Claims

1. A method for producing a cell mass comprising human pituitary tissue, comprising the following steps (1) and (2):

(1) a first step of suspension-culturing pluripotent stem cells to form a cell aggregate in the presence of a Wnt signal transduction pathway inhibiting substance,

(2) a second step of suspension-culturing the aggregate obtained in the first step in the presence of a BMP signal transduction pathway activating substance at a concentration showing BMP signal transduction promoting activity corresponding to that of 10 pM to 5 nM BMP4 within 0.5hr to 72 hr from the start of the suspension culturing in the first step and a Sonic hedgehog signal transduction pathway activating substance at a concentration having Sonic hedgehog signal transduction promoting activity corresponding to that of 100 nM to 3 μM SAG, thereby obtaining a cell mass comprising human pituitary tissue,

wherein the Wnt signal transduction pathway inhibiting substance is selected from Porcupine inhibitor (PORCN inhibitor), DKK inhibitor, Frizzled inhibitor, Dvl inhibitor, Tankyrase inhibitor (TANK inhibitor), casein kinase 1 inhibitor, catenin responsive transcription inhibitor, p300 inhibitor, CBP inhibitor and BCL-9 inhibitor,

wherein the BMP signal transduction pathway activating substance is selected from BMP2, BMP4, BMP7 and GDF7, and

wherein the Sonic hedgehog signal transduction pathway activating substance is selected from protein belonging to the Hedgehog family, Shh receptor, Shh receptor agonist, Smo

agonist, Purmorphamine, GSA-10, Hh-Ag1.5, 20(S)-hydroxycholesterol and SAG.

2. A method for producing a cell mass comprising human pituitary tissue, comprising the following steps (a), (1) and (2):
(a) step a of culturing pluripotent stem cells for 2hr to 96hr in the absence of feeder cells and in a medium containing 1) a TGFβsignal transduction pathway inhibiting substance at a concentration showing TGFβ signal transduction pathway inhibiting activity corresponding to that of 1 nM to 100 μM SB431542 and/or a Sonic hedgehog signal transduction pathway activating substance at a concentration having Sonic hedgehog signal transduction promoting activity corresponding to that of 10 nM to 700 nM SAG, and 2) a factor for maintaining an undifferentiated state,
(1) a first step of suspension-culturing the pluripotent stem cells, which were cultured in step a, to form a cell aggregate in the presence of a Wnt signal transduction pathway inhibiting substance,
(2) a second step of suspension-culturing the aggregate obtained in the first step in the presence of a BMP signal transduction pathway activating substance at a concentration showing BMP signal transduction promoting activity corresponding to that of 10 pM to 5 nM BMP4 within 0.5hr to 72 hr from the start of the suspension culturing in the first step and a Sonic hedgehog signal transduction pathway activating substance at a concentration having Sonic hedgehog signal transduction promoting activity corresponding to that of 100 nM to 3 μM SAG, thereby obtaining a cell mass comprising human pituitary tissue,

wherein the factor for maintaining undifferentiated state includes at least bFGF,

wherein the TGFβ signal transduction pathway inhibiting substance is Alk5/TGFβR1 inhibitor or SMAD3 inhibitor,

wherein the Wnt signal transduction pathway inhibiting substance is selected from Porcupine inhibitor (PORCN inhibitor), DKK inhibitor, Frizzled inhibitor, Dvl inhibitor, Tankyrase inhibitor (TANK inhibitor), casein kinase 1 inhibitor, catenin responsive transcription inhibitor, p300 inhibitor, CBP inhibitor and BCL-9 inhibitor,

wherein the BMP signal transduction pathway activating substance is selected from BMP2, BMP4, BMP7 and GDF7, and

wherein the Sonic hedgehog signal transduction pathway activating substance is selected from protein belonging to the Hedgehog family, Shh receptor, Shh receptor agonist, Smo agonist, Purmorphamine, GSA-10, Hh-Ag1.5, 20(S)-hydroxycholesterol and SAG.

