[0005] As such, there is a need to identify a marker of totipotency that allows a maximum degree of cellular plasticity to be achieved and maintained, thereby providing more options for efficient reprogramming and potential therapeutic avenues.

SUMMARY

[0005a] In one aspect, there is provided a method of determining a potency state of an embryonic stem cell (ESC) in culture comprising: a) detecting the expression level of Negative Elongation Factor Complex Member A (Nelfa) in said embryonic stem cell; and b) correlating the expression level of Nelfa in said embryonic stem cell with that of a reference sample to determine the potency state of said embryonic stem cell, wherein an increased level of Nelfa expression relative to the reference sample indicates that said embryonic stem cell has a totipotent-like state, wherein the reference sample is an embryonic stem cell that does not express Nelfa or expresses low levels of Nelfa.

[0006] In one ~~aspect~~example, there is provided a method of determining a potency state of an embryonic stem cell (ESC) in culture comprising:

a) detecting the expression level of Nelfa in said embryonic stem cell;

b) correlating the expression level of Nelfa in said embryonic stem cell with that of a reference sample to determine the potency state of said embryonic stem cell.

[0006a] In one aspect, there is provided a method of selecting totipotent-like embryonic stem cells (ESCs) from a population of embryonic stem cells in culture, comprising:

a) providing a population of ESCs that displays a predetermined phenotype when Nelfa is expressed;

b) identifying the ESCs that express the predetermined phenotype; and

c) selecting the ESCs that express the predetermined phenotype.

[0007] In one aspect, there is provided a method of selecting totipotent-like embryonic stem cells (ESCs) from a population of embryonic stem cells in culture, comprising:

a) contacting said population of ESCs with an anti-Nelfa antigen binding protein;

b) selecting the ESCs binding to said anti-Nelfa antigen binding protein; and

c) optionally isolating the ESCs from said anti-Nelfa antigen binding protein.

[0008] In one ~~aspect~~example, there is provided a method of inducing totipotency in an embryonic stem cell (ESC) in culture, comprising contacting and incubating said ESC with

one or more of a metabolic regulator, a small molecule compound, a chemical, a virus, a nucleic acid or a polypeptide, to induce expression of Nelfa.

[0008a] In one aspect, there is provided a method of inducing totipotency in an embryonic stem cell (ESC) in culture, comprising contacting and incubating said ESC with one or more of a metabolic regulator, wherein the metabolic regulator is a glycolytic inhibitor, or a nucleic acid, wherein the nucleic acid is a Max siRNA, a N-Myc siRNA or an Esrrb siRNA, to induce expression of Nelfa.

[0009] In one ~~aspect~~example, there is provided a use of a metabolic regulator, a nucleic acid, a small molecule, a chemical, a virus or a polypeptide for inducing totipotency in an embryonic stem cell.

[0009a]In one aspect, there is provided a use of a metabolic regulator, wherein the metabolic regulator is a glycolytic inhibitor, or a nucleic acid, wherein the nucleic acid is a Max siRNA, a N-Myc siRNA or an Esrrb siRNA, for inducing expression of Nelfa in an embryonic stem cell.

[0010] In one ~~aspect~~example, there is provided a cell comprising an expression vector encoding Nelfa operably linked to a promoter.

[0010a] In one aspect, there is provided an embryonic stem cell (ESC) comprising an expression vector encoding Nelfa operably linked to a promoter.

[0011] In one ~~aspect~~example, there is provided a kit comprising at least one of a metabolic regulator, a nucleic acid, a polypeptide, a small molecule, a virus or a chemical when used in the method as described herein.

[0011a] In one aspect, there is provided a kit comprising at least one of a metabolic regulator, wherein the metabolic regulator is a glycolytic inhibitor, or a nucleic acid, wherein the nucleic acid is a Max siRNA, a N-Myc siRNA or an Esrrb siRNA, when used in the method as described herein.

