Claims

1. A method for producing a cell mass comprising pituitary tissue, comprising the following steps (1) and (2):
(1) a first step of dispersing pluripotent stem cells into single cells and suspension-culturing the dispersed pluripotent stem cells for about 12 hr to 48 hr to form a cell aggregate in the presence of a non-canonical 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 and a Sonic hedgehog signal transduction pathway activating substance,
thereby obtaining a cell mass comprising pituitary tissue,
wherein the BMP signal transduction pathway activating substance in the step (2) is added within 0.5hr to 72 hr from the start of the suspension culturing of the pluripotent stem cells in the step (1),
wherein the non-canonical Wnt signal transduction pathway inhibiting substance is selected from a group consisting of PORCN inhibitor, DKK inhibitor, Frizzled inhibitor, Dvl inhibitor, Tankyrase 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 a group consisting of BMP2, BMP4, BMP7 and GDF7, and
wherein the Sonic hedgehog signal transduction pathway activating substance is selected from a group consisting of 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 pituitary

tissue, comprising the following steps (a), (1) and (2):
(a) step a of culturing pluripotent stem cells in the absence of feeder cells and in a medium containing 1) a TGFβ family 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 dispersing pluripotent stem cells into single cells and suspension-culturing the dispersed pluripotent stem cells, which were cultured in step a, for about 12 hr to 48 hr to form a cell aggregate in the presence of a non-canonical 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 and a Sonic hedgehog signal transduction pathway activating substance, thereby obtaining a cell mass comprising pituitary tissue,

wherein the BMP signal transduction pathway activating substance in the step (2) is added within 0.5hr to 72 hr from the start of the suspension culturing of the pluripotent stem cells in the step (1),

wherein the non-canonical Wnt signal transduction pathway inhibiting substance is selected from a group consisting of PORCN inhibitor, DKK inhibitor, Frizzled inhibitor, Dvl inhibitor, Tankyrase 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 a group consisting of BMP2, BMP4, BMP7 and GDF7,

wherein the Sonic hedgehog signal transduction pathway activating substance is selected from a group consisting of 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 TGFβ family signal transduction pathway inhibiting substance is selected from a group consisting of Alk5/TGFβR1 inhibitor and SMAD3 inhibitor.

3. A method for producing a cell mass comprising pituitary tissue, comprising the following steps (1), (2-1) and (2-2):
(1) a first step of dispersing pluripotent stem cells into single cells and suspension-culturing the dispersed pluripotent stem cells for about 12 hr to 48 hr to form a cell aggregate in the presence of a non-canonical 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 and a Sonic hedgehog signal transduction pathway activating substance,
(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, thereby obtaining a cell mass comprising pituitary tissue,

wherein the BMP signal transduction pathway activating substance in the step (2-1) is added within 0.5hr to 72 hr from the start of the suspension culturing of the pluripotent stem cells in the step (1),

wherein the non-canonical Wnt signal transduction pathway inhibiting substance is selected from a group consisting of PORCN inhibitor, DKK inhibitor, Frizzled inhibitor, Dvl inhibitor, Tankyrase 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 a group consisting of BMP2, BMP4, BMP7 and GDF7,

wherein the Sonic hedgehog signal transduction pathway activating substance is selected from a group consisting of

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 type I BMP receptor inhibitor.

4. A method for producing a cell mass comprising 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 family 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 dispersing pluripotent stem cells into single cells and suspension-culturing the dispersed pluripotent stem cells, which were cultured in step a, for about 12 hr to 48 hr to form a cell aggregate in the presence of a non-canonical 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 and a Sonic hedgehog signal transduction pathway activating substance,

(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, thereby obtaining a cell mass comprising pituitary tissue,

wherein the BMP signal transduction pathway activating substance in the step (2-1) is added within 0.5hr to 72 hr from the start of the suspension culturing of the pluripotent stem cells in the step (1),

wherein the non-canonical Wnt signal transduction

pathway inhibiting substance is selected from a group consisting of PORCN inhibitor, DKK inhibitor, Frizzled inhibitor, Dvl inhibitor, Tankyrase 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 a group consisting of BMP2, BMP4, BMP7 and GDF7,

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

wherein the TGFβ family signal transduction pathway inhibiting substance is selected from a group consisting of Alk5/TGFβR1 inhibitor and SMAD3 inhibitor, and

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

5. The production method according to any one of claims 1 to 4, wherein the non-canonical Wnt signal transduction pathway inhibiting substance is a PORCN inhibitor.

6. The production method according to claim 5, 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, ETC-159, GNF-6231 and Wnt-C59.

