

Non-patent Document 2: Steinbach, O.C., Wolffe, A.P. & Rupp, R.A. Somatic linker histones cause loss of mesodermal competence in *Xenopus*. *Nature* 389, 395-399 (1997)

Non-patent Document 3: Hebbar, P.B. & Archer, T.K. Altered histone H1 stoichiometry and an absence of nucleosome positioning on transfected DNA. *The Journal of biological chemistry* 283, 4595-4601 (2008).

Summary

Object to be Solved by the Disclosure

[0007]

An object of the present disclosure is to provide an agent for improving quality of an iPS cell, a method for producing an iPS cell, an iPS cell produced by such a method for production, and a composition for producing an iPS cell.

Means to Solve the Object

[0008]

The present inventors have studied diligently to improve quality of iPS cells and found as a result that in a method for producing an iPS cell by introducing a nuclear reprogramming substance into a somatic cell, introducing not only the nuclear reprogramming substance, but also "an H1foo gene or a gene product thereof" into the somatic cell allows the production of high-quality iPS cells with smaller variance in quality, thereby completing the present disclosure. It was unexpected for those skilled in the art that the combination of the "H1foo gene

or gene product thereof" and the nuclear reprogramming substance allowed the production of high-quality iPS cells with smaller variance in quality.

[0008a]

In one aspect, the present disclosure refers to an in vitro method for producing an iPS cell, comprising:

i) introducing (a) a nuclear reprogramming substance, wherein the nuclear reprogramming substance consists of an Oct3/4 gene or a gene product thereof, a Sox2 gene or a gene product thereof, and a Klf4 gene or a gene product thereof, and (b) an Hlfoo gene or a gene product thereof into a somatic cell, and

ii) culturing the somatic cell from i) to produce an iPS cell, wherein the iPS cell, when compared to an iPS cell produced without introducing the Hlfoo gene or a gene product thereof, is improved as shown in one or more selected from the group consisting of the following (1) to (11);

(1) an increase in a percentage of embryoid body formation when iPS cells have been cultured in a culture medium for embryoid body formation;

(2) an increase in a number of embryoid body formation when iPS cells have been cultured in the culture medium for embryoid body formation;

(3) a decrease in a size variance of embryoid bodies when iPS cells have been cultured in the culture medium for embryoid body formation;

(4) an increase in a percentage of viable cells when iPS cells have been cultured in the culture medium for embryoid body formation;

(5) a decrease in a percentage of apoptotic cells when iPS cells have been cultured in the culture medium for embryoid body formation;

(6) an increase in an expression level of a Ki67 gene or a PCNA gene when iPS cells have been cultured in the culture medium for embryoid body formation;

(7) an increase in a chimera competency of iPS cells;

(8) an increase in a germline transmission potential of iPS cells;

(9) an increase in an expression level of a SRF gene in iPS cells;

(10) an increase in an expression level of a ACTG2 gene in iPS cells; and

(11) a decrease in a variance of an expression level of a Oct3/4 gene in iPS cells.

[0008b]

In another aspect, the present disclosure refers to an in vitro method for producing an iPS cell, comprising:

i) introducing (a) a nuclear reprogramming substance, wherein the nuclear reprogramming substance consists of an Oct3/4 gene or a gene product thereof, a Sox2 gene or a gene product thereof, a Klf4 gene or a gene product thereof, and an L-Myc gene or a gene product thereof, and (b) an Hlfoo gene or a gene product thereof into a somatic cell, and

ii) culturing the somatic cell from i) to produce an iPS cell, wherein the iPS cell, when compared to an iPS cell produced without introducing the Hlfoo gene or a gene product thereof, is improved as shown in one or more

selected from the group consisting of the following (1) to (11);

(1) an increase in a percentage of embryoid body formation when iPS cells have been cultured in a culture medium for embryoid body formation;

(2) an increase in a number of embryoid body formation when iPS cells have been cultured in the culture medium for embryoid body formation;