[0012] In one ~~aspect~~examplet, there is provided a method of improving the reprogramming efficiency of a somatic cell into an induced pluripotent stem cell (iPSC), comprising contacting and incubating said somatic cell with one or more of a metabolic regulator, a small molecule compound, a chemical, a nucleic acid, a virus or a polypeptide to induce expression of Nelfa.

[0012a] In one aspect, there is provided a method of improving the reprogramming efficiency of a somatic cell into an induced pluripotent stem cell (iPSC), comprising contacting and incubating said somatic cell with one or more of a metabolic regulator, wherein the metabolic regulator is a glycolytic inhibitor, or a nucleic acid, wherein the nucleic acid is a Max siRNA, a N-Myc siRNA or an Esrrb siRNA, to induce expression of Nelfa.

[0013] In one example~~aspect~~, there is provided a method of reprogramming a somatic cell into a totipotent stem cell comprising contacting and incubating said somatic cell with one or more of a metabolic regulator, a small molecule compound, a chemical, a nucleic acid, a virus or a polypeptide to induce expression of Nelfa.

[0013a] In one aspect, there is provided a method of reprogramming a somatic cell into a totipotent stem cell comprising contacting and incubating said somatic cell with Oct4, Sox2, Klf4, c-myc, and Nelfa.

DEFINITIONS

[0014] The following words and terms used herein shall have the meaning indicated:

[0015] The term “stem cell” refers to a cell that has the ability to both self-renew and differentiate into other cell types.

[0016] As used herein, the term "potency" refers to the sum of all developmental options accessible to the cell (i.e., the developmental potency) and the term “potency state” of a stem cell refers to the ability of the stem cell to differentiate into various cell types. A stem cell that can differentiate into more cell types has a greater potency than a stem cell that can differentiate into fewer cell types.

[0017] The potency state of a cell can be determined by assessing characteristics of potency which include but are not limited to cell morphology, expression of markers of specific potency states or markers of specific lineages, ability of mouse stem cells to contribute to germline transmission in mouse chimeras, ability of stem cells to contribute to the embryo proper using tetraploid embryo complementation assays, teratoma formation of stem cells, formation of embryoid bodies and inactive X chromosome reactivation.

[0018] It will be understood to a person skilled in the art that cellular or developmental potency exists as a continuum, ranging from a cell with the highest development potency, a totipotent cell, to a cell with the least developmental potency, a terminally differentiated cell. Cells in this continuum can be broadly classified as being totipotent, pluripotent, multipotent, oligopotent, unipotent or terminally differentiated. As cellular or developmental potency exists as a continuum, it will be understood to a person skilled in the art that characteristics of potency would also be observed as a continuum.

[0019] A “totipotent cell”, “totipotent stem cell” or “totipotent-like cell” is a cell that has the developmental potential to make all of the cells in the adult body as well as the extra-embryonic tissues, including the placenta. The fertilized egg (zygote) is totipotent, as are the cells (blastomeres) of the morula (up to the 16-cell stage following fertilization).

Claims

1. A method of determining a potency state of an embryonic stem cell (ESC) in culture comprising:

   a) detecting the expression level of Negative Elongation Factor Complex Member A (Nelfa) in said embryonic stem cell; and

   b) correlating the expression level of Nelfa in said embryonic stem cell with that of a reference sample to determine the potency state of said embryonic stem cell, wherein an increased level of Nelfa expression relative to the reference sample indicates that said embryonic stem cell has a totipotent-like state, wherein the reference sample is an embryonic stem cell that does not express Nelfa or expresses low levels of Nelfa.

2. The method of claim 1 wherein the ESC is a mammalian ESC.

3. The method of claim 2, wherein the mammalian ESC is a human ESC.

~~4. The method as claimed in any one of claims 1 to 3, wherein the potency state is a totipotent-like state.~~

~~5. The method as claimed in any one of claims 1 to 4, wherein an increased level of Nelfa expression relative to the reference sample indicates that said embryonic stem cell has a totipotent-like state.~~

~~6.~~4. The method as claimed in any one of claims 1 to ~~5~~3, wherein the expression level of Nelfa is gene expression level, protein expression level or combinations thereof.

