

clarified that a motor neuron generated from an iPS cell derived from a patient with amyotrophic lateral sclerosis (ALS) using the present method autonomously undergoes cell death, and the cell death can be efficiently suppressed by an ALS therapeutic agent.

Furthermore, the present inventors discovered that, among the above three genes, an introduction of Ngn2 gene alone into a pluripotent stem cell followed by an induction of the expression of said gene causes a prompt and synchronized differentiation into a neuron. In addition, it was clarified that a neuron generated from an iPS cell derived from a patient with Alzheimer's type dementia produces A β peptide in a manner characteristic to the disease.

Based on those findings, the present inventors completed the invention.

[0012]

In one aspect, there is provided a method of generating a motor neuron from a pluripotent stem cell, comprising the following steps in order from (1) to (2):
(1) introducing one or more nucleic acids encoding Lhx3, Ngn2, and Isl1 into a pluripotent stem cell; and (2) maintaining expression of the Lhx3, Ngn2, and Isl1 in the pluripotent stem cell for three days or more.

In another aspect, there is provided a method of identifying a marker specific to a subject for whom an investigational agent for motor neuron diseases or nerve injury has been confirmed to be effective (namely, a responder) or ineffective (namely, a non-responder), comprising the following steps of (1) to (3):

(1) producing induced pluripotent stem cells from somatic cells isolated from a responder and a non-responder;
(2) generating motor neurons from the induced pluripotent stem cells obtained in step (1), by the method according to the present invention;
(3) measuring an amount of gene products in the motor neurons derived from the responder and the non-responder, which were obtained in step (2); and
(4) identifying a gene product which amount is higher in the motor neurons derived from the responder than in the motor neurons derived from the non-responder as the responder-specific marker, or a gene product which amount is lower in the motor neurons derived from the responder than in the motor neurons derived from the non-responder as the non-responder-specific marker.

In a further aspect, there is provided a method of selecting a subject for whom a therapeutic agent is effective, comprising the following steps of (1) to (3):

(1) producing induced pluripotent stem cells from somatic cells isolated from a subject;
(2) generating motor neurons from the induced pluripotent stem cells obtained in step (1), by the method according to the present invention;

(3) detecting the presence or absence of a marker in the motor neurons obtained in step (2), wherein the marker has been identified as a responder-specific or a non-responder-specific marker by the method according to the present invention; and (4) selecting a subject as the subject for whom the therapeutic agent corresponding to said marker is effective, when the responder-specific marker has been detected, or the non-responder-specific marker has not been detected in the motor neurons derived from said subject.

In yet a further aspect, there is provided a pluripotent stem cell in which one or more exogenous nucleic acids encoding Lhx3, Ngn2, and Isl1 have been inserted into the chromosome, wherein said nucleic acid is functionally connected to a drug-responsive promoter; and wherein said pluripotent stem cell is induced to differentiate into a motor neuron when expression of the Lhx3, Ngn2, and Isl1 is maintained for three days or more.

In one aspect, there is provided blood cells differentiated from the pluripotent stem cell according to the present invention.

In another aspect, there is provided a therapeutic composition for use in the treatment of motor neuron disease or nerve injury, comprising a pluripotent stem cell according to the present invention as an effective ingredient.

In yet another aspect, there is provided a therapeutic composition for use in the treatment of motor neuron disease or nerve injury, comprising blood cells according to the present invention as an effective ingredient.

In yet another aspect, there is provided a method for screening a therapeutic agent for amyotrophic lateral sclerosis, comprising the following steps of (1) to (5):

(1) generating motor neurons from induced pluripotent stem cells by the method according to the present invention, wherein the induced pluripotent stem cells have been produced from somatic cells isolated from a patient with amyotrophic lateral sclerosis;

(2) bringing a test substance into contact with the motor neurons obtained in step (1);

(3) culturing the motor neurons which have been contacted with the substance in step

(2) and the motor neurons which have not been contacted with the substance (i.e., control cells);

(4) measuring the cell number and/or the neurite length of the motor neurons obtained through step (3);

(5) selecting a test substance as the therapeutic agent for amyotrophic lateral sclerosis, when the value of the cell number and/or the neurite length of the motor neurons which have been contacted with said substance is higher than the value of the control cells.

That is, the present ~~invention~~disclosure also provides the following.

[1] A method of generating a motor neuron from a pluripotent stem cell, comprising the following steps in order from (1) to (2):

(1) introducing one or more nucleic acids encoding Lhx3, Ngn2, and Isl1 into a pluripotent stem cell;

(2) maintaining the expression of Lhx3, Ngn2, and Isl1 for three days or more.

