

Claims

1. A method for producing a ciliary marginal zone stem cell induced to differentiate from a pluripotent stem cell,
5 comprising either the following step (1) or step (2), or both of these steps:
(1) a step of floating culturing dispersed cells obtained from a cell aggregate comprising a ciliary marginal zone-like structure induced to differentiate from pluripotent stem cells,
10 thereby obtaining a retinosphere; and
(2) a step of collecting stage specific embryonic antigen-1 positive cells from dispersed cells obtained from a cell aggregate comprising a ciliary marginal zone-like structure induced to differentiate from pluripotent stem cells.
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2. The method according to claim 1, wherein the dispersed cells are cells obtained by dispersing the cell aggregate comprising a ciliary marginal zone-like structure induced to differentiate from pluripotent stem cells, cells obtained by dispersing a
20 ciliary marginal zone-like structure separated from the cell aggregate or cells obtained by dispersing cells collected from the cell aggregate.
3. The method according to claim 1 or 2, wherein the step (1)
25 is performed, and wherein the "dispersed cells obtained from a cell aggregate comprising a ciliary marginal zone-like structure induced to differentiate from pluripotent stem cells" are cells obtained by dispersing:
a cell aggregate comprising a ciliary marginal zone-like
30 structure induced to differentiate from pluripotent stem cells,
or
a ciliary marginal zone-like structure separated from the cell aggregate.
- 35 4. The method according to claim 1 or 2, wherein the step (2)

is performed, and wherein the "dispersed cells obtained from a cell aggregate comprising a ciliary marginal zone-like structure induced to differentiate from pluripotent stem cells" are cells obtained by dispersing:

5 a cell aggregate comprising a ciliary marginal zone-like structure induced to differentiate from pluripotent stem cells, or

 a ciliary marginal zone-like structure separated from the cell aggregate.

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5. The method according to claim 1 or 2, wherein the step (1) is performed followed by performing the step (2), and wherein the "dispersed cells obtained from a cell aggregate comprising a ciliary marginal zone-like structure induced to differentiate
15 from pluripotent stem cells" in step (1) are cells obtained by dispersing:

 a cell aggregate comprising a ciliary marginal zone-like structure induced to differentiate from pluripotent stem cells, or

20 a ciliary marginal zone-like structure separated from the cell aggregate; and

the "dispersed cells obtained from a cell aggregate comprising a ciliary marginal zone-like structure induced to differentiate from a pluripotent stem cell" in step (2) are cells obtained by
25 dispersing the retinosphere obtained in the step (1).

6. The method according to claim 1 or 2, wherein the step (2) is performed followed by performing the step (1), and wherein the "dispersed cells obtained from a cell aggregate comprising
30 a ciliary marginal zone-like structure induced to differentiate from pluripotent stem cells" in step (2) are cells obtained by dispersing:

 a cell aggregate comprising a ciliary marginal zone-like structure induced to differentiate from pluripotent stem cells,
35 or

a ciliary marginal zone-like structure separated from the cell aggregate; and
the "dispersed cells obtained from a cell aggregate comprising a ciliary marginal zone-like structure induced to differentiate
5 from pluripotent stem cells" in step (1) are cells obtained by dispersing cells collected in the step (2).

7. The method according to any one of claims 1, 2, 4, 5 and 6, wherein the stage specific embryonic antigen-1 positive cell is
10 further Rax positive.

8. The method according to any one of claims 1, 2, 4, 5, 6, and 7, wherein the stage specific embryonic antigen-1 positive cell is non-pigmented.

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9. The method according to any one of claims 1, 2, 3, 5 and 6, wherein the floating culturing in step (1) is performed in a serum-free medium or serum-containing medium each containing one or more substances selected from the group consisting of
20 substances capable of enhancing signal transduction mediated by FGF and substances capable of enhancing signal transduction mediated by EGF.

10. The method according to claim 9, wherein the serum-free
25 medium or serum-containing medium further comprises a ROCK inhibitor.

11. The method according to any one of claims 1, 2, 3, 5, 6, 9 and 10, wherein the retinosphere is non-pigmented.

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12. The method according to any one of claims 1 to 11, wherein the pluripotent stem cell is a primate pluripotent stem cell.

13. The method according to any one of claims 1 to 12, wherein
35 the pluripotent stem cell is a human pluripotent stem cell.

14. A method for producing a retinal layer-specific neuron,
comprising a step of culturing a ciliary marginal zone stem
cell obtained by the method according to any one of claims 1 to
5 13 in the presence of one or more substances selected from the
group consisting of Notch signal inhibitory substances,
retinoid and taurine.

15. A composition comprising a ciliary marginal zone stem cell
10 or retinal layer-specific neuron produced by the method
according to any one of claims 1 to 14.

16. A reagent for evaluation of toxicity or drug efficacy,
comprising a ciliary marginal zone stem cell or retinal layer-
15 specific neuron produced by the method according to any one of
claims 1 to 14.

17. A method of evaluating toxicity or drug efficacy of a test
substance, comprising bringing a ciliary marginal zone stem
20 cell or retinal layer-specific neuron produced by the method
according to any one of claims 1 to 14, into contact with the
substance, and assaying an influence of the substance on the
cell.

25 18. A retinosphere which contains 80% or more of Rax positive
and stage specific embryonic antigen-1 positive cells.