~~7.~~5. The method of claim ~~6~~4, wherein the step of detecting the protein expression level of Nelfa is by immunohistochemistry or flow cytometry.

~~8.~~6. The method of claim ~~6~~4, wherein the step of detecting the gene expression level of Nelfa comprises:

a) contacting a nucleic acid sequence obtained or derived from said embryonic stem cell with at least one primer and/or at least one probe for amplification of the Nelfa sequence;

b) amplifying said nucleic acid sequence using said at least one primer and/or said at least one probe, and

c) detecting the gene expression level of Nelfa.

~~9.~~7. The method of claim ~~8~~ 6 wherein said amplification step is performed by polymerase chain reaction (PCR).

~~10.~~8. The method of claim ~~9~~ 7 wherein said PCR is quantitative RT-PCR.

~~11.~~9. The method as claimed in any one of claims 4 to 8~~10~~, wherein the totipotent-like state is characterized by the Nelfa induced expression of Zscan4, Tcstv3, Gm4340, MERVL/HERVL family of retrotransposons, gsat_mm, Dux or combinations thereof in said ESC.

~~12.~~10. The method of claim 9~~11~~, wherein the totipotent-like state is further characterized in that Nelfa acts upstream of Zscan4 and MERVL/HERVL family of retrotransposons in said ESC.

~~13.~~11. The method of claim 9~~11~~ or 10~~12~~, wherein the Nelfa induced expression of MERVL/HERVL family of retrotransposons comprises the retrotransposons mt2_mm, Mervl-b4-int, MERVL_gag and Mervl-int.

~~14.~~12. The method as claimed in any one of claims 4 to 11~~13~~, wherein the totipotent-like state is further characterized by transient nuclear import of NAP1 in said ESC.

~~15.~~13. The method as claimed in any one of claims 4 to 12~~14~~, wherein the totipotent-like state is further characterized by chromatin decondensation and expression of MERVL/HERVL family of retrotransposons and Zscan4 in said ESC.

~~16.~~14. A method of selecting totipotent-like embryonic stem cells (ESCs) from a population of embryonic stem cells in culture, comprising:

a) providing a population of ESCs that displays a predetermined phenotype when Nelfa is expressed;

b) identifying the ESCs that express the predetermined phenotype; and

c) selecting the ESCs that express the predetermined phenotype.

~~17.~~15. The method of claim 14~~16~~, wherein the predetermined phenotype is expression of a reporter gene.

~~18.~~16. The method of claim 15~~17~~, wherein the reporter gene is green fluorescent protein (GFP).

~~19.~~17. A method of selecting totipotent-like embryonic stem cells (ESCs) from a population of embryonic stem cells in culture, comprising:

a) contacting said population of ESCs with an anti-Nelfa antigen binding protein;

b) selecting the ESCs binding to said anti-Nelfa antigen binding protein; and

c) optionally isolating the ESCs from said anti-Nelfa antigen binding protein.

~~20.~~18. The method as claimed in any one of claims 14~~16~~ to 17~~19~~, wherein said cells are selected using single cell sorting, fluorescent activated cell sorting or magnetic cell sorting.

~~21.~~19. The method as claimed in any one of claims 17~~19~~ or 18~~20~~, wherein the antigen binding protein is selected from the group consisting of a monoclonal antibody, a recombinant antibody, a polyclonal antibody, chimeric, a humanised antibody, a bispecific antibody, a heteroconjugate antibody, a single variable domain, a domain antibody, an antigen binding fragment, an immunologically effective fragment, a single chain Fv, a single chain antibody, a univalent antibody lacking a hinge region, a minibody, a diabody, and a tandem antibody.

~~22.~~20. A method of inducing totipotency in an embryonic stem cell (ESC) in culture, comprising contacting and incubating said ESC with one or more of a metabolic regulator, wherein the metabolic regulator is a glycolytic inhibitor, or a nucleic acid,

wherein the nucleic acid is a Max siRNA, a N-Myc siRNA or an Esrrb siRNA, to induce expression of Nelfa.

