gene and/or as a diagnostic marker to characterize a particular cell type. See, for example, WO 2006/094836.

SUMMARY

[0006] Disclosed herein are cells that are capable of stimulating neural recovery and/or neural regeneration after transplantation to sites of nervous system injury or disease.

[0006a] In a first aspect, the present invention refers to an *in vitro* method for altering the methylation state of one or more genes in a progenitor cell, wherein the progenitor cell is a marrow adherent stromal cell (MASC), by the process of:

transfecting the progenitor cell with a polynucleotide comprising sequences encoding a Notch intracellular domain;

wherein the alterations in methylation state comprise:

(a) increased methylation of the PITX2, DNMT3b, Igf2R and SDF4 genes, and

(b) decreased methylation of the ROPN1L and TMEM179 genes.

[0006b] In a second aspect, the present invention refers to an *in vitro* method for converting a progenitor cell to a cell that stimulates neural recovery and/or neural regeneration after transplantation to a site of nervous system injury or disease, the method comprising altering the methylation state of one or more genes in the progenitor cell, wherein the progenitor cell is a marrow adherent stromal cell;

further wherein the alterations in methylation state comprise:

(i) increased methylation of the PITX2, DNMT3b, Igf2R and SDF4 genes, and

(ii) decreased methylation of the ROPN1L and TMEM179 genes;

further wherein neither the progenitor cell nor any of its descendents were transfected with a polynucleotide comprising sequences encoding a Notch intracellular domain (NICD).

[0006c] In a third aspect, the present invention refers to a method for identifying a cell, descended from a marrow adherent stromal cell, that stimulates neural recovery and/or neural regeneration after transplantation to a site of nervous system injury or disease, the method comprising assaying the methylation state of one or more genes in the descendent cell;

wherein the one or more genes is selected from the group consisting of PITX2, DNMT3b, IGF2R, SDF4, ROPN1L and TMEM179;

further wherein:

(i) methylation of the PITX2, DNMT3b, IGF2R and SDF4 genes is increased, and

(ii) methylation of the ROPN1L and TMEM179 genes is decreased.

[0006d] In certain ~~embodiments~~exemplifications, the cells are descended from marrow adherent stem cells (MASCs), but have undergone alterations in the methylation status of certain genes after treatment and culture *in vitro*. Thus, the inventor has discovered that alteration of the methylation state of one or more genes can convert a progenitor cell into a descendent cell having neural regenerative properties not possessed by the progenitor cell.

[0007] As a result of this discovery, the present disclosure encompasses, *inter alia*, the following ~~embodiments~~exemplifications:

1. A method for altering the methylation state of a gene in a cell, the method comprising:

(a) transfecting the cell with a polynucleotide comprising sequences encoding a Notch intracellular domain; and

(b) culturing the transfected cell such that the methylation state of a gene in the cell or one or more descendents of the cell is altered as compared to the gene in an untransfected cell, thereby altering the methylation state of the gene.

2. The method of ~~embodiment~~exemplification 1, wherein the gene is the PITX2 gene.

3. The method of ~~embodiment~~exemplification 1, wherein the gene is the DNMT3b gene.

4. The method of ~~embodiment~~exemplification 1, wherein the gene is the IGF2R gene.

5. The method of ~~embodiment~~exemplification 1, wherein the gene is the SDF4 gene.

6. The method of ~~embodiment~~exemplification 1, wherein the gene is the ROPN1L gene.

7. The method of ~~embodiment~~exemplification 1, wherein the gene is the TMEM179 gene.

8. The method of any of ~~embodiments~~exemplifications 1-5, wherein methylation of the gene is increased in the descendent cell.

9. The method of any of ~~embodiments~~exemplifications 1 6 or 7, wherein methylation of the gene is decreased in the descendent cell.

10. The method of ~~embodiment~~exemplification 9, wherein the sequence C-A-T-C^{me}-G-C-C-C is converted to C-A-T-C-G-C-C-C.

11. The method of any of ~~embodiments~~exemplifications 1-10, wherein the cell is a marrow adherent stromal cell (MASC).

12. A method for making a descendent cell in which the methylation state of a gene is altered, the method comprising:

(a) transfecting a progenitor cell with a polynucleotide comprising sequences encoding a Notch intracellular domain;

(b) culturing the transfected cell; and

(c) obtaining, among the progeny of the transfected cell, one or more descendent cells in which the methylation state of the gene is altered.