3. A method for producing a cell mass comprising human pituitary tissue, comprising the following steps (1), (2-1) and (2-2):

(1) a first step of suspension-culturing pluripotent stem cells to form a cell aggregate in the presence of a Wnt signal transduction pathway inhibiting substance,

(2-1) a second step (1) of suspension-culturing the aggregate obtained in the first step in the presence of a BMP signal transduction pathway activating substance at a concentration showing BMP signal transduction promoting activity corresponding to that of 10 pM to 5 nM BMP4 within 0.5hr to 72 hr from the start of the suspension culturing in

the first step and a Sonic hedgehog signal transduction pathway activating substance at a concentration having Sonic hedgehog signal transduction promoting activity corresponding to that of 100 nM to 3 μM SAG,

(2-2) a second step (2) of suspension-culturing the aggregate suspension-cultured in the second step (1) in the presence of a BMP signal transduction pathway inhibiting substance after 12 hr and within 20 days from the start of the suspension culturing in the second step (1), thereby obtaining a cell mass comprising human pituitary tissue,

wherein the Wnt signal transduction pathway inhibiting substance is selected from Porcupine inhibitor (PORCN inhibitor), DKK inhibitor, Frizzled inhibitor, Dvl inhibitor, Tankyrase inhibitor (TANK inhibitor), casein kinase 1 inhibitor, catenin responsive transcription inhibitor, p300 inhibitor, CBP inhibitor and BCL-9 inhibitor,

wherein the BMP signal transduction pathway activating substance is selected from BMP2, BMP4, BMP7 and GDF7,

wherein the Sonic hedgehog signal transduction pathway activating substance is selected from protein belonging to the Hedgehog family, Shh receptor, Shh receptor agonist, Smo agonist, Purmorphamine, GSA-10, Hh-Ag1.5, 20(S)-hydroxycholesterol and SAG, and

wherein the BMP signal transduction pathway inhibiting substance is a type I BMP receptor inhibitor.

4. A method for producing a cell mass comprising human pituitary tissue, comprising the following steps (a), (1), (2-1) and (2-2):

(a) step a of culturing pluripotent stem cells in the absence of feeder cells and in a medium containing 1) a TGFβ

signal transduction pathway inhibiting substance and/or a Sonic hedgehog signal transduction pathway activating substance, and 2) a factor for maintaining an undifferentiated state, (1) a first step of suspension-culturing the pluripotent stem cells, which were cultured in step a, to form a cell aggregate in the presence of a Wnt signal transduction pathway inhibiting substance,
(2-1) a second step (1) of suspension-culturing the aggregate obtained in the first step in the presence of a BMP signal transduction pathway activating substance at a concentration showing BMP signal transduction promoting activity corresponding to that of 10 pM to 5 nM BMP4 within 0.5hr to 72 hr from the start of the suspension culturing in the first step and a Sonic hedgehog signal transduction pathway activating substance at a concentration having Sonic hedgehog signal transduction promoting activity corresponding to that of 100 nM to 3 μM SAG,
(2-2) a second step (2) of suspension-culturing the aggregate suspension-cultured in the second step (1) in the presence of a BMP signal transduction pathway inhibiting substance after 12 hr and within 20 days from the start of the suspension culturing in the second step (1), thereby obtaining a cell mass comprising human pituitary tissue,

wherein the factor for maintaining undifferentiated state includes at least bFGF,

wherein the TGFβ signal transduction pathway inhibiting substance is Alk5/TGFβR1 inhibitor or SMAD3 inhibitor,

wherein the Wnt signal transduction pathway inhibiting substance is selected from Porcupine inhibitor (PORCN inhibitor), DKK inhibitor, Frizzled inhibitor, Dvl inhibitor, Tankyrase inhibitor (TANK inhibitor), casein

kinase 1 inhibitor, catenin responsive transcription inhibitor, p300 inhibitor, CBP inhibitor and BCL-9 inhibitor,

wherein the BMP signal transduction pathway activating substance is selected from BMP2, BMP4, BMP7 and GDF7,

wherein the Sonic hedgehog signal transduction pathway activating substance is selected from protein belonging to the Hedgehog family, Shh receptor, Shh receptor agonist, Smo agonist, Purmorphamine, GSA-10, Hh-Ag1.5, 20(S)-hydroxycholesterol and SAG, and

wherein the BMP signal transduction pathway inhibiting substance is a type I BMP receptor inhibitor.

