GENERATION OF HUMAN iPS CELLS BY A SYNTHETIC SELF-REPLICATIVE RNA

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims priority to U.S. Provisional Application Serial Nos. 61/649,876, filed May 21, 2012 and 61/798,229, filed March 15, 2013, each of which are hereby incorporated by reference in their entirety.

TECHNICAL FIELD

[0002] Provided are methods and compositions useful for producing and propagating stem cells from fibroblasts. The disclosure relates to the production of induced pluripotent stem cells (iPS) and methods of use thereof.

BACKGROUND

[0003] Stems cells are a potential source from which organs may be regenerated, tissues may be repaired, biological factors prepared or delivered and disease or disorders treated.

SUMMARY

[0003a] In one aspect, there is provided a purified synthetic RNA replicon comprising: at least one non-structural replicase domain from an alphavirus and at least one non-alphavirus heterologous sequence encoding one or more factors for inducing the generation of pluripotent stem cells when expressed in a somatic cell.

[0003b] In another aspect, there is provided a composition comprising human cells transfected with a replicon as described herein.

[0003c] In yet another aspect, there is provided an in vitro method comprising: transfecting a human somatic cell with a purified synthetic ectopic self-replicating RNA replicon derived from an alphavirus, comprising polynucleotides encoding at least the following four de-differentiation factors (i) KLF4, (ii) OCT4, (iii) SOX2, (iv) c-MYC or n-MYC or L-MYC, and optionally (v) GLIS1 and (vi) NANOG, culturing the somatic cell to express the de-differentiation factor; selecting cells that display a stem cell morphology and/or stem cell markers; and subculturing the cells to obtain a population of induced stem cells.

29. The composition of claim 27, wherein the human cells are somatic cells.

30. The composition of claim 29, wherein the human cells are fibroblasts.

31. An *in vitro* method comprising:

transfecting a human somatic cell with a purified synthetic ectopic self-replicating RNA replicon derived from an alphavirus, comprising polynucleotides encoding at least the following four de-differentiation factors (i) KLF4, (ii) OCT4, (iii) SOX2, (iv) c-MYC or n-MYC or L-MYC, and optionally (v) GLIS1 and (vi) NANOG,

culturing the somatic cell to express the de-differentiation factor;

selecting cells that display a stem cell morphology and/or stem cell markers; and

subculturing the cells to obtain a population of induced stem cells.

32. The method of claim 31, wherein the cells are selected by detecting expression of a Tumor Rejection Antigen 1-60 and/or 1-81.

33. A vector system for producing human stem cells, comprising:

at least one purified synthetic self-replicating RNA replicon derived from an alphavirus, comprising one or more polynucleotides encoding de-differentiation factors selected from the group consisting of a KLF4, OCT4, SOX2, c-MYC or n-MYC or L-MYC, GLIS1, NANOG or any combination thereof.

34. The vector system of claim 33, wherein the replicon comprises:

(a)Oct4, Sox2, Klf4, and c-Myc, or

(b)Oct4, Sox2, Klf4, and Glis1.

35. The vector system of claim 33, wherein the one self-replicating RNA vector is derived from an alphavirus.

36. The vector system of claim 35, wherein the alphavirus is VEE.

37. An isolated human somatic cell comprising a purified synthetic ectopic RNA replicon derived from an alphavirus comprising one or more de-differentiation polynucleotide sequences.

38. The human somatic cell of claim 37, wherein the somatic cell de-differentiates when cultured under conditions to express at least the following four de-differentiation factors selected from the group consisting of a (i) KLF4, (ii) OCT4, (iii) SOX2, (iv) c-MYC or n-MYC or L-MYC, and optionally (v) GLIS1 and (vi) NANOG from the ectopic RNA replicon.

39. A cell population comprising the cells of claim 38.

40. A cell population of claims 39 or method of claim 31, wherein the cells or method further comprise culturing the cells in a medium conditioned with B18R so that the degradation of the ectopic RNA replicon is inhibited.

41. The cell population or method of claim 40, wherein the B18R conditioned media is produced by transfection of B18R mRNA into primary human fibroblasts.