~~BRIEF~~ SUMMARY

[0009] The present ~~invention~~disclosure is broadly summarized as relating to reprogramming of differentiated primate somatic cells to produce primate pluripotent cells.

[0010] ~~In a first aspect~~Disclosed is, ~~the invention is summarized in that~~ a method for producing a primate pluripotent cell includes the step of delivering into a primate somatic cell a set of transgenes sufficient to reprogram the somatic cell to a pluripotent state, the transgenes being carried on at least one episomal vector that does not encode an infectious virus, and recovering pluripotent cells. References herein to a "non-viral" vector or construct indicate that the vector or construct cannot encode an infectious virus.

[0011] ~~In a second aspect, the invention relates to~~Disclosed is an enriched population of replenishable reprogrammed pluripotent cells of a primate, including a human primate, wherein, in contrast to existing iPS cells, the at least one vector, including any element thereof having a viral source or derivation is substantially absent from the pluripotent cells. As used herein, this means that the reprogrammed cells contain fewer than one copy of the episomal vector per cell, and preferably no residual episomal vector in the cells. Because asymmetric partitioning during cell division dilutes the vector, one can readily obtain reprogrammed cells from which the vector has been lost. As noted elsewhere herein, on very rare occasions a reprogramming vector can integrate into the genome of the cell, but cells having an integrated vector can be avoided by screening for absence of the vector. Further, in contrast to existing ES cells, the primate pluripotent cells of the ~~invention~~ disclosure are substantially genetically identical to somatic cells from a fetal or post-natal individual. Fetal cells can be obtained from, e.g., amniotic fluid. The cells of the enriched population are not readily distinguished from existing primate ES and iPS cells morphologically (*i.e.*, round shape, large nucleoli and scant cytoplasm) or by growth properties (*i.e.*, doubling time; ES cells have a doubling time of about seventeen to eighteen hours). Like iPS cells and ES cells, the reprogrammed cells also express pluripotent cell-specific markers (*e.g.*, *OCT-4*, *SSEA-3*, *SSEA-4*, *TRA-1-60*, *TRA-1-81*, but not *SSEA-1*). Unlike ES cells, the reprogrammed cells are not immediately derived from embryos. As used herein, "not immediately derived from embryos" means that the starting cell type for producing the pluripotent cells is a non-pluripotent cell, such as a multipotent cell or terminally differentiated cell, such as somatic cells obtained from a fetal or post-natal individual. Like iPS cells, the pluripotent cells

produced in the method can transiently express one or more copies of selected potency-determining factors during their derivation.

[0011a] In one aspect, there is provided an enriched population of primate induced pluripotent cells substantially genetically identical to a post-natal individual and substantially free of any vector component associated with induction of pluripotency, wherein the primate-induced pluripotent cells express pluripotent cell-specific markers, have a cell morphology characteristic of undifferentiated cells, and can differentiate into cells of all three germ layers. In another aspect, there is provided a primate induced pluripotent cells substantially free of any component associated with induction of pluripotency.

[0012] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this ~~invention~~ disclosure belongs. Although suitable materials and methods for the practice or testing of the present ~~invention~~ disclosure are described below, other materials and methods similar or equivalent to those described herein, which are well known in the art, can be used.

[0013] Other objectives, advantages and features of the present ~~invention~~ disclosure will become apparent from the following specification taken in conjunction with the accompanying drawings.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

[0014] FIG. 1A-B illustrate the effect on reprogramming efficiency of different nucleotide sequences that link transgenes on the vector(s) delivered during the reprogramming methods.

[0015] FIG. 2A-C illustrate the effect on reprogramming efficiency of *c-Myc*, *KLF-4*, and SV40 large T antigen gene expression in human newborn foreskin fibroblasts.

[0016] FIG. 3A-C illustrate a suitable construct for carrying transgenes into somatic cells in accord with the method, temporal expression of an episomal vector-mediated transgene, and the effect of vector quantity on cell survival after nucleofection.

[0017] FIG. 4A-D illustrate reprogramming of human newborn foreskin fibroblasts with episomal vector-mediated transgene expression.

[0018] FIG. 5A-B illustrate related constructs harboring an expression cassette useful in the reprogramming methods of the invention.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

[0019] The present invention broadly relates to novel methods for reprogramming differentiated primate somatic cells into reprogrammed primate cells that are substantially free of the vectors used in their production by introducing potency-determining factors on a non-viral vector that is present during reprogramming, but is substantially absent from the reprogrammed cells. As used herein, "reprogramming" refers to a genetic process whereby differentiated somatic cells are converted into de-differentiated cells having a higher potency than the cells from which they were derived.

CLAIM OR CLAIMS

WE CLAIM:

1. An enriched population of primate induced pluripotent cells substantially genetically identical to a post-natal individual and substantially free of any vector component associated with induction of pluripotency, wherein the primate-induced pluripotent cells express pluripotent cell-specific markers, have a cell morphology characteristic of undifferentiated cells, and can differentiate into cells of all three germ layers.

2. The population of claim 1, wherein the population is produced according to a method comprising the steps of:

introducing at least one non-viral episomal vector encoding one or more potency-determining factors into primate somatic cells, wherein the at least one vector encodes at least potency-determining factors OCT4, SOX2, and at least one of LIN28 and NANOG, wherein the cells express the introduced OCT4, SOX2, and at least one of LIN28 and NANOG, and further comprise SV40 T Antigen, under conditions sufficient to express the potency-determining factors, thereby reprogramming the somatic cells to produce the primate induced pluripotent cells;

the pluripotent cells being substantially free of any vector component associated with induction of pluripotency and substantially genetically identical to a post-natal individual.

