METHODS OF GENERATING AND ISOLATING MIDBRAIN DOPAMINE NEURONS

CROSS REFERENCE TO RELATED APPLICATIONS

The present application claims priority to United States Provisional Application No. 62/893,674 filed August 29, 2019, the content of which is incorporated by reference in its entirety herein, and priority to which is claimed.

1. INTRODUCTION

The present disclosure provides methods for generating midbrain dopamine neurons (mDAs) and precursors thereof, mDAs and precursors thereof generated by such methods and composition comprising such cells. The present disclosure also provides uses of the mDAs and composition comprising thereof for preventing and/or treating neurological disorders. The present disclosure further provides methods of isolating mDAs and precursors thereof from a cell population using novel surface markers.

2. BACKGROUND

Previously, embryonic and somatic stem cells were used as therapeutics and model systems for neurodegenerative diseases. Research and technological developments relating to directed differentiation of embryonic and somatic stem cells has taken place in the field of diseases of the central nervous system (CNS), such as for Huntington's, Alzheimer's, Parkinson's, and multiple sclerosis. However the results of these studies showed little *in vivo* capability to restore neuronal function and often resulted in the growth of unwanted tumors in the patients.

Therefore, there is still a need for improved methods for generating midbrain dopamine neurons that are suitable for treating neurodegenerative disorders such as Parkinson's disease.

3. SUMMARY ~~OF THE INVENTION~~

The present disclosure provides methods for generating midbrain dopamine neurons (mDAs) and precursors thereof, mDAs and precursors thereof generated by such methods, compositions comprising such cells, and uses of such cells and compositions for preventing and/or treating neurological disorders. In addition, the present disclosure provides methods of isolating mDAs and precursors thereof from a cell population using novel surface markers.

In certain aspects~~embodiments~~, the present disclosure provides an *in vitro* method for inducing differentiation of stem cells, comprising: contacting the stem cells with at least one inhibitor of Small Mothers Against Decapentaplegic (SMAD) signaling, at least one activator of Sonic hedgehog (SHH) signaling, and at least one activator of wingless (Wnt) signaling; and contacting the cells with at least one activator of fibroblast growth factor (FGF) signaling to obtain a population of differentiated cells expressing at least one marker indicating a midbrain dopamine neuron (mDA) or a precursor thereof, wherein the at least one activator of FGF signaling is selected from FGF18, FGF17, FGF8a, and combination thereof.

In certain aspects~~embodiments~~, the present disclosure provides an *in vitro* method for inducing differentiation of stem cells, comprising: contacting the stem cells with at least one inhibitor of Small Mothers Against Decapentaplegic (SMAD) signaling, at least one activator of Sonic hedgehog (SHH) signaling, and at least one activator of wingless (Wnt) signaling; and contacting the cells with at least one activator of fibroblast growth factor (FGF) signaling to obtain a population of differentiated cells expressing at least one marker indicating a midbrain dopamine neuron (mDA) or a precursor thereof, wherein the initial contact of the cells with the at least one activator of FGF signaling is at least about 5 days from the initial contact of the cells with the at least one inhibitor of SMAD signaling.

In certain examples~~embodiments~~, the cells are contacted with the at least one activator of FGF signaling for at least about 1 day. In certain examples~~embodiments~~, the cells are contacted with the at least one activator of FGF signaling for up to about 15 days. In certain examples~~embodiments~~, the cells are contacted with the at least one activator of FGF signaling for about 5 days.

In certain examples~~embodiments~~, the initial contact of the cells with the at least one activator of FGF signaling is at least about 5 days from the initial contact of the cells with the at least one inhibitor of SMAD signaling.

In certain examples~~embodiments~~, the initial contact of the cells with the at least one activator of FGF signaling is about 10 days from the initial contact of the cells with the at least one inhibitor of SMAD signaling. In certain examples~~embodiments~~, the initial contact of the cells with the at least one activator of FGF signaling is 12 days from the initial contact of the cells with the at least one inhibitor of SMAD signaling.

In certain examples~~embodiments~~, the cells are contacted with the at least one inhibitor of SMAD signaling for about 5 days. In certain examples~~embodiments~~, the cells are contacted with the at least one inhibitor of SMAD signaling for 7 days.

In certain examples~~embodiments~~, the cells are contacted with the at least one activator of SHH signaling for about 5 days. In certain examples~~embodiments~~, the cells are contacted with the at least one activator of SHH signaling for 7 days.

