

Claims

1. A method of producing pancreatic hormone-producing cells,
comprising subjecting human iPS cells or human ES cells~~stem cells~~ to
5 the following steps (1) - (6):
- (1) a step of cultivating ~~stem cells~~ human iPS cells or human ES
cells in a medium containing a Rho kinase inhibitor
- (2) a step of cultivating the cells obtained in the aforementioned
step (1) in a medium containing a GSK3 inhibitor
- 10 (3) a step of cultivating the cells obtained in the aforementioned
step (2) in a medium containing GSK3 inhibitor and an activator of
activin receptor-like kinase-4,7
- (4) a step of forming a cell mass from the cells obtained in the
aforementioned (3), and cultivating the cell mass in a suspension
15 state in a medium
- (5) a step of cultivating the cells obtained in the aforementioned
step (4) in a medium containing a retinoic acid receptor agonist, an
inhibitor of AMP-activated protein kinase and/or activin receptor-
like kinase-2,3,6, an inhibitor of activin receptor-like kinase-
20 4,5,7 and a cell growth factor
- (6) a step of cultivating the cells obtained in the aforementioned
step (5), wherein
the activator of of activin receptor-like kinase-4,7 in step (3) is
activin;
- 25 the Rho kinase inhibitor in step (1) is (+)-(R)-trans-4-(1-
aminoethyl)-N-(4-pyridyl)cyclohexanecarboxamide dihydrochloride;
the GSK3 inhibitor in step (2) and (3) is (i) 6-[[2-[[4-(2,4-
dichlorophenyl)-5-(4-methyl-1H-imidazol-2-yl)-2-
pyrimidinyl]amino]ethyl]amino]nicotinonitrile and/or (ii) (2'E,3'E)-
30 6-bromoindirubin-3'-oxime;
- the retinoic acid receptor agonist in step (5) is retinoic acid;
the inhibitor of AMP-activated protein kinase and/or activin
receptor-like kinase-2,3,6 in step (5) is dorsomorphin;
the inhibitor of activin receptor-like kinase-4,5,7 in step (5) is

4-[4-(1,3-benzodioxol-5-yl)-5-(2-pyridinyl)-1H-imidazol-2-yl]-benzamide;

the cell growth factor in step (5) is basic fibroblast growth factor.

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2. The production method according to claim 1, wherein the human iPS cells or human ES cells is human iPS cells.

~~2. The production method according to claim 1, wherein the activator
10 of activin receptor-like kinase-4,7 in step (3) is activin.~~

~~3. The production method according to claim 1, wherein the Rho-kinase inhibitor in step (1) is (+)-(R)-trans-4-(1-aminoethyl)-N-(4-pyridyl)cyclohexanecarboxamide dihydrochloride.~~

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~~4. The production method according to claim 1, wherein the GSK3-inhibitor in steps (2) and (3) is (i) 6-[[2-[[4-(2,4-~~

~~dichlorophenyl)-5-(4-methyl-1H-imidazol-2-yl)-2-pyrimidinyl]amino]ethyl]amino]nicotinonitrile and/or (ii) (2'Z,3'E)-6-bromoindirubin-3'-oxime.~~

5 ~~5. The production method according to claim 1, wherein the retinoic acid receptor agonist in step (5) is retinoic acid.~~

~~6. The production method according to claim 1, wherein the inhibitor of AMP-activated protein kinase and/or activin receptor-like kinase~~
10 ~~2,3,6 in step (5) is dorsomorphin.~~

~~7. The production method according to claim 1, wherein the inhibitor of activin receptor-like kinase-4,5,7 in step (5) is 4-[4-(1,3-benzodioxol-5-yl)-5-(2-pyridinyl)-1H-imidazol-2-yl]-benzamide.~~

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~~8. The production method according to claim 1, wherein the cell growth factor in step (5) is basic fibroblast growth factor.~~

~~9. The production method according to claim 1, wherein the steps~~
20 ~~(1) - (6) do not substantially use a feeder cell.~~

~~10. The production method according to claim 1, wherein the medium in steps (1) - (6) does not substantially contain a serum.~~

25 ~~11. The production method according to claim 1, wherein the stem cells are induced pluripotent stem cells (iPS cells), embryonic stem cells (ES cells) or human somatic stem cells.~~

~~12. The production method according to claim 1, wherein the~~
30 ~~pancreatic hormone-producing cells are any selected from the group consisting of insulin-producing cells, glucagon-producing cells, somatostatin-producing cells, pancreatic~~

polypeptide (PP)-producing cells and ghrelin-producing cells.