(3) a decrease in a size variance of embryoid bodies when iPS cells have been cultured in the culture medium for embryoid body formation;

(4) an increase in a percentage of viable cells when iPS cells have been cultured in the culture medium for embryoid body formation;

(5) a decrease in a percentage of apoptotic cells when iPS cells have been cultured in the culture medium for embryoid body formation;

(6) an increase in an expression level of a Ki67 gene or a PCNA gene when iPS cells have been cultured in the culture medium for embryoid body formation;

(7) an increase in a chimera competency of iPS cells;

(8) an increase in a germline transmission potential of iPS cells;

(9) an increase in an expression level of a SRF gene in iPS cells;

(10) an increase in an expression level of a ACTG2 gene in iPS cells; and

(11) a decrease in a variance of an expression level of a Oct3/4 gene in iPS cells.

[0008c]

In yet another aspect, the present disclosure refers to an iPS cell produced by the in vitro method for producing an iPS cell as described herein [0008a], wherein the iPS cell, when compared to an iPS cell produced without introducing the Hlfoo gene or a gene product thereof, is improved as shown in one or more selected from the group consisting of the following (1) to (11);

(1) an increase in a percentage of embryoid body formation when iPS cells have been cultured in a culture medium for embryoid body formation;

(2) an increase in a number of embryoid body formation when iPS cells have been cultured in the culture medium for embryoid body formation;

(3) a decrease in a size variance of embryoid bodies when iPS cells have been cultured in the culture medium for embryoid body formation;

(4) an increase in a percentage of viable cells when iPS cells have been cultured in the culture medium for embryoid body formation;

(5) a decrease in a percentage of apoptotic cells when iPS cells have been cultured in the culture medium for embryoid body formation;

(6) an increase in an expression level of a Ki67 gene or a PCNA gene when iPS cells have been cultured in the culture medium for embryoid body formation;

(7) an increase in a chimera competency of iPS cells;

(8) an increase in a germline transmission potential of iPS cells;

(9) an increase in an expression level of a SRF gene in iPS cells;

(10) an increase in an expression level of a ACTG2 gene in iPS cells; and

(11) a decrease in a variance of an expression level of a Oct3/4 gene in iPS cells.

[0008d]

In yet another aspect, the present disclosure refers to an iPS cell produced by the in vitro method for producing an iPS cell as described herein [0008b], wherein the iPS cell, when compared to an iPS cell produced without introducing the Hlfoo gene or a gene product thereof, is improved as shown in one or more selected from the group consisting of the following (1) to (11);

(1) an increase in a percentage of embryoid body formation when iPS cells have been cultured in a culture medium for embryoid body formation;

(2) an increase in a number of embryoid body formation when iPS cells have been cultured in the culture medium for embryoid body formation;

(3) a decrease in a size variance of embryoid bodies when iPS cells have been cultured in the culture medium for embryoid body formation;

(4) an increase in a percentage of viable cells when iPS cells have been cultured in the culture medium for embryoid body formation;

(5) a decrease in a percentage of apoptotic cells when iPS cells have been cultured in the culture medium for embryoid body formation;

(6) an increase in an expression level of a Ki67 gene or a PCNA gene when iPS cells have been cultured in the culture medium for embryoid body formation;

(7) an increase in a chimera competency of iPS cells;

(8) an increase in a germline transmission potential of iPS cells;

(9) an increase in an expression level of a SRF gene in iPS cells;

(10) an increase in an expression level of a ACTG2 gene in iPS cells; and

(11) a decrease in a variance of an expression level of a Oct3/4 gene in iPS cells.

[0009]

Accordingly, the disclosure provides:

(1) an agent for improving quality of an iPS cell, comprising an Hlfoo gene or a gene product thereof; and

(2) the agent for improving quality of an iPS cell according to (1), wherein the agent comprises an expression vector comprising the Hlfoo gene.

