Claims

[Claim 1]

A method for producing neural crest cells and maintaining their multipotency, comprising the steps of:

(1) obtaining neural crest cells by inducing the differentiation of stem cells into the 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~~ 3.0 μM ~~or higher and lower than~~ to 4.5 μM, ~~wherein the effect is evaluated on the basis of GSK3β inhibitory activity~~thereby maintaining multipotent neural crest cells over 63 days or longer, wherein the cultured neural crest cells comprise a ratio of at least 75% or more of neural crest cells that maintain multipotency to cultured cell population~~; 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~~9]

A method for proliferating neural crest cells and maintaining their multipotency, 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~~ 3.0 μM ~~or higher and lower than~~ to 4.5 μM, thereby maintaining multipotent neural crest cells over 63 days or longer, wherein the cultured neural crest cells comprise a ratio of at least 75% or more of neural crest cells that maintain multipotency to cultured cell population~~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~~10]

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~~ 3.0 μM or higher and lower than 4.5 μM, thereby maintaining multipotent neural crest cells over 63 days or longer, wherein the cultured neural crest cells comprise a ratio of at least 75% or more of neural crest cells that maintain multipotency to cultured cell population~~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~~; 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~~11]

A method for culturing neural crest cells and extending their ~~having~~ multipotency for a period of ~~35~~ 63 days or longer, comprising the steps of:

(1) obtaining neural crest cell by inducing the differentiation of stem cells into the neural crest cells; 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~~ 3.0 μM ~~or higher and lower than~~ to 4.5 μM, thereby maintaining multipotent neural crest cells over 63 days or longer, wherein the cultured neural crest cells comprise a ratio of at least 75% or more of neural crest cells that maintain multipotency to cultured cell population~~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.~~

[Claim 12]

The production method according to claim 1, wherein the cultured neural crest cells that maintain multipotency over 77 days or longer, and wherein the cultured neural crest cells comprise a

ratio of at least 80% or more of neural crest cells that maintain multipotency to cultured cell population.

[Claim 13]
The method according to claim 9, wherein the cultured neural crest cells that maintain multipotency over 77 days or longer, and wherein the cultured neural crest cells comprise a ratio of at least 80% or more of neural crest cells that maintain multipotency to cultured cell population.

[Claim 14]
The method according to claim 11, wherein the cultured neural crest cells that maintain multipotency over 77 days or longer, and wherein the cultured neural crest cells comprise a ratio of at least 80% or more of neural crest cells that maintain multipotency to cultured cell population.

[Claim 15]
The production method according to claim 1, wherein the cultured neural crest cells that maintain multipotency over 121 days or longer, and wherein the cultured neural crest cells comprise a ratio of at least 90% or more of neural crest cells that maintain multipotency to cultured cell population.

[Claim 16]
The method according to claim 9, wherein the cultured neural crest cells that maintain multipotency over 121 days or longer, and wherein the cultured neural crest cells comprise a ratio of

at least 90% or more of neural crest cells that maintain multipotency to cultured cell population.

[Claim 17]

The method according to claim 11, wherein the cultured neural crest cells that maintain multipotency over 121 days or longer, and wherein the cultured neural crest cells comprise a ratio of at least 90% or more of neural crest cells that maintain multipotency to cultured cell population.