[2] The method according to [1], wherein said nucleic acids are introduced into the cell using a transposon.

[3] The method according to [2], wherein said transposon is a piggyBac transposon.

[CLAIMS]

What is claimed is:

1. A method of generating a motor neuron from a pluripotent stem cell, comprising the following steps in order from (1) to (2):

(1) introducing one or more nucleic acids encoding Lhx3, Ngn2, and Isl1 into a pluripotent stem cell; and

(2) maintaining expression of the Lhx3, Ngn2, and Isl1 in the pluripotent stem cell for three days or more.
2. The method according to claim 1, wherein said nucleic acids are introduced into the cell using a transposon.
3. The method according to claim 2, wherein said transposon is a piggyBac transposon.
4. The method according to any of claims 1 to 3, wherein the expression of Lhx3, Ngn2, and Isl1 is driven by a drug-responsive promoter.
5. The method according to claim 4, wherein said drug-responsive promoter is a tetracycline-responsive promoter.

6. The method according to claim 1, wherein Lhx3, Ngn2, and Isl1 are polycistronically expressed from the nucleic acid.
7. The method according to claim 6, wherein the nucleic acid encoding Lhx3, Ngn2, or Isl1 is connected via a nucleic acid encoding a 2A sequence.
8. The method according to any of claims 1 to 7, wherein the expression of Lhx3, Ngn2, and Isl1 is maintained for 7 days or more in step (2).
9. The method according to any of claims 1 to 8, wherein step (1) further comprises a step of inducing differentiation of the pluripotent stem cell in which the nucleic acid has been introduced, into blood cells.
10. The method according to claim 9, wherein said blood cells are monocytes and/or macrophages.
11. The method according to claim 9 or 10, wherein said step of inducing differentiation of the pluripotent stem cell into blood cells consists of culturing the pluripotent stem cell together with bone marrow-derived stromal cells in the presence of

a stem cell factor, a macrophage-colony stimulating factor, and an interleukin-3.

12. The method according to any of claims 1 to 11, wherein said pluripotent stem cell is an induced pluripotent stem cell derived from a human.

13. The method according to any of claims 1 to 12, wherein said pluripotent stem cell has one or more mutant SOD1 genes.

14. A method of identifying a marker specific to a subject for whom an investigational agent for motor neuron diseases or nerve injury has been confirmed to be effective (namely, a responder) or ineffective (namely, a non-responder), comprising the following steps of (1) to (3):

(1) producing induced pluripotent stem cells from somatic cells isolated from a responder and a non-responder;

(2) generating motor neurons from the induced pluripotent stem cells obtained in step (1), by the method according to any of claims 1-8;

(3) measuring an amount of gene products in the motor neurons derived from the responder and the non-responder, which were obtained in step (2); and

(4) identifying a gene product which amount is higher in the motor neurons derived from the responder than in the motor neurons derived from the non-responder as the

responder-specific marker, or a gene product which amount is lower in the motor neurons derived from the responder than in the motor neurons derived from the non-responder as the non-responder-specific marker.

15. A method of selecting a subject for whom a therapeutic agent is effective, comprising the following steps of (1) to (3):

- (1) producing induced pluripotent stem cells from somatic cells isolated from a subject;
- (2) generating motor neurons from the induced pluripotent stem cells obtained in step (1), by the method according to any of claims 1-8;
- (3) detecting the presence or absence of a marker in the motor neurons obtained in step (2), wherein the marker has been identified as a responder-specific or a non-responder-specific marker by the method according to claim 14; and
- (4) selecting a subject as the subject for whom the therapeutic agent corresponding to said marker is effective, when the responder-specific marker has been detected, or the non-responder-specific marker has not been detected in the motor neurons derived from said subject.

16. A pluripotent stem cell in which one or more exogenous nucleic acids encoding Lhx3, Ngn2, and Isl1 have been inserted into the chromosome, wherein said nucleic acid is functionally connected to a drug-responsive promoter; and

wherein said pluripotent stem cell is induced to differentiate into a motor neuron when expression of the Lhx3, Ngn2, and Isl1 is maintained for three days or more.

17. The pluripotent stem cell according to claim 16, wherein said nucleic acids express Lhx3, Ngn2, and Isl1 in a polycistronic manner under the control of the drug-responsive promoter.

18. The pluripotent stem cell according to claim 17, wherein said nucleic acids have been inserted into the chromosome by a transposon.

19. The pluripotent stem cell according to claim 18, wherein said transposon is a piggyBac transposon.

20. The pluripotent stem cell according to any of claims 16 to 19, wherein said drug-responsive promoter is a tetracycline-responsive promoter.

