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 Aβ 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 in a basal medium supplemented with a retinoic acid, SHH signal stimulants, and neurotrophic factors 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 in the pluripotent stem cell in a basal medium supplemented with a retinoic acid, SHH signal stimulants, and neurotrophic factors 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 use of the pluripotent stem cell according to the present inventionin the manufacture of a medicament for the treatment of motor neuron disease or nerve injury.
In yet another aspect, there is provided use of the blood cells according to the present invention in the manufacture of a medicament for the treatment of motor neuron disease or nerve injury.
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.
In a further aspect, there is provided a composition for use in therapy, comprising the pluripotent stem cell as described herein as an effective ingredient.
In yet a further aspect, there is provided a composition for use in therapy, comprising the blood cells as described herein as an effective ingredient.
That is, the present 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.

The present invention further provides a method of inducing a prompt and synchronized differentiation of a pluripotent stem cell into a neuron, by introducing a nucleic acid encoding Ngn2 into a pluripotent stem cell using a transposon and maintaining the expression of said gene for 3 days or more. The expression of Ngn2 gene can be induced, either under culture or in an animal body.

Hereafter, a technique of constituting these methods will be described in detail.

[0017]

< Pluripotent stem cells >

In the present invention, the pluripotent stem cell means a stem cell that has a pluripotency of differentiating into all types of cells present in a living body and also has a proliferating ability. Examples of the pluripotent stem cells include, but are not limited to, embryonic stem (ES) cells, nuclear transfer embryonic stem (ntES) cells derived from a cloned embryo obtained by nuclear transfer, spermatogonial stem cells ("GS cells"), induced pluripotent stem (iPS) cells, and pluripotent cells derived from cultured fibroblasts or bone marrow stem cells (Muse cells). Among these cells, pluripotent stem cells preferably used in the present invention include ES cells, ntES cells, and iPS cells. Hereinafter, each of these stem cells will be described.

[0018]

(A) Embryonic stem cells

ES cell is a stem cell having a pluripotency and a proliferating ability associated with self-replication, which has been established from an inner cell mass of an early embryo

(e.g., a blastocyst) of a mammal such as a human or a mouse.

The ES cell is a stem cell derived from an embryo from the inner cell mass of a blastocyst that is an embryo after the 8-cell stage and morula stage of a fertilized egg, and this cell has an ability to differentiate into all types of cells constituting an adult body, namely, differentiation pluripotency, and proliferating ability associated with self-replication. The ES cells were discovered in a mouse in 1981 (M.J. Evans and M.H. Kaufman (1981), Nature 292:154-156), and thereafter, the ES cell line was also established in primates such as a human and a monkey (J.A. Thomson et al. (1998), Science 282:1145-1147; J.A. Thomson et al. (1995), Proc. Natl. Acad. Sci. USA, 92:7844-7848;J.A. Thomson et al. (1996), Biol. Reprod., 55:254-259; J.A. Thomson and V.S. Marshall (1998), Curr. Top. Dev. Biol., 38:133-165).

[0019]

The ES cells can be established by extracting an inner cell mass from the blastocyst of the fertilized egg of a target animal and then culturing the inner cell mass on a fibroblast feeder. In addition, retention of the cells by subculture can be carried out using a culture, to which a substance such as a leukemia inhibitory factor (LIF) or a basic fibroblast growth factor (bFGF) has been added. Methods for establishing and retaining the ES cells of a human and a monkey are described, for example, in USP5,843,780; Thomson JA, et al. (1995), Proc Natl. Acad. Sci. U S A. 92:7844-7848 ; Thomson JA, et al. (1998), Science. 282:1145-1147 ; H. Suemori et al. (2006), Biochem. Biophys. Res. Commun., 345:926-932 ; M. Ueno et al. (2006), Proc. Natl. Acad. Sci. USA, 103:9554-9559 ; H. Suemori et al. (2001), Dev. Dyn., 222:273-279; H. Kawasaki et al. (2002), Proc. Natl. Acad. Sci. USA, 99:1580-1585 ; Klimanskaya I, et al. (2006), Nature.

444:481-485; etc.

[0020]

As a culture used to produce ES cells, for example, a DMEM/F-12 culture, to which 0.1 mM 2-mercaptoethanol, 0.1 mM non-essential amino acid, 2 mM L-glutamic acid, 20% KSR and 4ng/ml bFGF have been replenished, is used, and human ES cells can be retained at 37 · C in a wet atmosphere of 2% CO_2/98% air (O. Fumitaka et al. (2008), Nat. Biotechnol., 26:215-224). Moreover, the ES cells need to be subcultured every 3 or 4 days, and the subculture can be carried out, for example, using 0.25% trypsin and 0.1 mg/ml collagenase IV in PBS that contains 1 mM $CaCl_2$ and 20% KSR.

