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(54) Title: PLURIPOTENTIAL STEM CELLS

(57) Abstract: Described are mammalian stem cells derived from somatic stem cells of a lower relative potency, pluripotent stem cells are obtained from somatic stem cells of lower relative potency. Also described are mammalian stem cell lines that are capable of stable regeneration in vitro, which cell lines are derived from lower relative potency somatic stems cells. In addition, methods are provided for the production of mammalian stem cells, cell lines and non-human mammals derived from somatic stem cells of a lower relative potency. The invention also provides methods for reprogramming a nucleus of a somatic cell so as to become a less differentiated stem cell.

PLURIPOTENTIAL STEM CELLS

The invention relates to pluripotential stem cells that are capable of forming cells of all three of the somatic lineages of ectoderm, mesoderm and endoderm in the adult animal. Also provided are methods for generating these pluripotential stem cells. The invention further relates to derivatives and uses of such pluripotential stem cells.

In multicellular organisms the ultimate cell is the fertilised egg cell which is totipotent, in that as well as giving rise to all cells of the embryo and afterwards the organism, it also gives rise to the cells forming the placenta. Embryonal stem cells are stem cells found in the embryo and which are able to give rise to all cells of the adult but have a dramatically reduced ability to form placenta - these cells are known as pluripotent stem cells. Embryonal stem cells give rise to three specialised cell lineages which in combination form all of the cells and tissues in the body except the gametes. These lineages are known as ectodermal, endodermal and mesodermal cells - while the gametes are derived from a fourth and separate cell lineage known as the germline. Ectoderm, endoderm and mesoderm cells supply all of the cell types comprising somatic tissue and are termed multipotent, as each lineage precursor or stem cell can produce a range, although not all, of the cell types found in the body.

Some of the cells formed from the embryonal stem cells are progenitor lineage stem cells and are capable both of forming a differentiated cell and of self-renewal, While other cells are terminally differentiated cells which no longer divide. Cells which can self-renew or differentiate are known as stem cells, precursor cells or progenitor cells and play crucial roles in regeneration and homeostasis in virtually every tissue in the body.

Stem cells are found to have greater levels of potency the higher they are in the developmental cascade - i.e. the closer they are developmentally to the fertilised egg the more different cell types they are capable of forming. In addition, it is a dogmatic fundamental of developmental biology that the differentiation of a stem cell results in a cell of reduced developmental capacity - that is to say that cells progress only downwards in the developmental cascade.

Embryonic Stem (ES) cells were originally derived from the pluripotential inner cell mass (ICM) of the preimplantation mouse embryo, and retain the capacity

for multilineage differentiation both in vitro and in vivo. With their potential for leading to all cell types, there is a desire to obtain and maintain embryonic stem cells from many species, in particular from humans.

There are considerable difficulties in obtaining ES cells from mammalian embryos. ES cells are typically isolated from the ICM by sorting, but even after careful segregation of cell types, these stem cell preparations consist of mixed cell types and while enriched for stem cells, include high proportions of more differentiated cells which are not categorised as ES cells. Further difficulties arise with regards culturing of these ES cells outside of the embryo, for example whilst there are various reports describing the isolation of ES cells from diverse species, ES cells from only two inbred strains of mice (129 and Black 6) can be efficiently cultured in vitro. Thus, a significant problem that exists in the art is that the range of ES cells that can be propagated efficiently in culture is limited to just a few types, mainly mouse ES cells, despite efforts to derive ES cells from other species.

The term "ES cell" has also been widely adopted and used to describe a variety of pluripotent stem cells including those which have been derived from cells of the mammalian germline, that is cells capable of giving rise to gametes. As mentioned above, ES cells are typically derived from the inner cell mass or primitive ectoderm, but other ES cells, which are more correctly described as EG (embryo-gonadal) cells, are isolated from primordial germ cells of the developing foetus.

Some confusion arises when efforts are made to define an "ES cell". An ES cell in its truest form is a cell which can be maintained indefinitely in culture whilst still retaining normal chromosome content and the ability to form any cell type found in the body, including the germline. While ES (and EG) cells have been isolated and definitively proven for some strains of mice, the term ES cell is also widely used to describe pluripotent cells derived from other species, including humans, which have not been shown or tested for germline competence.