[0010]

Moreover, the present disclosure provides:

(3) a method for producing an iPS cell, comprising the step of introducing (a) a nuclear reprogramming substance and (b) an Hlfoo gene or a gene product thereof into a somatic cell;

(4) the method for producing an iPS cell according to (3), wherein the nuclear reprogramming substance comprises at least one selected from the group consisting of a gene of Oct gene family, a gene of Sox gene family, a gene of Klf

gene family, a gene of Myc gene family, a gene of Lin gene family, a Nanog gene, and gene products thereof;

(5) the method for producing an iPS cell according to (3) or (4), wherein the nuclear reprogramming substance consists of a gene of Oct gene family or a gene product thereof, a gene of Sox gene family or a gene product thereof, and a gene of Klf gene family or a gene product thereof;

(6) the method for producing an iPS cell according to any one of (3) to (5), wherein the nuclear reprogramming substance consists of an Oct3/4 gene or a gene product thereof, a Sox2 gene or a gene product thereof, and a Klf4 gene or a gene product thereof; and

(7) the method for producing an iPS cell according to any one of (3) to (5), wherein the nuclear reprogramming substance consists of an Oct3/4 gene or a gene product thereof, a Sox2 gene or a gene product thereof, a Klf4 gene or a gene product thereof, and an L-Myc gene or a gene product thereof.

[0011]

Furthermore, the disclosure provides:

(8) an iPS cell produced by the method for producing an iPS cell according to any one of (1) to (7); and

(9) a composition for producing an iPS cell, comprising (a) a nuclear reprogramming substance and (b) an Hlfoo gene or a gene product thereof.

Effect of the Invention

[0012]

The present invention can provide an agent for improving quality of an iPS cell, a method for producing an iPS cell, an iPS cell produced by such a method for production, and a composition for producing an iPS cell. According to the present invention, high-quality iPS cells with smaller variance in quality can be produced.

Brief Description of Drawings

[0013]

[Figure 1] Figure 1 is a set of microscopic images of tail tip fibroblasts (hereinafter simply referred to as "mouse

CLAIMS

1. An in vitro method for producing an iPS cell, comprising:

i) introducing (a) a nuclear reprogramming substance, wherein the nuclear reprogramming substance consists of an Oct3/4 gene or a gene product thereof, a Sox2 gene or a gene product thereof, and a Klf4 gene or a gene product thereof, and (b) an Hlfoo gene or a gene product thereof into a somatic cell, and

ii) culturing the somatic cell from i) to produce an iPS cell, wherein the iPS cell, when compared to an iPS cell produced without introducing the Hlfoo gene or a gene product thereof, is improved as shown in one or more selected from the group consisting of the following (1) to (11);

(1) an increase in a percentage of embryoid body formation when iPS cells have been cultured in a culture medium for embryoid body formation;

(2) an increase in a number of embryoid body formation when iPS cells have been cultured in the culture medium for embryoid body formation;

(3) a decrease in a size variance of embryoid bodies when iPS cells have been cultured in the culture medium for embryoid body formation;

(4) an increase in a percentage of viable cells when iPS cells have been cultured in the culture medium for embryoid body formation;

(5) a decrease in a percentage of apoptotic cells

when iPS cells have been cultured in the culture medium for embryoid body formation;

(6) an increase in an expression level of a Ki67 gene or a PCNA gene when iPS cells have been cultured in the culture medium for embryoid body formation;

(7) an increase in a chimera competency of iPS cells;

(8) an increase in a germline transmission potential of iPS cells;

(9) an increase in an expression level of a SRF gene in iPS cells;

(10) an increase in an expression level of a ACTG2 gene in iPS cells; and

(11) a decrease in a variance of an expression level of a Oct3/4 gene in iPS cells.