21. The pluripotent stem cell according to any of claims 16 to 20, wherein the nucleic acid encoding Lhx3, Ngn2, or Isl1 is connected via a nucleic acid encoding 2A sequence.

22. The pluripotent stem cell according to any of claims 16 to 21, wherein said pluripotent stem cell is an induced pluripotent stem cell derived from a human.
23. The pluripotent stem cell according to any of claims 16 to 22, wherein said pluripotent stem cell has one or more mutant SOD1 genes.
24. Blood cells differentiated from the pluripotent stem cell according to any of claims 16 to 22.
25. The blood cells according to claim 24, wherein said blood cells are monocytes and/or macrophages.
26. A therapeutic composition for use in the treatment of motor neuron disease or nerve injury, comprising a pluripotent stem cell according to any of claims 16 to 22 as an effective ingredient.
27. The therapeutic composition according to claim 26, wherein said motor neuron disease is amyotrophic lateral sclerosis.
28. A therapeutic composition for use in the treatment of motor neuron disease or

nerve injury, comprising blood cells according to claim 24 or 25 as an effective ingredient.

29. The therapeutic composition according to claim 28, wherein said motor neuron disease is amyotrophic lateral sclerosis.

30. A method for screening a therapeutic agent for amyotrophic lateral sclerosis, comprising the following steps of (1) to (5):

(1) generating motor neurons from induced pluripotent stem cells by the method according to any of claims 1-8, wherein the induced pluripotent stem cells have been produced from somatic cells isolated from a patient with amyotrophic lateral sclerosis;

(2) bringing a test substance into contact with the motor neurons obtained in step (1);

(3) culturing the motor neurons which have been contacted with the substance in step (2) and the motor neurons which have not been contacted with the substance (i.e., control cells);

(4) measuring the cell number and/or the neurite length of the motor neurons obtained through step (3);

(5) selecting a test substance as the therapeutic agent for amyotrophic lateral sclerosis, when the value of the cell number and/or the neurite length of the motor neurons which have been contacted with said substance is higher than the value of the control cells.

31. The method according to claim 30, wherein said somatic cells have one or more mutations in SOD1 gene.

[CLAIMS]

What is claimed is:

1. A method of generating a motor neuron from a pluripotent stem cell, comprising the following steps in order from (1) to (2):

(1) introducing one or more nucleic acids encoding Lhx3, Ngn2, and Isl1 into a pluripotent stem cell; and

(2) maintaining expression of the Lhx3, Ngn2, and Isl1 for three days or more.
2. The method according to claim 1, wherein said nucleic acids are introduced into the cell using a transposon.
3. The method according to claim 2, wherein said transposon is a piggyBac transposon.
4. The method according to any of claims 1 to 3, wherein the expression of Lhx3, Ngn2, and Isl1 is driven by a drug-responsive promoter.
5. The method according to claim 4, wherein said drug-responsive promoter is a tetracycline-responsive promoter.

6. The method according to claim 1, wherein Lhx3, Ngn2, and Isl1 are polycistronically expressed from the nucleic acid.
7. The method according to claim 6, wherein the nucleic acid encoding Lhx3, Ngn2, or Isl1 is connected via a nucleic acid encoding a 2A sequence.
8. The method according to any of claims 1 to 7, wherein the expression of Lhx3, Ngn2, and Isl1 is maintained for 7 days or more in step (2).
9. The method according to any of claims 6 to 8, wherein step (2) further comprises steps of introducing the cell obtained in step (1) into an animal body, and bringing the cell into contact with an agent corresponding to the drug-responsive promoters in the animal body.
10. The method according to any of claims 1 to 9, wherein step (1) further comprises a step of inducing differentiation of the pluripotent stem cell in which the nucleic acid has been introduced, into blood cells.
11. The method according to claim 10, wherein said blood cells are monocytes

and/or macrophages.

12. The method according to claim 10 or 11, wherein said step of inducing differentiation of the pluripotent stem cell into blood cells consists of culturing the pluripotent stem cell together with bone marrow-derived stromal cells in the presence of a stem cell factor, a macrophage-colony stimulating factor, and an interleukin-3.

13. The method according to any of claims 1 to 12, wherein said pluripotent stem cell is an induced pluripotent stem cell derived from a human.