So while germline competence has not been demonstrated for any species other than mice, and the term "ES cell" is now readily used to describe chromosomally normal pluripotent stem cells capable of extended growth in vitro and differentiation into all of the three germ layers of ectoderm, mesoderm

and endoderm.

EP-A-0695351 provides a method of isolating and/or enriching and/or selectively propagating pluripotential animal cells, and especially ES cells obtained from embryonic tissue. Whilst this represents a powerful method of selecting for stem cells of any given potential, it does not address the issue of the source of the starting material.

It is thus a problem that current efforts to obtain ES cells remain generally unreliable, though the quantity of embryonic tissue available for this work is greater than hitherto. In relation to obtaining human ES cells there is the additional problem that obtaining pluripotent stem cells from the embryo is not only technically difficult but fraught with ethical considerations.

It would therefore be desirable, and it is an object of the present invention, to provide alternative and/or improved methods of obtaining pluripotential stem cells with a level of potency equivalent to that of an ES cell. It is a further object of the invention to overcome or at least ameliorate the aforementioned deficiencies in the art.

The present invention is based upon techniques developed by the inventors which have enabled cells having limited developmental potential to be taken back up the differentiation cascade, to yield cells having greater developmental potential. Specifically the methods of the invention enable production of a cell having the developmental capacity of an ES cell to be derived from a starting material that is not itself an embryo- or even germline-derived cell.

The present invention thus provides for the conversion of a somatic cell, having restricted developmental capacity, into a pluripotent stem cell that is able to form cells of the mesodermal, endodermal and ectodermal lineages.

Accordingly, a first aspect of the invention provides:-

a mammalian stem cell, capable of acting as a progenitor for generating a plurality of mammalian cell types, wherein the stem cell is derived from a second stem cell of lower relative potency.

This cell has the capacity to form many different differentiated cells, but has

been obtained from a cell hitherto believed to have the capacity to form fewer cell types - the parent of the cell of the invention is a cell that has been reversed up its developmental pathway, it has been de-differentiated. Thus, a particular cell of the invention is a pluripotent stem cell, capable of stable regeneration in in vitro culture conditions, obtained by dedifferentiation of a somatic cell.

The invention further provides a method of generating a stem cell comprising:

(a) obtaining a mammalian somatic stem cell; and

(b) exposing the cell of (a) to conditions that promote conversion of the cell into a stem cell of greater relative potency.

In a specific embodiment, the invention provides a somatic cell-derived pluripotential stem cell.

Unless apparent from the context, terms herein have the definitions set out below.

A somatic cell is a cell is not part of the germline, that is, does not have the ability to form gametes of either sex.

In developmental terms, a fertilised oocyte develops into an embryo, which in turn develops into a foetus which is then born as a neonate and develops into an adult. As the pluripotent stem cells of the developing embryo differentiate to form the foetus, cells are committed to either one of three somatic cell lineages (ectoderm, mesoderm and endoderm), or to the germline.

Undifferentiated pluripotent stem cells of the embryo have the capacity to form gametes and are therefore part of the germline. Similarly, the more specialised cells of the developing germline, such as primordial germ cells, have the ability to form gametes and are also part of the germline. Somatic cells are not part of the germline and are derived from one of three somatic cell lineages.

Thus, for example a skin cell removed from the arm of an adult human is a somatic cell and not a germ cell. Likewise, a cell removed from non-gonadal adult or foetal tissue is a somatic cell and not a germ cell. ES cells as hitherto described in the art are derived from germline cells either from the ICM (or

primitive ectoderm) of embryos - usually by simply physically removing them from the ICM - or from the primordial germ cells of the developing foetal gonad. These prior art ES and EG cells whilst functionally similar to cells of the present invention are clearly germline derived ES cells. The cells of the present invention are termed 'soma-derived' ES cells or soma-derived pluripotential cells to clearly distinguish them from all prior art cells.

Pluripotential in relation to a stem cell refers to the ability of the stem cell to form cells of all three of the somatic cell lineages of mesodermal, endodermal and ectodermal. A pluripotential stem cell is therefore capable of acting as a progenitor for all cell types found in the adult organism though capacity to form germ cells is often unproven or untested. This definition, it is emphasised, is not to be confused with multipotential, which in relation to a stem cell indicates it has the capacity to form daughter cells of a plurality but restricted number of somatic cell types.. Logically speaking, a pluripotential cell is a multipotential cell, but a multipotential cell is not a pluripotential cell.
A stem cell has the capacity to self renew and also to form at least one type of differentiated progeny.