The ES cells can be generally selected by a Real-Time PCR method, using the expression of a gene marker such as alkaline phosphatase, Oct-3/4 or Nanog, as an indicator. In particular, when human ES cells are selected, the expression of a gene marker specific to undifferentiated cells, such as OCT-3/4, NANOG, or ECAD, can be used as an indicator (E. Kroon et al. (2008), Nat. Biotechnol., 26:443-452).

[0021]

Human ES cell lines, for example WA01 (H1) and WA09 (H9) are available from WiCell Reserch Institute, and KhES-1, KhES-2 and KhES-3 are available from the Kyoto University Institute for Frontier Medical Sciences (Kyoto, Japan).

[0022]

(B) Spermatogonial stem cells

Spermatogonial stem cell is a pluripotent stem cell derived from testis, which serves as

an origin for formation of the sperm. The cell can be induced to differentiate into cells of various lines, as well as ES cells, and has properties such that if the cell is transplanted, for example, in a mouse blastocyst, a chimeric mouse can be produced (M. Kanatsu-Shinohara et al, Biol Reprod, 69:.. 612-616, 2003; K. Shinohara et al, Cell, 119: 1001-1012, 2004). The spermatogonial stem cells are able to self-replicate in a culture medium containing glial cell line-derived neurotrophic factor (GDNF), and such spermatogonial stem cells can also be obtained by repeating subculture under the same culture conditions as those of ES cells cells (Takebayashi Masanori et al. (2008), Experimental Medicine, Vol. 26, No. 5 (special edition), 41-46 pages, Yodosha (Tokyo, Japan), 2008).

[0023]

(C) Induced pluripotent stem cells

Induced pluripotent stem (iPS) cell is an artificial stem cell derived from a somatic cell, which can be produced by introducing a specific reprogramming factor in the form of a DNA or protein into a somatic cell, and show almost equivalent property (e.g.,

pluripotent differentiation and proliferation potency based on self-renewal) as ES cells (K. Takahashi and S. Yamanaka (2006) Cell, 126:663-676; K. Takahashi et al. (2007), Cell, 131:861-872; J. Yu et al. (2007), Science, 318:1917-1920; Nakagawa, M. et al., Nat. Biotechnol. 26:101-106 (2008); WO2007/069666). The reprogramming factor may be constituted with a gene specifically expressed by ES cell, a gene product or non-coding RNA thereof, a gene playing an important role for the maintenance of undifferentiation of ES cell, a gene product or non-coding RNA thereof, or a low molecular weight compound. Examples of the gene contained in the reprogramming factor include Oct3/4, Sox2, Sox1, Sox3, Sox15, Sox17, Klf4, Klf2, c-Myc, N-Myc, L-Myc, Nanog, Lin28, Fbx15, ERas, ECAT15-2, Tcl1, beta-catenin, Lin28b, Sall1, Sall4, Esrrb, Nr5a2, Tbx3, Glis1 and the like. These reprogramming factors may be used alone or in combination. Examples of the combination of the reprogramming factors include combinations described in WO2007/069666, WO2008/118820, WO2009/007852, WO2009/032194, WO2009/058413, WO2009/057831, WO2009/075119, WO2009/079007, WO2009/091659, WO2009/101084, WO2009/101407, WO2009/102983, WO2009/114949, WO2009/117439, WO2009/126250, WO2009/126251, WO2009/126655, WO2009/157593, WO2010/009015, WO2010/033906, WO2010/033920, WO2010/042800, WO2010/050626, WO2010/056831, WO2010/068955, WO2010/098419, WO2010/102267, WO2010/111409, WO2010/111422, WO2010/115050, WO2010/124290, WO2010/147395, WO2010/147612, Huangfu D, et al. (2008), Nat. Biotechnol., 26: 795-797, Shi Y, et al. (2008), Cell Stem Cell, 2: 525-528, Eminli S, et al. (2008), Stem Cells. 26:2467-2474, Huangfu D, et al. (2008), Nat Biotechnol. 26:1269-1275, Shi Y, et al. (2008), Cell Stem Cell, 3, 568-574, Zhao Y, et al. (2008),

Cell Stem Cell, 3:475-479, Marson A, (2008), Cell Stem Cell, 3, 132-135, Feng B, et al. (2009), Nat Cell Biol. 11:197-203, R. L. Judson et al., (2009), Nat. Biotech., 27:459-461, Lyssiotis C A, et al. (2009), Proc Natl Acad Sci USA. 106:8912-8917, Kim J B, et al. (2009), Nature. 461:649-643, Ichida J K, et al. (2009), Cell Stem Cell. 5:491-503, Heng J C, et al. (2010), Cell Stem Cell. 6:167-74, Han J, et al. (2010), Nature. 463:1096-100, Mali P, et al. (2010), Stem Cells. 28:713-720, and Maekawa M, et al. (2011), Nature. 474:225-9.