~~23.~~21. The method of claim 20~~22~~, wherein the glycolytic inhibitor is selected from the group consisting of 2-deoxy-D-glucose, 3-bromopyruvate and imatinib.

~~24.~~22. Use of a metabolic regulator, wherein the metabolic regulator is a glycolytic inhibitor, or a nucleic acid, wherein the nucleic acid is a Max siRNA, a N-Myc siRNA or an Esrrb siRNA, for inducing expression of Nelfa in an embryonic stem cell.

~~25.~~23. An embryonic stem cell (ESC) comprising an expression vector encoding Nelfa operably linked to a promoter.

~~26.~~24. The embryonic stem cell of claim 23~~25~~ wherein the ESC is a mammalian ESC.

~~27.~~25. The embryonic stem cell of claim 24~~26~~, wherein the mammalian ESC is a human ESC.

~~28.~~26. The embryonic stem cell as claimed in any one of claims 23~~25~~ to 25~~27~~, wherein the promoter is an inducible promoter.

~~29.~~27. The embryonic stem cell as claimed in any one of claims 23~~25~~ to 26~~28~~, wherein the vector further comprises a reporter gene.

~~30.~~28. The embryonic stem cell of claim 27~~29~~, wherein the reporter gene is selected from streptavidin or green fluorescent protein.

~~31.~~29. A kit comprising at least one of a metabolic regulator, wherein the metabolic regulator is a glycolytic inhibitor, or a nucleic acid, wherein the nucleic acid is a Max siRNA, a N-Myc siRNA or an Esrrb siRNA, when used in the method as claimed in claims 20~~22~~ or 21~~23~~.

~~32.~~30. A method of improving the reprogramming efficiency of a somatic cell into an induced pluripotent stem cell (iPSC), comprising contacting and incubating said

somatic cell with one or more of a metabolic regulator, wherein the metabolic regulator is a glycolytic inhibitor, or a nucleic acid, wherein the nucleic acid is a Max siRNA, a N-Myc siRNA or an Esrrb siRNA, to induce expression of Nelfa.

~~33.~~31. The method of claim 30~~32~~, wherein the somatic cell is a mouse or human cell.

~~34.~~32. The method of claim 31~~33~~, wherein the mouse or human cell is selected from the group consisting of an embryonic fibroblast cell, a neonatal foreskin fibroblast cell, a neuronal progenitor and a B-cell.

~~35.~~33. The method as claimed in claims 30~~32~~ to 32~~34~~, wherein the improvement of reprogramming efficiency is determined by comparing a preselected phenotype in an iPSC where Nelfa expression was induced to an iPSC where Nelfa expression was not induced, wherein an increased level of expression or increased rate of expression of the preselected phenotype indicates an improvement in reprogramming efficiency.

~~36.~~34. The method of claim 33~~35~~, wherein the preselected phenotype is selected from the group consisting of pluripotent marker gene expression, surface marker expression, alkaline phosphatase staining and teratoma formation.

~~37.~~35. The method of claim 34~~36~~, wherein the pluripotent marker gene is Oct4 and/or Nanog.

~~38.~~36. The method of claim 34~~36~~, wherein the surface marker is selected from the group consisting of SSEA-1, SSEA-4, TRA-1-60, TRA-1-81, CD73, CD49d, CD54 and CD326.

~~39.~~37. A method of reprogramming a somatic cell into a totipotent stem cell comprising contacting and incubating said somatic cell with Oct4, Sox2, Klf4, c-myc, and Nelfa .

~~40.~~38. The method of claim 37~~39~~, wherein the somatic cell is further contacted with a metabolic regulator, wherein the metabolic regulator is a glycolytic inhibitor, or a nucleic acid, wherein the nucleic acid is a Max siRNA, a N-Myc siRNA or an Esrrb siRNA.