5. The production method according to any one of claims 1 to 4, wherein the BMP signal transduction pathway activating substance in the step (2) or step (2-1) is added during a period when not less than 10% of the cells of the surface layer of the aggregate formed in the step (1) form a tight junction.

6. The production method according to any one of claims 1 to 5, wherein the Wnt signal transduction pathway inhibiting substance is a PORCN inhibitor, TANK inhibitor, KY-02111 or KY-03-I.

7. The production method according to claim 6, wherein the PORCN inhibitor is at least one kind of compound selected from the group consisting of IWP-2, IWP-3, IWP-4, IWP-L6, IWP-12, LGK-974, Wnt-C59, ETC-159, and GNF-6231, and the TANK inhibitor is at least one kind of compound selected from the group consisting of IWR1-endo, XAV939, and MN-64.

8. The production method according to any one of claim 1 to 7, wherein the Sonic hedgehog signal transduction pathway activating substance is at least one kind of compound selected from the group consisting of SAG, Purmorphamine, and GSA-10.

9. The production method according to any one of claims 1 to 8, wherein the suspension culturing in the steps (1), (2), (2-1) and (2-2) is performed in the further presence of Alk5/TGFβR1 inhibitor.

10. The production method according to any one of claims 2 and 4 to 8, wherein the Alk5/TGFβR1 inhibitor is at least one kind of compound selected from the group consisting of SB431542, SB505124, SB525334, LY2157299, GW788388, LY364947, SD-208, EW-7197, A 83-01, and RepSox, and the SMAD3 inhibitor is SIS3.

11. The production method according to any one of claims 3 to 9, wherein the type I BMP receptor inhibitor is at least one selected from the group consisting of K02288, Dorsomorphin, LDN-193189, LDN-212854, LDN-214117, ML347, DMH1, and DMH2.

12. The production method according to any one of claims 1 to 11, wherein at least one step selected from the group consisting of the steps (1), (2), (2-1) and (2-2) is performed in the presence of an FGF signal transduction pathway activating substance comprising at least one selected from the group consisting of FGF2 and FGF8, and variants of these.

13. The production method according to any one of claims 1 to 12, wherein at least one step selected from the group consisting of the steps (1), (2), (2-1) and (2-2) is performed in the presence of a Wnt signal transduction pathway activating substance,

wherein the Wnt signal transduction pathway activating substance is a substance that activates a canonical Wnt pathway.

14. The production method according to claim 13, wherein the substance that activates the canonical Wnt pathway is a GSK3 inhibitor.

15. The production method according to claim 14, wherein the GSK3 inhibitor comprises at least one selected from the group consisting of CHIR99021, CHIR98014, TWS119, SB216763, SB415286, BIO, AZD2858, AZD1080, AR-A014418, TDZD-8, LY2090314, IM-12, Indirubin, Bikinin, A 1070722, 3F8, Kenpaullone, 10Z-Hymenialdisine, Indirubin-3'-oxime, NSC 693868, TC-G 24, TCS 2002, TCS 21311, CP21R7 and derivatives of these compounds.

16. The production method according to any one of claims 1 to 15, wherein the suspension culturing in at least one step selected from the group consisting of the steps (1), (2), (2-1) and (2-2) is performed in the further presence of a γ secretase inhibitor.

17. The production method according to any one of claims 1 to 16, wherein the suspension culturing in at least one step selected from the group consisting of the steps (1), (2),

(2-1) and (2-2) is performed in the further presence of dexamethasone.

18. A method for producing a human pituitary tissue, comprising the following step (1) and (2):
(1) a step of obtaining a cell mass comprising human pituitary tissue by the production method according to any one of claims 1 to 17,
(2) a second step of collecting human pituitary tissue from the cell mass obtained in the first step.