A soma-derived pluripotential stem cell is a chromosomally normal, pluripotential stem cell that has the ability to self renew and is also a progenitor for cells of all three of the mesodermal, endodermal and ectodermal lineages in the body of the adult, but which has not been obtained from an embryo or germline cell. The soma-derived pluripotential stem cell also expresses the Oct4 gene, unlike other somatic stem cells, and specific cells of the invention have been found capable of stable culture in vitro for at least 20 cell divisions.

Somatic cells are cells of the adult that are not germ cells. Somatic stem cells are readily obtained from either foetal or adult tissue, in which sense foetal tissue refers to tissue that is post embryonic but pre-parturition.

Animal tissue encompasses all tissue in the fully formed organism and thus includes all tissues present in the organism post-parturition. Animal tissue therefore includes tissue taken from neonates and other animals through to fully grown as well as ageing adults.

The potency of a stem cell refers to its level of developmental commitment or

how restricted the cell is to a particular call fate, and thus its ability to act as a progenitor for different derivative cell types. The higher the potency of a cell the lower the restriction to a developmental pathway or fate. A cell that is more restricted to a developmental fate is further down its developmental pathway and has a lower level of potency compared to a cell higher up the pathway which is less restricted. In this context we refer to a cell which is of relatively higher potency.

The developmental dogma of unidirectional differentiation teaches that cells higher up the developmental cascade become more and more restricted in developmental capacity or potency. Some tissue types are able to regenerate themselves (if they contain tissue specific stem cells) or simply progress towards a defined non-regenerative cell type. However, it is generally accepted that normal cells do not progress back up the differentiation pathway to a less differentiated state.

Dedifferentiation is a term used to describe a process whereby a cell moves back up the developmental pathway and thus becomes less restricted to a particular cell fate. In the case of stem cells, dedifferentiation expands their potency thereby increasing the number of cell types that they are capable of acting as progenitors for. In the specific case of dedifferentiation of somatic stem cells into pluripotent stem cells, this process is characterised by reversion to a stem cell capable of forming differentiated cells of all three germ layers. Dedifferentiation of a somatic stem to a cell of higher potency typically results in a change of endogenous gene expression which in many instances is accompanied by a change in gross morphology.

A further aspect of the present invention provides methods to take cells having limited developmental potential back up the differentiation cascade, to yield cells having greater developmental potential.

Hence, the invention includes a method a method of generating a stem cell comprising:

(a) obtaining a mammalian somatic stem cell; and

(b) exposing the cell of (a) to conditions that promote conversion of the cell into a stem cell of greater relative potency.

This has been done, in one example, by culturing the somatic stem cell under conditions that promote propagation and maintenance of the stem cell of greater potency. More specifically, the invention achieves this conversion by exposing the somatic stem cell to media and/or culture conditions that are believed to be suitable for the cell of greater potency but not in fact well tolerated by the somatic stem cell starting material. The methods thus comprise "abusing" the starting material, using conditions believed in the art to be unfavourable to its growth. The cells being cultured can be spread out on culture plates and even replated a number of times, each time spreading the progeny. It then becomes possible to isolate from the progeny, sometimes towards the outside of clusters of cells, cells that have been dedifferentiated by this process.

In a specific embodiment the invention provides for the conversion of a neural cell into a pluripotential stem cell. This is carried out by culturing the neural stem cell in the presence of medium that does not promote differentiation, and preferably medium that contains factors promoting growth only of pluripotential stem cells. In examples of the invention described in detail below, good results have been obtained using medium containing serum and optionally Leukocyte Inhibitory Factor (LIF).

Other somatic cells and particularly somatic stem cells are suitably used as starting material in the method of the invention. For example, haemopoietic cells and/or stem cells or even epithelial cells and/or stem cells.

Using the methods described, specific embodiments of the invention enable isolation and thereafter maintenance stably over many generations of soma-derived pluripotent stem cells or soma-derived ES cells.

A further aspect of the invention provides derivation of a pluripotent stem cell line, by:-

(a) obtaining a first stem cell from somatic tissue;

(b) exposing the first stem cell to conditions that promote conversion of the first stem cell into a second stem cell that exhibits greater relative potency compared to the first stem cell; and

(c) maintaining the second stem cell in conditions that promote stable regeneration and/or propagation of the second stem cell.