In certain examples~~embodiments~~, the cells are contacted with the at least one activator of Wnt signaling for about 10 days. In certain examples~~embodiments~~, the cells are contacted with the at least one activator of Wnt signaling for 12 days.

In certain examples~~embodiments~~, the concentration of the at least one activator of Wnt signaling is increased about 4 days from its initial contact with the stem cells. In certain examples~~embodiments~~, the concentration of the at least one activator of Wnt signaling is increased by between about 300 % and about 1000 % from the initial concentration of the at least one activator of Wnt signaling. In certain examples~~embodiments~~, the concentration of the at least one activator of Wnt signaling is increased to a concentration of between about 3 μM and 10 μM. In certain examples~~embodiments~~, the concentration of the at least one activator of Wnt signaling is increased to a concentration of about 3 μM. In certain examples~~embodiments~~, the concentration of the at least one activator of Wnt signaling is increased to a concentration of about 7.5 μM.

In certain examples~~embodiments~~, the at least one activator of FGF signaling comprises FGF18.

In certain examples~~embodiments~~, the at least one inhibitor of SMAD signaling is selected from inhibitors of TGFβ/Activin-Nodal signaling, inhibitors of bone morphogenetic protein (BMP) signaling, and combinations thereof.

In certain examples~~embodiments~~, the at least one inhibitor of TGFβ/Activin-Nodal signaling is comprises an inhibitor of ALK5.

In certain examples~~embodiments~~, the at least one inhibitor of TGFβ/Activin-Nodal signaling comprises SB431542, or a derivative, or a mixture thereof. In certain examples~~embodiments~~, the derivative of SB431542 is A83-01. In certain examples~~embodiments~~, the at least one inhibitor of TGFβ/Activin-Nodal signaling comprises SB431542.

In certain examples~~embodiments~~, the at least one inhibitor of BMP signaling comprises LDN193189, Noggin, dorsomorphin, a derivative thereof, or a mixture thereof. In certain examples~~embodiments~~, the at least one inhibitor of BMP comprises LDN-193189.

In certain examples~~embodiments~~, the at least one activator of Wnt signaling comprises an inhibitor of glycogen synthase kinase 3β (GSK3β) signaling.

In certain examples~~embodiments~~, the at least one activator of Wnt signaling is selected from CHIR99021, BIO, CHIR98014, Lithium, 3F8,Wnt3A, Wnt1, Wnt5a, derivatives thereof, and mixtures thereof. In certain examples~~embodiments~~, the at least one activator of Wnt signaling comprises CHIR99021.

In certain examples~~embodiments~~, the at least one activator of SHH signaling is selected from SHH protein, Smoothened agonists (SAG), derivatives thereof, and mixtures thereof. In certain examples~~embodiments~~, the SHH protein comprises a recombinant SHH, a purified SHH, or a combination of the foregoing.

In certain examples~~embodiments~~, the recombinant SHH comprises a recombinant protein that is at least about 80% identical to a mouse Sonic Hedgehog N-terminal fragment. In certain examples~~embodiments~~, the recombinant SHH comprises SHH C25II. In certain examples~~embodiments~~, the SAG comprises purmorphamine.

In certain examples~~embodiments~~, the at least one marker indicating a midbrain dopamine neuron or a precursor thereof is selected from EN1, OTX2, TH, NURR1, FOXA2, PITX3, LMX1A, LMO3, SNCA, ADCAP1, CHRNA4, GIRK2, and combinations thereof.

In certain examples~~embodiments~~, the differentiated cells have a detectable level of expression of the at least one marker indicating a midbrain dopamine neuron or a precursor thereof at least about 10 days from the initial contact of the stem cells with the at least one inhibitor of SMAD signaling.

In certain examples~~embodiments~~, the differentiated cells have a detectable level of expression of EN1 about 30 days from the initial contact of the stem cells with the at least one inhibitor of SMAD signaling. In certain examples~~embodiments~~, the differentiated cells have a detectable level of expression of EN1 about 40 days from the initial contact of the stem cells with the at least one inhibitor of SMAD signaling.

In certain examples~~embodiments~~, the differentiated cells do not express at least one marker selected from PAX6, EMX2, LHX2, SMA, SIX1, PITX2, SIM1, POU4F1, PHOX2A, BARHL1, BARHL2, GBX2, HOXA2, HOXB2, POU5F1, NANOG, and combinations thereof.