3. The population of Claim 2, wherein the primate somatic cells are obtained from a fetal or post-natal individual.

4. The population of Claim 2, wherein the primate is a human.

5. The population of Claim 2, wherein the one or more potency-determining factors comprise human OCT-4, human SOX2, human LIN28, human NANOG, human c-Myc, and human KLF4, wherein when the vector comprises c-Myc, the method further comprises the step of exposing the primate somatic cells to SV40 T Antigen.

6. Primate induced pluripotent cells substantially free of any component associated with induction of pluripotency.

WE CLAIM:

1. A method of reprogramming primate somatic cells, the method comprising the steps of:

exposing the primate somatic cells to a plurality of potency-determining factors under conditions sufficient to reprogram the cells; and

culturing the exposed cells to obtain reprogrammed cells having a higher potency level than the primate somatic cells, the reprogrammed cells being substantially free of any component associated with exposing the potency-determining factors to the somatic cells.

2. The method of Claim 1, wherein the primate somatic cells are obtained from a fetal or post-natal individual.

3. The method of Claim 1, wherein the primate is a human.

4. The method of Claim 1, wherein the reprogrammed cells are genetically identical to a fetal or post-natal individual.

5. The method of Claim 1, wherein the exposing step includes the step of introducing at least one vector encoding one or more potency-determining factors into the primate somatic cells.

6. The method of Claim 5, wherein the vector is a non-viral episomal vector.

7. The method of Claim 5, wherein the one or more potency-determining factors comprise human OCT-4, human SOX2, human LIN28, human NANOG, human c-Myc, and human KLF4, wherein when the vector comprises c-Myc, the method further comprises the step of exposing the primate somatic cells to SV40 T Antigen.

8. The method of Claim 5, wherein the at least one vector is a single vector.

9. The method of Claim , wherein the single vector comprises at least one internal ribosomal entry site.

10. The method of Claim 8, wherein the single vector comprises, in order, a first promoter, *OCT4*, IRES2, *SOX2,* a second promoter, *SV40 T* antigen, IRES2, and *KLF4*.

11. The method of Claim 8, wherein the vector comprises, in order, a first promoter, *OCT4*, IRES2, *SOX2,* a second promoter, *c-Myc*, IRES 2, *KLF4*, a third promoter, *NANOG*, IRES2, and *LN28*.

12. The method of Claim 5, wherein a first vector comprises, in order, a first promoter, *OCT4*, IRES2, *SOX2,* a second promoter, *SV40 T* antigen, IRES2, and *KLF4*, wherein a second vector comprises, in order, a third promoter, *OCT4*, IRES2, *SOX2,* a fourth promoter, *NANOG*, IRES2, and *KLF4*, and wherein a third vector comprises, in order, a fifth promoter, *c-Myc*, IRES 2, and *LN28*, wherein the promoters need not be identical.

13. The method of Claim 12, wherein each of the first, second, third, and fourth promoters is an elongation factor 1α (EF1α) gene promoter.

14. The method of Claim 5, wherein a first vector comprises, in order, a first promoter, *OCT4*, IRES2, *SOX2,* a second promoter, *KLF4*, IRES2, *c-Myc*, a third promoter, *NANOG*, IRES2, and *LN28*, wherein a second vector comprises, in order, a fourth promoter, *OCT4*, IRES2, *SOX2,* a fifth promoter, *SV40 T* antigen, IRES2, and *KLF4*, wherein the promoters need not be identical

15. The method of Claim 14, wherein each of the first, third, fourth, and fifth promoters is an EF1α gene promoter and wherein the second promoter is a cytomegalovirus immediate early gene (CMV) promoter.

16. The method of Claim 5, wherein a first vector comprises, in order, a first promoter, *OCT4*, IRES2, *SOX2*, a second promoter, *NANOG*, IRES2, and *LN28*, wherein a second vector comprises, in order, a third promoter, *OCT4*, IRES2, *SOX2*, a fourth promoter, *SV40 T* antigen, IRES2, and *KLF4*, and wherein a third vector comprises, in order, a fifth promoter, *OCT4*, IRES2, *SOX2*, a sixth promoter, *c-Myc*, IRES 2, and *KLF4*, wherein the promoters need not be identical.

17. An enriched population of primate pluripotent cells produced according to a method comprising the step of:

exposing primate somatic cells to a plurality of potency-determining factors under conditions sufficient to express the potency-determining factors, thereby reprogramming the somatic cells to produce the primate pluripotent cells;

the cells being substantially free of any component associated with exposing the potency-determining factors to the somatic cells.

18. The population of Claim 17, wherein the primate somatic cells are obtained from a fetal or post-natal individual.

19. The population of Claim 17, wherein the primate is a human.

20. The population of Claim 17, wherein the pluripotent cells are substantially genetically identical to a post-natal individual.

21. The population of Claim 17, wherein the exposing step includes the step of introducing at least one vector encoding one or more potency-determining factors into the primate somatic cells.

22. The population of Claim 21, wherein the vector is a non-viral episomal vector.

23. The population of Claim 21, wherein the one or more potency-determining factors comprise human OCT-4, human SOX2, human LIN28, human NANOG, human c-Myc, and human KLF4, wherein when the vector comprises c-Myc, the method further comprises the step of exposing the primate somatic cells to SV40 T Antigen.

24. Primate induced pluripotent cells substantially free of any component associated with induction of pluripotency.