13. The method of ~~embodiment~~exemplification 12, wherein the gene is the PITX2 gene.

14. The method of ~~embodiment~~exemplification 12, wherein the gene is the DNMT3b gene.

15. The method of ~~embodiment~~exemplification 12, wherein the gene is the IGF2R gene.

16. The method of ~~embodiment~~exemplification 12, wherein the gene is the SDF4 gene.

17. The method of ~~embodiment~~exemplification 12, wherein the gene is the ROPN1L gene.

18. The method of ~~embodiment~~exemplification 12, wherein the gene is the TMEM179 gene.

19. The method of any of ~~embodiments~~exemplifications 12-16, wherein methylation of the gene is increased in the descendent cell compared to the progenitor cell.

20. The method of any of ~~embodiments~~exemplifications 12 or 17 or 18, wherein methylation of the gene is decreased in the descendent cell compared to the progenitor cell.

21. The method of ~~embodiment~~exemplification 20, wherein the sequence C-A-T-C^{me}-G-C-C-C is converted to C-A-T-C-G-C-C-C.

22. The method of any of ~~embodiments~~exemplifications 12-21, wherein the progenitor cell is a marrow adherent stromal cell (MASC).

23. A method for converting a progenitor cell to a neural regenerating cell, the method comprising altering the methylation state of one or more genes in the progenitor cell.

24. The method of ~~embodiment~~exemplification 23, wherein the gene is the PITX2 gene.

25. The method of ~~embodiment~~exemplification 23, wherein the gene is the DNMT3b gene.

26. The method of ~~embodiment~~exemplification 23, wherein the gene is the IGF2R gene.

27. The method of ~~embodiment~~exemplification 23, wherein the gene is the SDF4 gene.

28. The method of ~~embodiment~~exemplification 23, wherein the gene is the ROPN1L gene.

29. The method of ~~embodiment~~exemplification 23, wherein the gene is the TMEM179 gene.

30. The method of any of ~~embodiments~~exemplifications 23-27, wherein methylation of the gene is increased in the neural regenerating cell, compared to the progenitor cell.

31. The method of any of ~~embodiments~~exemplifications 23, 28 or 29, wherein methylation of the gene is decreased in the neural regenerating cell, compared to the progenitor cell.

32. The method of ~~embodiment~~exemplification 31, wherein the sequence C-A-T-C^{me}-G-C-C-C is converted to C-A-T-C-G-C-C-C.

33. The method of any of ~~embodiments~~exemplifications 23-32, wherein the progenitor cell is a marrow adherent stromal cell (MASC).

34. The method of ~~embodiment~~exemplification 23, wherein the methylation state of the gene is altered by:

(a) transfecting the progenitor cell with a polynucleotide comprising sequences encoding a Notch intracellular domain;

(b) culturing the transfected cell; and

(c) obtaining, among the progeny of the transfected cell, one or more descendent cells in which the methylation state of the gene is altered;

wherein said descendent cell in which the methylation state of the gene is altered is a neural regenerating cell.

35. The method of ~~embodiment~~exemplification 30, wherein the methylation state of the gene is altered by contacting the progenitor cell with a fusion protein comprising a methylation domain and a DNA-binding domain, or with a nucleic acid encoding a fusion protein comprising a methylation domain and a DNA-binding domain, wherein the DNA binding domain is engineered to bind to one or more sequences in the gene.

36. The method of ~~embodiment~~exemplification 31, wherein the methylation state of the gene is altered by contacting the progenitor cell with a fusion protein comprising a demethylation domain and a DNA-binding domain, or with a nucleic acid encoding a fusion protein comprising a demethylation domain and a DNA-binding domain, wherein the DNA binding domain is engineered to bind to one or more sequences in the gene.

37. A cell that is descended from a progenitor cell through culture *in vitro*, wherein:

(a) the cell supports the growth and/or regeneration of neural tissue;

(b) the methylation state of one or more genes in the cell is altered compared to the progenitor cell; and

(c) during the culture *in vitro*, neither the progenitor cell nor any of its descendents were transfected with a polynucleotide comprising sequences encoding the Notch intracellular domain (NICD).