~~613.~~ A method of producing pancreatic hormone-producing cells,
comprising subjecting endodermal cells to the following steps (4')

5 and (5')):

(4') a step of forming a cell mass from endodermal cells, and
cultivating the cell mass in a suspension state in a medium

(5') a step of cultivating the cells obtained in the aforementioned
step (4') in a medium containing a retinoic acid receptor agonist,

10 an inhibitor of AMP-activated protein kinase and/or activin
receptor-like kinase-2,3,6, an inhibitor of activin receptor-like
kinase-4,5,7 and a cell growth factor

wherein the retinoic acid receptor agonist in step (5) is retinoic
acid;

15 the inhibitor of AMP-activated protein kinase and/or activin
receptor-like kinase-2,3,6 in step (5) is dorsomorphin;

the inhibitor of activin receptor-like kinase-4,5,7 in step (5) is
4-[4-(1,3-benzodioxol-5-yl)-5-(2-pyridinyl)-1H-imidazol-2-yl]-
benzamide;

20 the cell growth factor in step (5) is basic fibroblast growth
factor.

~~14. A medicament comprising pancreatic hormone-producing cells
obtained by the production method of claim 1 or 13.~~

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~~157.~~ A method of screening for a therapeutic drug for diabetes,
comprising using the cells obtained by any one or more steps
selected from the group consisting of the following steps (1) - (6):

(1) a step of cultivating human iPS cells or human ES cells~~stem-~~

30 ~~cells~~ in a medium containing a Rho kinase inhibitor

(2) a step of cultivating the cells obtained in the aforementioned
step (1) in a medium containing a GSK3 inhibitor

(3) a step of cultivating the cells obtained in the aforementioned
step (2) in a medium containing GSK3 inhibitor and an activator of

activin receptor-like kinase-4,7

(4) a step of forming a cell mass from the cells obtained in the
aforementioned (3), and cultivating the cell mass in a suspension
state in a medium

- 5 (5) a step of cultivating the cells obtained in the aforementioned
step (4) in a medium containing a retinoic acid receptor agonist, an
inhibitor of AMP-activated protein kinase and/or activin receptor-
like kinase-2,3,6, an inhibitor of

activin receptor-like kinase-4,5,7 and a cell growth factor
 (6) a step of cultivating the cells obtained in the aforementioned
 step (5), wherein
 the activator of of activin receptor-like kinase-4,7 in step (3) is
 5 activin;
 the Rho kinase inhibitor in step (1) is (+)-(R)-trans-4-(1-
aminoethyl)-N-(4-pyridyl)cyclohexanecarboxamide dihydrochloride;
 the GSK3 inhibitor in step (2) and (3) is (i) 6-[[2-[[4-(2,4-
dichlorophenyl)-5-(4-methyl-1H-imidazol-2-yl)-2-
 10 pyrimidinyl]amino]ethyl]amino]nicotinonitrile and/or (ii) (2'Z,3'E)-
6-bromoindirubin-3'-oxime;
 the retinoic acid receptor agonist in step (5) is retinoic acid;
 the inhibitor of AMP-activated protein kinase and/or activin
receptor-like kinase-2,3,6 in step (5) is dorsomorphin;
 15 the inhibitor of activin receptor-like kinase-4,5,7 in step (5) is
4-[4-(1,3-benzodioxol-5-yl)-5-(2-pyridinyl)-1H-imidazol-2-yl]-
benzamide;
 the cell growth factor in step (5) is basic fibroblast growth
factor.