An optional additional step, useful in removing non-desired cells from the culture utilises the selectable marker techniques described in EP-A-0695351. In a preferred embodiment, cells in the starting material posses a selectable marker that is differentially expressed in the desired target cells, the cells of higher relative potency, compared to its expression in the starting material. After culture, typically under the "abusing" conditions described above, selection for the selectable marker is used to purify the culture in respect of the target cells, and preferably substantially to remove all other cells.

Selectable markers suitable for application in this optional step may be present in the starting material or optionally introduced into the starting material by a range of techniques including but not limited to electroporation, lipofection, calcium phosphate precipitation, direct injection or ballistic missile.

A method used by the inventors and explained in more detail below resulted in a culture of cells containing cells of sufficiently high potency to act as progenitors for ectodermal, mesodermal and endodermal cell types. More generally, however, the invention is of application in obtaining cell lines of greater relative potency than the precursor cells.

In a further aspect the invention also provides for production of non-human animals using the pluripotential stem cells obtained as described.

Accordingly, there is provided a method of producing a non-human mammal, comprising:

(a) obtaining a somatic mammalian stem cell;

(b) exposing the somatic stem cell to conditions that promote conversion of the somatic stem cell into a pluripotent stem cell; and

(c) implanting or aggregating the pluripotent stem cell with an embryo to form a chimeric non-human embryo.

The resultant embryo can then be allowed to go to term, with birth of chimeric offspring having a range of cell types derived from that pluripotential cell including gametes. A chimeric non-human animal, and a non-human mammal obtained by the fusion of gametes or the reproductive cloning of a cell produced by the method is a further feature of the invention.

The invention further relates to production of cells and organs and tissue, derived from cells and/or non-human embryos or animals obtained using the methods described.

Accordingly, the invention provides a method of generating a somatic mammalian cell or tissue, comprising:

(a) obtaining a first somatic stem cell from a first mammalian tissue;

(b) exposing the first stem cell of (a) to conditions that promote conversion of the first stem cell into a second stem cell of higher relative potency; and

(c) exposing the second stem cell to conditions that promote its differentiation into a second mammalian cell or tissue.

The first and second cells are of different types. It is thus possible to start with a somatic cell of a selected patient mammal, dedifferentiate the cell to a second stem cell of higher relative potency and use that cell to derive a variety of different cells and/or tissues having the same genotype of the patient. This is of particular importance for human cell therapies, as rejection and disease transmission are thereby minimised and even potentially eliminated.

Currently, a hypothetical means of isolating pluripotent ES cells from a patient is to reprogramme a somatic nucleus from the patient using a Nuclear Transfer (NT) procedure wherein the nucleus is introduced into an enucleated human oocyte. ES cells derived from any resulting embryos are used to isolate tissue of various types suitable for transplantation back into the original patient. Other methods proposed include the introduction of oocyte or egg cytoplasm into somatic cells in an effort to reprogramme the somatic nucleus. However, although potentially overcoming the problem of host rejection, both the nuclear and cytoplasmic transfer/exchange procedures suffer from the disadvantage of the risk of disease transmission.

More specifically, a preferred embodiment of the invention comprises:
(a) obtaining a first somatic stem cell from a first mammalian tissue;
(b) exposing the first stem cell of (a) to conditions that promote conversion of the first stem cell into a second stem cell which is a pluripotent stem cell; and
(c) exposing the pluripotent stem cell to conditions that promote differentiation into a second mammalian cell or tissue.

The differentiation step can be suitably undertaken either in vitro or in vivo.

A further aspect of the invention provides a soma-derived pluripotential stem cell or soma-derived ES cell. This cell is not obtained from an embryo or cells of the germline, but is obtained from a somatic cell and is nevertheless able to form all cells of the mesodermal, endodermal and ectodermal lineages. This cell is a somatic cell-derived pluripotential stem cell. These pluripotential stem cells of the invention are animal cells, preferably mammalian, and the term mammal is intended to encompass all such animals and include higher mammals, especially mice, rats, sheep, goats, cows, pigs, primates and humans.

A yet further aspect provides a somatic cell derived from a somatic cell-derived pluripotent stem cell. This differentiated cell can be of mesodermal, endodermal or ectodermal type.

Another aspect of the invention is the conversion of the nucleus of a somatic cell into the nucleus of a cell that has greater developmental potential.