2. An in vitro method for producing an iPS cell, comprising:

i) introducing (a) a nuclear reprogramming substance, wherein the nuclear reprogramming substance consists of an Oct3/4 gene or a gene product thereof, a Sox2 gene or a gene product thereof, a Klf4 gene or a gene product thereof, and an L-Myc gene or a gene product thereof, and (b) an Hlfoo gene or a gene product thereof into a somatic cell, and

ii) culturing the somatic cell from i) to produce an iPS cell, wherein the iPS cell, when compared to an iPS cell produced without introducing the Hlfoo gene or a gene product thereof, is improved as shown in one or more

selected from the group consisting of the following (1) to (11);

(1) an increase in a percentage of embryoid body formation when iPS cells have been cultured in a culture medium for embryoid body formation;

(2) an increase in a number of embryoid body formation when iPS cells have been cultured in the culture medium for embryoid body formation;

(3) a decrease in a size variance of embryoid bodies when iPS cells have been cultured in the culture medium for embryoid body formation;

(4) an increase in a percentage of viable cells when iPS cells have been cultured in the culture medium for embryoid body formation;

(5) a decrease in a percentage of apoptotic cells when iPS cells have been cultured in the culture medium for embryoid body formation;

(6) an increase in an expression level of a Ki67 gene or a PCNA gene when iPS cells have been cultured in the culture medium for embryoid body formation;

(7) an increase in a chimera competency of iPS cells;

(8) an increase in a germline transmission potential of iPS cells;

(9) an increase in an expression level of a SRF gene in iPS cells;

(10) an increase in an expression level of a ACTG2 gene in iPS cells; and

(11) a decrease in a variance of an expression level of a Oct3/4 gene in iPS cells.

3. An iPS cell produced by the in vitro method for producing an iPS cell according to claim 1, wherein the iPS cell, when compared to an iPS cell produced without introducing the Hlfoo gene or a gene product thereof, is improved as shown in one or more selected from the group consisting of the following (1) to (11);

(1) an increase in a percentage of embryoid body formation when iPS cells have been cultured in a culture medium for embryoid body formation;

(2) an increase in a number of embryoid body formation when iPS cells have been cultured in the culture medium for embryoid body formation;

(3) a decrease in a size variance of embryoid bodies when iPS cells have been cultured in the culture medium for embryoid body formation;

(4) an increase in a percentage of viable cells when iPS cells have been cultured in the culture medium for embryoid body formation;

(5) a decrease in a percentage of apoptotic cells when iPS cells have been cultured in the culture medium for embryoid body formation;

(6) an increase in an expression level of a Ki67 gene or a PCNA gene when iPS cells have been cultured in the culture medium for embryoid body formation;

(7) an increase in a chimera competency of iPS cells;

(8) an increase in a germline transmission potential of iPS cells;

(9) an increase in an expression level of a SRF gene in iPS cells;

(10) an increase in an expression level of a ACTG2 gene in iPS cells; and

(11) a decrease in a variance of an expression level of a Oct3/4 gene in iPS cells.

4. An iPS cell produced by the in vitro method for producing an iPS cell according to claim 2, wherein the iPS cell, when compared to an iPS cell produced without introducing the Hlfoo gene or a gene product thereof, is improved as shown in one or more selected from the group consisting of the following (1) to (11);

(1) an increase in a percentage of embryoid body formation when iPS cells have been cultured in a culture medium for embryoid body formation;

(2) an increase in a number of embryoid body formation when iPS cells have been cultured in the culture medium for embryoid body formation;

(3) a decrease in a size variance of embryoid bodies when iPS cells have been cultured in the culture medium for embryoid body formation;

(4) an increase in a percentage of viable cells when iPS cells have been cultured in the culture medium for embryoid body formation;

(5) a decrease in a percentage of apoptotic cells when iPS cells have been cultured in the culture medium for embryoid body formation;

(6) an increase in an expression level of a Ki67 gene or a PCNA gene when iPS cells have been cultured in the culture medium for embryoid body formation;

(7) an increase in a chimera competency of iPS cells;

(8) an increase in a germline transmission potential of iPS cells;

(9) an increase in an expression level of a SRF gene in iPS cells;

(10) an increase in an expression level of a ACTG2 gene in iPS cells; and

(11) a decrease in a variance of an expression level of a Oct3/4 gene in iPS cells.