14. The method according to any of claims 1 to 13, wherein said pluripotent stem cell has one or more mutant SOD1 genes.

15. A method of identifying a marker specific to a subject for whom an investigational agent for motor neuron diseases or nerve injury has been confirmed to be effective (namely, a responder) or ineffective (namely, a non-responder), comprising the following steps of (1) to (3):

(1) producing induced pluripotent stem cells from somatic cells isolated from a responder and a non-responder;

(2) generating motor neurons from the induced pluripotent stem cells obtained in step

(1), by the method according to any of claims 1-8;

(3) measuring an amount of gene products in the motor neurons derived from the responder and the non-responder, which were obtained in step (2); and

(4) identifying a gene product which amount is higher in the motor neurons derived from the responder than in the motor neurons derived from the non-responder as the responder-specific marker, or a gene product which amount is lower in the motor neurons derived from the responder than in the motor neurons derived from the non-responder as the non-responder-specific marker.

16. A method of selecting a subject for whom a therapeutic agent is effective, comprising the following steps of (1) to (3):

(1) producing induced pluripotent stem cells from somatic cells isolated from a subject;

(2) generating motor neurons from the induced pluripotent stem cells obtained in step

(1), by the method according to any of claims 1-8;

(3) detecting the presence or absence of a marker in the motor neurons obtained in step

(2), wherein the marker has been identified as a responder-specific or a non-responder-specific marker by the method according to claim 15; and

(4) selecting a subject as the subject for whom the therapeutic agent corresponding to said marker is effective, when the responder-specific marker has been detected, or the non-responder-specific marker has not been detected in the motor neurons derived from said subject.

17. A pluripotent stem cell in which one or more exogenous nucleic acids encoding Lhx3, Ngn2, and Isl1 have been inserted into the chromosome.
18. The pluripotent stem cell according to claim 17, wherein said nucleic acids express Lhx3, Ngn2, and Isl1 in a polycistronic manner under the control of an inducible promoter.
19. The pluripotent stem cell according to claim 18, wherein said nucleic acids have been inserted into the chromosome by a transposon.
20. The pluripotent stem cell according to claim 19, wherein said transposon is a piggyBac transposon.
21. The pluripotent stem cell according to any of claims 17 to 20, wherein said nucleic acid is functionally connected to a drug-responsive promoter.
22. The pluripotent stem cell according to claims 21, wherein said drug-responsive promoter is a tetracycline-responsive promoter.

23. The pluripotent stem cell according to any of claims 18 to 22, wherein the nucleic acid encoding Lhx3, Ngn2, or Isl1 is connected via a nucleic acid encoding 2A sequence.
24. The pluripotent stem cell according to any of claims 17 to 23, wherein said pluripotent stem cell is an induced pluripotent stem cell derived from a human.
25. The pluripotent stem cell according to any of claims 17 to 24, wherein said pluripotent stem cell has one or more mutant SOD1 genes.
26. Blood cells differentiated from the pluripotent stem cell according to any of claims 17-24.
27. The blood cells according to claim 26, wherein said blood cells are monocytes and/or macrophages.
28. A therapeutic composition for motor neuron disease or nerve injury, comprising a pluripotent stem cell according to any of claims 17-24 as an effective ingredient.

29. The therapeutic composition according to claim 28, wherein said motor neuron disease is amyotrophic lateral sclerosis.

30. A therapeutic composition for motor neuron disease or nerve injury, comprising blood cells according to claim 26 or 27 as an effective ingredient.

31. The therapeutic composition according to claim 30, wherein said motor neuron disease is amyotrophic lateral sclerosis.

32. A method for screening a therapeutic agent for amyotrophic lateral sclerosis, comprising the following steps of (1) to (5):

(1) generating motor neurons from induced pluripotent stem cells by the method according to any of claims 1-8, wherein the induced pluripotent stem cells have been produced from somatic cells isolated from a patient with amyotrophic lateral sclerosis;

(2) bringing a test substance into contact with the motor neurons obtained in step (1);

(3) culturing the motor neurons which have been contacted with the substance in step (2) and the motor neurons which have not been contacted with the substance (i.e., control cells);

(4) measuring the cell number and/or the neurite length of the motor neurons obtained through step (3);

(5) selecting a test substance as the therapeutic agent for amyotrophic lateral sclerosis, when the value of the cell number and/or the neurite length of the motor neurons which have been contacted with said substance is higher than the value of the control cells.

33. The method according to claim 32, wherein said somatic cells have one or more mutations in SOD1 gene.

34. A method of generating a neuron from a pluripotent stem cell, comprising the following steps in order from (1) to (2):

(1) introducing a nucleic acid encoding Ngn2 into a pluripotent stem cell using a transposon;

(2) inducing an activation of the promotor and maintaining expression of the Ngn2 for three days or more.