Accordingly, the invention provides a method of reprogramming a nucleus of a somatic cell, comprising:
(1) obtaining a somatic stem cell containing the nucleus; and
(2) converting said somatic stem cell into a stem cell that is less differentiated.

This can be carried out by culturing said somatic cell under conditions designed for maintenance of a cell of increased developmental potency; and isolating therefrom a stem cell nucleus having increased developmental potency.
A specific method described below in more detail comprises culturing said nuclear donor cell in the presence of conditions designed for maintenance of pluripotential stem cells; and isolating a cell or cells containing a nucleus or nuclei that have been reprogrammed. These conditions are preferably designed also to be relatively unsuitable for culture of somatic cells, even specifically unsuitable.

The methods described herein are thus suitable for reprogramming a nucleus of a somatic stem cell so as to be a nucleus of a soma-derived pluripotential stem cell or soma-derived ES cell. The methods are characterised by steps comprising culturing said somatic stem cell in the presence of conditions designed for

maintenance of an embryonic stem cell; and isolating a nucleus that has been reprogrammed. In an example described in more detail below, a nucleus of a neural stem cell is reprogrammed so as to be a nucleus of a soma-derived pluripotential stem cell or soma-derived ES cell, by culturing the neural nuclear donor cell under conditions designed for maintenance of an ES cell and which conditions are not suitable for propagation of the neural stem cell.

Also provided is a method of reprogramming a somatic cell so as to be a less differentiated somatic cell, comprising culturing said somatic cell under conditions designed for maintenance of the less differentiated somatic cell. In a specific example a method of reprogramming a neural stem cell so as to be a non embryonic or soma-derived pluripotential stem cell or soma-derived ES cell, comprises culturing said cell under conditions for maintenance of a pluripotential stem cell.

Still additionally, the invention provides the use of media for maintenance of an embryonic stem cell in conversion of a somatic stem cell into a soma-derived pluripotential stem cell or soma-derived ES cell, and use of embryonic stem cell culture conditions in conversion of a somatic stem cell into a soma-derived pluripotential stem cell or soma-derived ES cell.

The invention is now described in specific embodiments with reference to the following examples.

Example 1

Dedifferentiation of neural cells from embryo brains

The brains except forebrains were dissected from E14.5 embryos of Oct4-GFP mice (total 4 embryos) and were mechanically disassociated. The cells were centrifuged for 5 min at 1000 rpm and resuspended in N2B27 medium. 5 x 105 cells were plated into PDL-laminin coated 5 cm Nunc. dish in 5 ml N2B27 medium with 20ng/ml bFGF, 100U/ml penicillin and 100µg/ml streptomycin. Two days after culture, the medium was replaced with GMEM + 10%FCS medium supplemented with LIF and 20ng/ml bFGF. The cells were left without changing medium for 5 days, then the medium as replaced with fresh GMEM + 10%FCS medium supplemented with LIF. At this stage, there were in total only three "circles" and along the circles there were some "cells

aggregates". One day later, 7 of these aggregates were picked and trypsinized and plated in 0.1% gelatin-coated 24-well plate in GMEM + 10%FCS medium supplemented with LIF. One of these "cells aggregates" grew to be ES cell-like cells.

After picking the "cells aggregates", the cells in the 5cm dish were then trypsinized for 3 min at 37°C and the dish was washed once with GMEM + 10%FCS medium. After trypsinization and washing, there were still some cells adhered to the bottom. These cells were then cultured in N2B27 medium with 20ng/ml bFGF without changing medium, and expanded to form numerous cell colonies.

Cells were tested and found to be pluripotent.

Example 2

Dedifferentiation of neural cells from embryo brains using feeder/co-culture cells

Forebrains were dissected from E14.5 embryos of Oct4-GFP mice (total 4 embryos) and were mechanically disassociated. The cells were centrifuged for 5 min at 1000 rpm and resuspended in N2B27 medium. 5 x 105 cells were plated into PDL-laminin coated 5 cm Nunc dish in 5 ml N2B27 medium with 20ng/ml bFGF, 100U/ml penicillin and 100µg/ml streptomycin. Two days after culture, the cells were trypsinized and 1X106 cells were replated into non-coated 5cm petri dish in N2B27medium with bFGF. The cells grew in suspension and formed neurospheres (or aggregates).

