CHIR99021 with a concentration of 2 μM or higher and lower than 5 μM, wherein the effect is evaluated on the basis of GSK3β inhibitory activity; or

(b) CHIR99021 with a concentration of 2 μM or higher and lower than 5 μM as the GSK3β inhibitor; or

(c) CP21R7 with a concentration of 0.5 μM or higher and 1 μM or lower as the GSK3β inhibitor.

[Claim 2]

The production method according to claim 1, wherein the medium further comprises a TGFβ inhibitor.

[Claim 3]

The production method according to claim 1, wherein the medium is Chemically Defined Medium (CDM).

[Claim 4]

The production method according to claim 1, wherein the medium further comprises an epidermal growth factor.

[Claim 5]

The production method according to claim 1, wherein in the medium
comprising (a), the GSK3β inhibitor is at least one member selected from the group consisting of CHIR99021, CP21R7, CHIR98014, LY2090314, kenpaullone, AR-AO144-18, TDZD-8, SB216763, BIO, TWS-119 and SB415286.

[Claim 6]

The production method according to claim 5, wherein the GSK3β inhibitor is CHIR99021.

[Claim 7]

The production method according to claim 2, wherein the TGFβ inhibitor is at least one member selected from the group consisting of SB431542, A83-01, LDN193189, Wnt3a/BIO, BMP4, GW788388, SM16, IN-1130, GW6604 and SB505124.

[Claim 8]

The production method according to claim 1, wherein in the step (2), the neural crest cells are passaged every 5 to 8 days after inoculation.

[Claim 9]

The production method according to claim 1, wherein the step (1) is the step of inducing the differentiation of stem cells into the neural crest cells.

[Claim 10]

A method for proliferating neural crest cells, comprising the step of:

(I) suspension-culturing the neural crest cells for a period of 35 days or longer in a medium comprising a GSK3β inhibitor and a basic fibroblast growth factor, wherein the medium comprises

(a) the GSK3β inhibitor with a concentration that exhibits an effect equivalent to that exhibited by CHIR99021 with a concentration of 2 μM or higher and lower than 5 μM, wherein the effect is evaluated on the basis of GSK3β inhibitory activity; or

(b) CHIR99021 with a concentration of 2 μM or higher and lower than 5 μM as the GSK3β inhibitor; or

(c) CP21R7 with a concentration of 0.5 μM or higher and 1 μM or lower as the GSK3β inhibitor.

[Claim 11]

A medium for producing and proliferating neural crest cells for a period of 35 days or longer comprising a GSK3β inhibitor,

a basic fibroblast growth factor and neural crest cells, wherein the medium comprises

(a) the GSK3β inhibitor with a concentration that exhibits an effect equivalent to that exhibited by CHIR99021 with a concentration of 2 μM or higher and lower than 5 μM, wherein the effect is evaluated on the basis of GSK3β inhibitory activity; or

(b) CHIR99021 with a concentration of 2 μM or higher and lower than 5 μM as the GSK3β inhibitor; or

(c) CP21R7 with a concentration of 0.5 μM or higher and 1 μM or lower as the GSK3β inhibitor.

[Claim 12]

The medium according to claim 11, further comprising a TGFβ inhibitor.

[Claim 13]

The medium according to claim 11, wherein the medium is Chemically Defined Medium (CDM).

[Claim 14]

The medium according to claim 11, further comprising an epidermal growth factor.

[Claim 15]

The medium according to claim 11, wherein in the medium comprising (a), the GSK3β inhibitor is at least one member selected from the group consisting of CHIR99021, CP21R7, CHIR98014, LY2090314, kenpaullone, AR-AO144-18, TDZD-8, SB216763, BIO, TWS-119 and SB415286.

[Claim 16]

The medium according to claim 15, wherein the GSK3β inhibitor is CHIR99021.

[Claim 17]

The medium according to claim 12, wherein the TGFβ inhibitor is at least one member selected from the group consisting of SB431542, A83-01, LDN193189, Wnt3a/BIO, BMP4, GW788388, SM16, IN-1130, GW6604 and SB505124.

[Claim 18]

A method for producing nerve cells, glial cells, mesenchymal stromal cells, bone cells, chondrocytes, corneal cells or pigment cells, comprising the steps of:

(i) suspension-culturing neural crest cells for a period of 35 days or longer in a medium comprising a GSK3β inhibitor and a basic fibroblast growth factor, wherein the medium comprises

(a) the GSK3β inhibitor with a concentration that exhibits an effect equivalent to that exhibited by CHIR99021 with a concentration of 2 μM or higher and lower than 5 μM, wherein the effect is evaluated on the basis of GSK3β inhibitory activity; or

(b) CHIR99021 with a concentration of 2 μM or higher and lower than 5 μM as the GSK3β inhibitor; or

(c) CP21R7 with a concentration of 0.5 μM or higher and 1 μM or lower as the GSK3β inhibitor; and

(ii) differentiating the neural crest cells obtained in the step (i) into cells of at least one lineage selected from the group consisting of nerve cells, glial cells, mesenchymal stromal cells, bone cells, chondrocytes, corneal cells and pigment cells.

[Claim 19]

A method for culturing neural crest cells having multipotency for a period of 35 days or longer, comprising the steps of:

(1) obtaining neural crest cell; and

(2) suspension-culturing the neural crest cells in a medium comprising a GSK3β inhibitor and a basic fibroblast growth factor, wherein the medium comprises

(a) the GSK3β inhibitor with a concentration that exhibits an effect equivalent to that exhibited by CHIR99021 with a concentration of 2 μM or higher and lower than 5 μM, wherein the effect is evaluated on the basis of GSK3β inhibitory activity; or

(b) CHIR99021 with a concentration of 2 μM or higher and lower than 5 μM as the GSK3β inhibitor; or

(c) CP21R7 with a concentration of 0.5 μM or higher and 1 μM or lower as the GSK3β inhibitor.

[Claim 20]

Use of a medium for culturing neural crest cells having multipotency
for a period of 35 days or longer, wherein the medium comprises a basic fibroblast growth factor and

(a) a GSK3β inhibitor with a concentration that exhibits an effect equivalent to that exhibited by CHIR99021 with a concentration of 2 μM or higher and lower than 5 μM, wherein the effect is evaluated on the basis of GSK3β inhibitory activity; or

(b) CHIR99021 with a concentration of 2 μM or higher and lower than 5 μM as the GSK3β inhibitor; or

(c) CP21R7 with a concentration of 0.5 μM or higher and 1 μM or lower as the GSK3β inhibitor.