[0024]

Examples of the above-mentioned reprogramming factor include, but are not limited to, factors used for enhancing the establishment efficiency, such as histone deacetylase (HDAC) inhibitors [e.g., low-molecular inhibitors such as valproic acid (VPA), trichostatin A, sodium butyrate, MC 1293, and M344, nucleic acid-based expression inhibitors such as siRNAs and shRNAs against HDAC (e.g., HDAC1 siRNA Smartpool® (Millipore), HuSH 29mer shRNA Constructs against HDAC1 (OriGene) and the like), and the like], MEK inhibitor (e.g., PD184352, PD98059, U0126, SL327 and PD0325901), Glycogen synthase kinase-3 inhibitor (e.g., Bio and CHIR99021), DNA methyl transferase inhibitors (e.g., 5-azacytidine), histone methyl transferase inhibitors [for example, low-molecular inhibitors such as BIX-01294, and nucleic acid-based expression inhibitors such as siRNAs and shRNAs against Suv39h1, Suv39h2, SetDB1 and G9a], L-channel calcium agonist (e.g., Bayk8644), butyric acid, TGFβ inhibitor or ALK5 inhibitor (e.g., LY364947, SB431542, 616453 and A-83-01), p53 inhibitor (e.g., siRNA and shRNA against p53), ARID3A inhibitor (e.g., siRNA and shRNA against ARID3A), miRNA such as miR-291-3p, miR-294, miR-295, mir-302

and the like, Wnt Signaling activating agent (e.g., soluble Wnt3a), neuropeptide Y, prostaglandins (e.g., prostaglandin E2 and prostaglandin J2), hTERT, SV40LT, UTF1, IRX6, GLIS1, PITX2, DMRTB1 and the like. In the present specification, these factors used for enhancing the establishment efficiency are not particularly 5 distinguished from the reprogramming factor.

[0025]

When in the form of a protein, a reprogramming factor may be introduced into a somatic cell by a method, for example, lipofection, fusion with cell penetrating peptide (e.g., TAT derived from HIV and polyarginine), microinjection and the like.

[0026]

When in the form of a DNA, a reprogramming factor may be introduced into a somatic cell by the method of, for example, vector of virus, plasmid, artificial chromosome and the like, lipofection, liposome, microinjection and the like. Examples of the virus vector include retrovirus vector, lentivirus vector (Cell, 126, pp. 663-676, 2006; Cell, 131, pp. 861-872, 2007; Science, 318, pp. 1917-1920, 2007), adenovirus vector (Science, 322, 945-949, 2008), adeno-associated virus vector, vector of Hemagglutinating Virus of Japan (WO 2010/008054) and the like. Examples of the artificial chromosome vector include human artificial chromosome (HAC), yeast artificial chromosome (YAC), bacterial artificial chromosome (BAC, PAC) and the like. As the plasmid, plasmids for mammalian cells can be used (Science, 322:949-953, 2008). The vector can contain regulatory sequences of promoter, enhancer, ribosome binding sequence, terminator, polyadenylation site and the like so that a nuclear reprogramming substance can be expressed and further, where necessary, a selection marker sequence of a drug resistance

gene (e.g., kanamycin resistance gene, ampicillin resistance gene, puromycin resistance gene and the like), thymidine kinase gene, diphtheria toxin gene and the like, a reporter gene sequence of green fluorescent protein (GFP), β glucuronidase (GUS), FLAG and the like, and the like. Moreover, the above-mentioned vector may have a LoxP sequence before and after thereof to simultaneously cut out a gene encoding a reprogramming factor or a gene encoding a reprogramming factor bound to the promoter, after introduction into a somatic cell.

[0027]

When in the form of RNA, for example, it may be introduced into a somatic cell by a means of lipofection, microinjection and the like, and RNA incorporating 5-methylcytidine and pseudouridine (TriLink Biotechnologies) may be used to suppress degradation (Warren L, (2010) Cell Stem Cell. 7:618-630).