38. The cell of ~~embodiment~~exemplification 37, wherein the gene is the PITX2 gene.

39. The cell of ~~embodiment~~exemplification 37, wherein the gene is the DNMT3b gene.

40. The cell of ~~embodiment~~exemplification 37, wherein the gene is the IGF2R gene.

41. The cell of ~~embodiment~~exemplification 37, wherein the gene is the SDF4 gene.

42. The cell of ~~embodiment~~exemplification 37, wherein the gene is the ROPN1L gene.

43. The cell of ~~embodiment~~exemplification 37, wherein the gene is the TMEM179 gene.

44. The cell of any of ~~embodiments~~exemplifications 37-41, wherein methylation of the gene is increased in the neural regenerating cell, compared to the progenitor cell.

45. The cell of any of ~~embodiments~~exemplifications 37, 42 or 43, wherein methylation of the gene is decreased in the neural regenerating cell compared to the progenitor cell.

46. The cell of ~~embodiment~~exemplification 45, wherein the sequence C-A-T-C^{me}-G-C-C-C is converted to C-A-T-C-G-C-C-C.

47. The cell of any of ~~embodiments~~exemplifications 37-46, wherein the progenitor cell is a marrow adherent stromal cell (MASC).

48. The cell of ~~embodiment~~exemplification 37, wherein the methylation state of the gene is altered by contacting the progenitor cell with a fusion protein comprising a methylation domain and a DNA-binding domain, or with a nucleic acid encoding a fusion protein comprising a methylation domain and a DNA-binding domain, wherein the DNA binding domain is engineered to bind to one or more sequences in the gene.

49. The cell of ~~embodiment~~exemplification 37, wherein the methylation state of the gene is altered by contacting the progenitor cell with a fusion protein comprising a demethylation domain and a DNA-binding domain, or with a nucleic acid encoding a fusion protein comprising a demethylation domain and a DNA-binding domain, wherein the DNA binding domain is engineered to bind to one or more sequences in the gene.

50. A method for identifying a neural regenerating cell, the method comprising assaying the methylation state of one or more genes in the cell, wherein a change in the methylation state of the assayed genes is indicative of a neural regenerating cells.

51. The method of ~~embodiment~~exemplification 50, wherein the assay is for increased methylation of the one or more genes.

52. The method of ~~embodiment~~exemplification 50, wherein the assay is for decreased methylation of the one or more genes.

53. The method of ~~embodiment~~exemplification 50, wherein the assay is for increased methylation of one or more first genes and for decreased methylation of one or more second genes.

54. The method of ~~embodiment~~exemplification 50, wherein the one or more genes is/are selected from the group consisting of PITX2, ROPN1L, DNMT3b, IGF2R, TMEM179 and SDF4.

BRIEF DESCRIPTION OF THE DRAWINGS

[0008] Not applicable.

DETAILED DESCRIPTION

Methylation State as a Diagnostic

[0009] Analysis of changes in the DNA methylation state of specific CpG sequences in or near one or more genes of interest can be used to identify a cell and to distinguish it from other cells with different DNA methylation patterns. For example, if, in a stem or other type of progenitor cell, a particular CpG sequence is methylated on its C residue, and upon further differentiation, the C residue becomes demethylated; the demethylation of that C residue can be used as a marker for that differentiation step. Conversely, methylation of a C residue may serve as a marker for differentiation. Total, all-or-none changes in methylation state are not required; a change in methylation frequency at a particular CpG sequence can also be diagnostic.

[0010] A number of methods, known in the art, can be used to distinguish methylated from unmethylated cytosine residues. These include, but are not limited to, treatment of DNA with bisulfite, and assay of DNA cleavage with methylation-sensitive and methylation-dependent restriction enzymes. Bisulfite (SO_3^-) treatment deaminates unmethylated cytosine, converting it to deoxyuridine which, upon replication, templates an adenosine residue in the nascent DNA strand. Thus, bisulfite treatment results in eventual conversion of a C-G base pair into a T-A base pair; and such changes can be detected by standard DNA sequencing methods. Methylated C residues are unaffected by bisulfite treatment; therefore C^{me}-G base pairs remain unchanged.

[0011] For assay of methylation status using restriction enzymes, enzymes with the sequence CG in their recognition site can be used. For certain recognition sites which contain the sequence CG, an enzyme which recognizes the site will fail to