7. The production method according to any one of claims 1 to 6, wherein the suspension culturing in the steps (1), (2), (2-1) and (2-2) is performed in the further presence of a TGFβ signal transduction pathway inhibiting substance.

8. The production method according to any one of claims 1 to 7, 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,

wherein the FGF signal transduction pathway activating substance is selected from a group consisting of FGF1, FGF2 and FGF8.

9. The production method according to any one of claims 1 to 8, 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 non-canonical Wnt signal transduction pathway activating substance.

10. The production method according to any one of claims 1 to 9, 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~~Notch signal~~ inhibitor.

11. The production method according to any one of claims 1 to 10, 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.

12. The production method according to any one of claims 1 to 11, wherein the cell mass comprising pituitary tissue comprises 1) nonneural epithelium, 2) neuroepithelium, and 3) mesenchymal cells,

wherein the cell mass has an outside covered by the nonneural epithelium, ventral diencephalon or hypothalamus-like neuroepithelial tissue having a ventricle-like structure in the inside, and cranial mesenchymal cell present between the nonneural epithelium and the neuroepithelium, and

wherein a part of the outer nonneural epithelium is pituitary placode.

13. A method for producing a pituitary tissue, comprising the following steps (1) - (3):
(1) a first step of dispersing pluripotent stem cells into single cells and suspension-culturing the dispersed pluripotent stem cells for about 12 hr to 48 hr to form a cell aggregate in the presence of a non-canonical 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 and a Sonic hedgehog signal transduction pathway activating substance, thereby obtaining a cell mass comprising pituitary tissue,
(3) a third step of collecting pituitary tissue from the cell mass obtained in the second step,
wherein the BMP signal transduction pathway activating substance in the step (2) is added within 0.5hr to 72 hr from the start of the suspension culturing of the pluripotent stem cells in the step (1).

14. A method for producing a pituitary tissue, comprising the following steps (1), (2-1), (2-2) and (3):
(1) a first step of dispersing pluripotent stem cells into single cells and suspension-culturing the dispersed pluripotent stem cells for about 12 hr to 48 hr to form a cell aggregate in the presence of a non-canonical 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 and a Sonic hedgehog signal transduction pathway activating substance,
(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, thereby obtaining a cell mass comprising

pituitary tissue,
(3) a third step of collecting pituitary tissue from the cell mass obtained in the second step (2),
wherein the BMP signal transduction pathway activating substance in the step (2-1) is added within 0.5hr to 72 hr from the start of the suspension culturing of the pluripotent stem cells in the step (1).

~~15. A pituitary tissue obtained by the production method according to claim 13 or 14.~~

~~16~~15. A cell mass comprising 1) neural cells or neural tissue, 2) nonneural epithelial tissue comprising pituitary tissue and 3) mesenchymal cells,
wherein the mesenchymal cells are present between nonneural epithelial tissue coating a surface of the cell mass and the neural cells or neural tissue present inside the cell mass.

~~17~~16. A cell mass comprising 1) neural cells or neural tissue, 2) nonneural epithelial tissue comprising pituitary tissue and 4) neural crest cells ~~and~~ or neural crest-derived cells,
wherein the neural crest cells or neural crest-derived cells are present between nonneural epithelial tissue coating a surface of the cell mass and the neural cells or neural tissue present inside the cell mass.

17. The cell mass according to claim 15, wherein the mesenchymal cells express at least one marker selected from a group consisting of Nestin, Vimentin, Cadherin-11, Laminin, CD44, CD90 and CD105.

18. The cell mass according to claim 16, wherein the neural crest cells or neural crest-derived cells express at least one marker selected from a group consisting of Nestin,

Sox10, Slug, Snail, Pax3, Zic1, FoxD3, p75NTR, HNK-1, CHD7, Numb and Ascl1.

~~18. A therapeutic drug for a disease due to a disorder of pituitary gland, comprising a cell mass produced by the method according to any one of claims 1 to 12 or a pituitary tissue produced by the method according to claim 13 or 14.~~

19. ~~A~~ The cell mass according to claim ~~16~~15, ~~wherein the cell mass comprises 1) nonneural epithelium, 2) neuroepithelium and 3) mesenchymal cells,~~

wherein the cell mass has an outside covered by the nonneural epithelium, ventral diencephalon-like or hypothalamus-like neuroepithelial tissue having a ventricle-like structure in the inside, and cranial mesenchymal cell present between the nonneural epithelium and the neuroepithelium, and

wherein a part of the outer nonneural epithelium is pituitary placode.

20. A therapeutic drug for a disease due to a disorder of pituitary gland, comprising the cell mass according to any one of claims 15 to 19.

21. A method for producing a pituitary tissue comprising a step of collecting pituitary tissue from the cell mass according to any one of claims 15 to 19.