[0028]

Examples of the culture medium for inducing iPS cell include 10 to 15% FBS-containing DMEM, DMEM/F12 or DME culture medium (these culture media can further contain LIF, penicillin/streptomycin, puromycin, L-glutamine, nonessential amino acids, β-mercaptoethanol and the like as appropriate) or a commercially available culture medium [for example, culture medium for mouse ES cell culture (TX-WES culture medium, Thromb-X), culture medium for primate ES cell (culture medium for primate ES/iPS cell, Reprocell), serum-free medium (mTeSR, Stemcell Technologies)] and the like.

[0029]

Examples of the culture method include contacting a somatic cell with a reprogramming factor on 10% FBS-containing DMEM or DMEM/F12 culture medium at 37° C. in the presence of 5% CO2 and culturing for about 4 to 7 days, thereafter reseeding the cells on feeder cells (e.g., mitomycin C-treated STO cells, SNL cells etc.), and culturing the cells in a bFGF-containing culture medium for primate ES cell from about 10 days after the contact of the somatic cell and the reprogramming factor, whereby ES-like colonies can be obtained after about 30 to about 45 days or longer from the contact.

[0030]

Alternatively, the cells are cultured on feeder cells (e.g., mitomycin C-treated STO cells, SNL cells etc.) at 37° C. in the presence of 5% CO2 in a 10% FBS-containing DMEM culture medium (which can further contain LIF, penicillin/streptomycin, puromycin, L-glutamine, nonessential amino acids, β-mercaptoethanol and the like as appropriate), whereby ES-like colonies can be obtained after about 25 to about 30 days or longer. Desirably, a method using a somatic cell itself to be reprogrammed, or an extracellular substrate (e.g., Laminin-5 (WO2009/123349) and Matrigel (BD)), instead of the feeder cells (Takahashi K, et al. (2009), PLoS One. 4:e8067 or WO2010/137746).

[0031]

Besides the above, a culture method using a serum-free medium can also be recited as an example (Sun N, et al. (2009), Proc Natl Acad Sci USA. 106:15720-15725). Furthermore, to enhance establishment efficiency, an iPS cell may be established under hypoxic conditions (oxygen concentration of not less than 0.1% and not more than 15%) (Yoshida Y, et al. (2009), Cell Stem Cell. 5:237-241 or WO2010/013845), can be mentioned.

[0032]

The culture medium is exchanged with a fresh culture medium once a day between the above-mentioned cultures, from day 2 from the start of the culture. While the cell number of the somatic cells used for nuclear reprogramming is not limited, it is about 5×103 to about 5×106 cells per 100 cm2 culture dish.

[0033]

The iPS cell can be selected based on the shape of the formed colony. When a drug resistance gene which is expressed in association with a gene (e.g., Oct3/4, Nanog) expressed when a somatic cell is reprogrammed is introduced as a marker gene, an established iPS cell can be selected by culturing in a culture medium (selection culture medium) containing a corresponding drug. When the marker gene is a fluorescent protein gene, iPS cell can be selected by observation with a fluorescence microscope, when it is a luminescent enzyme gene, iPS cell can be selected by adding a luminescent substrate, and when it is a chromogenic enzyme gene, iPS cell can be selected by adding a chromogenic substrate.

[0034]

The term "somatic cells" used in the present specification means any animal cells (preferably, cells of mammals inclusive of human) excluding germ line cells and totipotent cells such as ovum, oocyte, ES cells and the like. Somatic cells unlimitatively encompass any of somatic cells of fetuses, somatic cells of neonates, and mature healthy or pathogenic somatic cells, and any of primary cultured cells, passage cells, and established lines of cells. Specific examples of the somatic cells include (1) tissue stem cells (somatic stem cells) such as neural stem cell, hematopoietic stem cell, mesenchymal stem cell, dental pulp stem cell and the like, (2) tissue progenitor cells, (3)

[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 in a basal medium supplemented with a retinoic acid, SHH signal stimulants, and neurotrophic factors 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 in the pluripotent stem cell in a basal medium supplemented with a retinoic acid, SHH signal stimulants, and neurotrophic factors 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. Use of the pluripotent stem cell according to any of claims 16 to 22 in the manufacture of a medicament for the treatment of motor neuron disease or nerve injury.

27. The use according to claim 26, wherein said motor neuron disease is amyotrophic lateral sclerosis.

28. Use of the blood cells according to claim 24 or 25 in the manufacture of a medicament for the treatment of motor neuron disease or nerve injury.

29. The use 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.

32. A composition for use in therapy, comprising the pluripotent stem cell according to any of claims 16 to 22 as an effective ingredient.

33. A composition for use in therapy, comprising the blood cells according to claim 24 or 25 as an effective ingredient.