Two days after growing in suspension, some neurospheres were collected and trypsinized. 5 x 104 cells (containing some small aggregates which were difficult to be disassociated) were used for co-culture with 1 x 105 E14TG2a ES cells in 0.1% gelatin-coated 6-well plate in GMEM + 10%FCS medium supplemented with LIF. Three days after co-culture, the cells were trypsinized and replated into 0.1% gelatin-coated 75mm flask in GMEM + 10%FCS medium supplemented with LIF and 1µg/ml puromycin. The medium was changed every two days with the same supplements. Ten days after selection a total 5 colonies had survived and all other cells were killed.

Thus, co-culture of ES cells and neural cells resulted in conversion of some

neural cells into GFP-green colonies with ES cell morphology and a higher pluripotency consistent with pluripotent stem cells or so termed soma-derived pluripotent stem cells or soma-derived ES cells..

Example 3

Dedifferentiation of neural cells from embryo brains

The brains except forebrains were dissected from E14.5 embryos of Oct4-GFP mice (total 4 embryos) and were mechanically disassociated. The cells were centrifuged for 5 min at 1000 rpm and resuspended in N2B27 medium. 5 x 105 cells were plated into a PDL-laminin coated 5 cm Nunc. dish in 5 ml N2B27 medium with 20ng/ml bFGF, 100U/ml penicillin and 100µg/ml streptomycin. Two days after culture, the medium was replaced with GMEM + 10%FCS medium supplemented with LIF and 20ng/ml bFGF. The cells were left without changing medium for 5 days, then the medium was replaced with fresh GMEM + 10%FCS medium supplemented with LIF. At this stage, there were in total only three "circles" and along the circles there were some "cells aggregates". One day later, 7 of these aggregates were picked and trypsinized and plated in 0.1% gelatin-coated 24-well plates in GMEM + 10%FCS medium supplemented with LIF. One of these "cells aggregates" grew to be pluripotent stem cells.

After picking the "cells aggregates", the cells in the 5cm dish were then trypsinized for 3 min at 37°C and the dish was washed once with GMEM + 10%FCS medium. After trypsinization and washing, there were still some cells adhered to the bottom. These cells were then cultured in N2B27 medium with 20ng/ml bFGF without changing medium, and expanded to form numerous cell colonies.

The cells displayed morphological characteristics and reporter gene expression profiles consistent with soma-derived pluripotent stem cells, or soma-derived pluripotent ES cells. The cells were further tested for differentiation capacity in in vitro cell culture and found to be pluripotent. Significantly, the soma-derived pluripotent ES cells were shown to be capable of spontaneous differentiation into all three germ layers in the absence of additional cells including feeder or other co-culture cells frequently required for the induction of somatic cell differentiation in vitro.

Thus, provided by the invention, is a pluripotent mammalian stem cell, capable of acting as a progenitor for generating a plurality of mammalian cell types, wherein the stem cell is derived from a second somatic stem cell of lower relative potency.

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CLAIMS

1. A mammalian stem cell wherein the stem cell is derived from a somatic stem cell of lower relative potency.

2. A stem cell according to claim 1, wherein the stem cell is a pluripotent stem cell.

3. A stem cell according to claim 1, wherein the stem cell is a multipotent stem cell.

4. A stem cell according to any previous claim, wherein the stem cell is a human stem cell.

5. A stem cell according to any previous claim, further comprising a selectable marker.

6. A stem cell according to claim 5, wherein the selectable marker selects in favour of a more undifferentiated stem cell state than the stem cell from which it is derived.

7. A stem cell according to claims 5 and 6, wherein the selectable marker is under control of the Oct 4 promoter.

8. A mammalian stem cell line, capable of stable regeneration in in vitro culture conditions, wherein the stem cell line is derived from a mammalian somatic stem cell of lower relative potency.

9. A stem cell line according to claim 8, wherein the stem cell line is a pluripotent stem cell line.

10. A stem cell line according to claims 8 and 9, wherein the somatic stem cell is a neural stem cell.

11. A stem cell line according to claims 8 and 9, wherein the somatic stem cell is a haematopoietic stem cell.

12. A stem cell line according to claims 8-11, wherein the stem cell line is a human stem cell line.

13. A non-germline derived pluripotential stem cell.

14. A cell according to claim 13 which is a mammalian cell.

15. A somatic cell-derived pluripotential stem cell.

16. A cell according to claim 15 which is a mammalian cell.

17. A somatic cell-derived embryonic stem cell.

18. A cell according to claim 17 which is a mammalian cell.

19. An in vitro method of generating a stem cell comprising:
(a) obtaining a mammalian somatic stem cell; and
(b) converting the somatic stem cell into a stem cell of relative higher potency

20. A method according to claim 19, comprising
(c) exposing the cell of (a) to conditions that promote conversion of the cell into a stem cell of greater relative potency.