35. The method according to claim 34, wherein said transposon is a piggyBac transposon.

36. The method according to claim 34 or 35, wherein the expression of Ngn2 is driven by a drug-responsive promoter.

37. The method according to claim 36, wherein said drug-responsive promoter is a tetracycline-responsive promoter.

38. The method according to any of claims 34 to 37, wherein the expression of Ngn2 is maintained for 7 days or more in step (2).

39. The method according to any of claims 34 to 38, wherein step (2) further comprises steps of introducing the cells obtained in step (1) into an animal body, and subsequently bringing an agent corresponding to the drug-responsive promoter into contact with the cells in the animal body.

40. The method according to any of claims 34 to 39, wherein said pluripotent stem cells are induced pluripotent stem cells derived from a human.

41. The method according to claim 40, wherein said human-derived induced pluripotent stem cells have been produced from somatic cells isolated from a patient with Alzheimer's disease.

42. The method according to claim 40, wherein said human-derived induced pluripotent stem cells have one or more mutations in presenilin 1 gene.

43. A method of identifying a marker specific to a subject for whom an investigational agent for Alzheimer's disease has been confirmed to be effective (namely, a responder) or ineffective (namely, a non-responder), comprising the following steps in order (1) to (3):

(1) producing induced pluripotent stem cells from somatic cells isolated from a responder and a non-responder;

(2) generating neurons from the induced pluripotent stem cells obtained in step (1), by the method according to any of claims 34-38;

(3) measuring the amount of gene products in the neurons derived from the responder and the non-responder, which were obtained in step (2); and

(4) identifying a gene product which amount is higher in the neurons derived from the responder than in the neurons derived from the non-responder as the responder-specific marker, or a gene product which amount is lower in the neurons derived from the responder than in the neurons derived from the non-responder as the non-responder-specific marker.

44. A method of selecting a subject for whom a therapeutic agent is effective, comprising the following steps of (1) to (3):

(1) producing induced pluripotent stem cells from somatic cells isolated from a subject;

(2) generating neurons from the induced pluripotent stem cells obtained in step (1), by

the method according to any of claims 34-38;

(3) detecting the presence or absence of the a marker in the neurons obtained in step (2), wherein the marker has been identified as a responder-specific or a non-responder-specific marker by the method according to claim 43; and

(4) selecting the subject as the subject for whom the therapeutic agent corresponding to said marker is effective, when the responder-specific marker is detected, or the non-responder-specific marker is not detected in the neurons derived from said subject.

45. A pluripotent stem cell in which an exogenous nucleic acid encoding Ngn2 has been inserted in the chromosome by a transposon.

46. The pluripotent stem cell according to claim 45, wherein said transposon is a piggyBac transposon.

47. The pluripotent stem cell according to claim 45 or 46, wherein said nucleic acid is functionally connected to a drug-responsive promoter.

48. The pluripotent stem cell according to claims 47, wherein said drug-responsive promoter is a tetracycline-responsive promoter.

49. The pluripotent stem cell according to any of claims 45 to 48, wherein said pluripotent stem cell is an induced pluripotent stem cell derived from a human.

50. The pluripotent stem cell according to claim 49, wherein said human-derived induced pluripotent stem cell has been produced from a somatic cell isolated from a patient with Alzheimer's disease.

51. The pluripotent stem cell according to claim 49, wherein said human-derived induced pluripotent stem cell has one or more mutations in presenilin 1 gene.

52. A method for screening a therapeutic agent for Alzheimer's disease, comprising the following steps in order (1) to (4):

(1) generating neurons from induced pluripotent stem cells by the method according to any of claims 34-38, wherein the induced pluripotent stem cells have been produced from somatic cells isolated from a patient with Alzheimer's disease;

(2) bringing a test substance into contact with the neurons obtained in step (1);

(3) culturing the neurons which have been contacted with the substance in step (2) and the neurons which have not been contacted with the substance (i.e., control cells), and measuring the amount of A β 42 in the medium;

(4) selecting a test substance as the therapeutic agent for Alzheimer's disease, when the

amount of A β 42 in the medium of the neurons which have been contacted with said substance is lower than the amount of A β 42 in the medium of control cells.

53. The method according to claim 52, wherein

step (3) further comprises a step of measuring the amount of A β 40 in the medium, and

step (4) comprises a step of selecting a test substance as the therapeutic agent for Alzheimer's disease, when the ratio of the amount of A β 42 to that of A β 40 (i.e., the amount of A β 42/the amount of A β 40) in the medium of the neurons which have been contacted with said substance is lower than that of control cells.