21. A method according to claims 19 and 20, for generating a pluripotent stem cell.

22. A method according to claims 19 to 21, wherein the conditions that promote conversion of the cell of (a) into a stem cell of greater relative potency comprise conditions that promote propagation and maintenance of the stem cell of greater potency.

23. A method according to claim 22, where said conditions are unsuitable for maintenance of the mammalian somatic stem cell.

24. A method according to claim 20 to 23, wherein the conditions comprise culture medium comprising serum.

25. A method according to claims 20 to 24, wherein the conditions comprise culture medium comprising LIF.

26. A method of generating a stem cell comprising:

(a) obtaining a mammalian somatic stem cell; and

(b) converting the somatic stem cell into a stem cell of relative higher potency by culturing the cell in vitro in the presence of feeder cells which support the maintenance of the stem cell of higher potency

27. A method of generating a stem cell comprising:

(a) obtaining a mammalian somatic stem cell; and

(b) converting the somatic stem cell into a stem cell of relative higher potency by culturing the cell in vitro in the presence of co-culture cells which support the dedifferentiation of the somatic stem cell to a stem cell of higher potency.

28. A method of generating a stable stem cell line, comprising:

(a) obtaining a first stem cell from somatic tissue;

(b) exposing the first stem cell to conditions that promote conversion of the first stem cell into a second stem cell that exhibits greater relative potency compared to the first stem cell; and

(c) maintaining the second stem cell in conditions that promote stable regeneration and/or propagation.

29. A method according to claim 28, wherein the second stem cell is of sufficiently high potency that it is able to act as a progenitor for ectodermal, mesodermal and endodermal cell types.

30. A method according to claims 28 and 29 wherein the stable stem cell line comprises pluripotent stem cells.

31. A method according to claims 28 to 30, wherein the conditions that promote conversion of the first stem cell into the second stem cell of greater relative potency comprise conditions that promote propagation and maintenance of the second stem cell.

32. A method according to claims 28 to 31, wherein the conditions of part (c)

comprise culturing in medium supplemented with serum.

33. A method according to claims 28 to 32, wherein the conditions of part (c) comprise culturing in medium supplemented with LIF.

34. A method according to claims 28 to 33 wherein the first stem cell is a mammalian stem cell, and preferably a human stem cell

35. A method of producing a non-human mammal, comprising:
(a) obtaining a somatic mammalian stem cell;
(b) exposing the somatic stem cell to conditions that promote conversion of the somatic stem cell into a pluripotent stem cell; and
(c) implanting or aggregating the pluripotent stem cell/s with an embryo in order to generate a mammal composed in part or in total from the pluripotent stem cell/s.

36. A non-human mammal obtained by the method of claim 35 and composed in part or in whole of cells derived from pluripotent stem cells.

37. A non-human mammal descendent from a pluripotent stem cell or a decendent of a pluripotent stem cell of claim 36.

38. A non-human mammal produced by transfer of a nucleus from a cell of the method of claim 35 to an oocyte, or early embryo cell.

39. A method of generating a somatic mammalian cell or tissue, comprising:
(a) obtaining a somatic stem cell from a first mammalian tissue;
(b) exposing the stem cell of (a) to conditions that promote conversion of the stem cell into a pluripotent stem cell; and
(c) exposing the pluripotent stem cell to conditions that promote differentiation into a second mammalian cell or tissue.

40. A method according to claim 39, wherein the second mammalian tissue is a different type of tissue to the first mammalian cell or tissue.

41. A method according to claims 39 and 40, wherein the first mammalian cell or tissue is a human cell or tissue.

42. A method of reprogramming a nucleus of a somatic cell, comprising
(a) obtaining a somatic stem cell containing the nucleus; and
(b) converting said somatic stem cell into a stem cell that is less differentiated by culturing said somatic cell in conditions conducive to dedifferentiation.

43. A method of reprogramming a somatic cell so as to be a less differentiated stem cell, comprising culturing said somatic cell under conditions designed for maintenance of the less differentiated stem cell.