In certain examples~~embodiments~~, the method further comprises subjecting the population of differentiated cells to conditions favoring differentiation of midbrain dopamine neuron precursors to midbrain dopamine neurons.

In certain examples~~embodiments~~, the conditions comprise exposing the cells to at least one of brain-derived neurotrophic factor (BDNF), glial cell-derived neurotrophic factor (GDNF), Cyclic adenosine monophosphate (cAMP), Transforming growth factor beta 3 (TGFP3), ascorbic acid (AA), and DAPT.

In certain examples~~embodiments~~, the stem cells are selected from human, nonhuman primate or rodent nonembryonic stem cells; human, nonhuman primate or rodent embryonic stem cells; human, nonhuman primate or rodent induced pluripotent stem cells; and human, nonhuman primate or rodent recombinant pluripotent cells. In certain examples~~embodiments~~, the stem cells are human stem cells. In certain examples~~embodiments~~, the stem cells are pluripotent or multipotent stem cells. In certain examples~~embodiments~~, the stem cells are pluripotent stem cells. In certain examples~~embodiments~~, the pluripotent stem cells are selected from embryonic stem cells, induced pluripotent stem cells, and combinations thereof.

In another aspect, the present disclosure provides a cell population of *in vitro* differentiated cells, wherein said *in vitro* differentiated cells are obtained by any preceding methods.

In another aspect, the present disclosure provides a cell population of *in vitro* differentiated cells, wherein at least about 50% of the cells express at least one marker indicating a midbrain dopamine neuron or a precursor thereof, and less than about 50% of the differentiated cells express at least one marker selected from PAX6, EMX2, LHX2, SMA, SIX1, PITX2, SIM1, POU4F1, PHOX2A, BARHL1, BARHL2, GBX2, HOXA2, HOXB2, POU5F1, NANOG, and combinations thereof. In certain examples~~embodiments~~, the at least one marker indicating a midbrain dopamine neuron or a precursor thereof is selected from EN1, OTX2, TH, NURR1, FOXA2, LMX1A, PITX3, LMO3, SNCA, ADCAP1, CHRNA4, GIRK2.

In another aspect, the present disclosure provides a composition comprising the cell population disclosed herein. In certain examples~~embodiments~~, the composition is a pharmaceutical composition further comprising a pharmaceutically acceptable carrier.

In another aspect, the present disclosure provides a method for isolating midbrain dopamine neurons and precursors thereof from a population of cells, comprising

isolating cells that do not express a detectable level of at least one negative surface marker and express a detectable level of at least one positive surface marker.

In another aspect, the present disclosure provides a method for isolating midbrain dopamine neurons and precursors thereof from a population of cells, comprising isolating cells that (a) do not express a detectable level or express a reduced level of at least one negative surface marker as compared to the mean expression level of the at least one negative surface marker in the population of cells; and (b) an increased level of at least one positive surface marker as compared to the mean expression level of the at least one positive marker in the population of cells.

In certain examples~~embodiments~~, the at least one positive surface marker is selected from CD171, CD184, and combinations thereof. In certain examples~~embodiments~~, the at least one positive surface marker comprises CD184. In certain examples~~embodiments~~, the at least one negative surface marker is selected from CD49e, CD99, CD340, and combinations thereof. In certain examples~~embodiments~~, the at least one negative surface marker comprises CD49e. In certain examples~~embodiments~~, the method comprises isolating cells that do not express a detectable level of CD49e and express a detectable level of CD184.

In certain examples~~embodiments~~, the method comprises isolating cells that do not express a detectable level or express a reduced level of CD49e as compared the mean expression level of CD49e in the population of cells; and express an increased level of CD184 as compared to the mean expression level of CD184 in the population of cells.

In another aspect, the present disclosure provides a cell population of *in vitro* differentiated cells, wherein at least about 50% of the cells express a detectable level of at least one positive surface marker and do not express a detectable level of at least one negative surface marker.

In another aspect, the present disclosure provides a cell population of in vitro differentiated cells, wherein at least about 50% of the cells express an increased level of at least one positive surface marker as compared to the mean expression level of the at least one positive marker in the population of cells; and do not express a detectable level or express a reduced level of at least one negative surface marker as compared to the mean expression level of the at least one negative surface marker in the population of cells.

In certain examples~~embodiments~~, the at least one positive surface marker is selected from CD171, CD184, and combinations thereof. In certain examples~~embodiments~~, the at least one

positive surface marker comprises CD184. In certain examples~~embodiments~~, the at least one negative surface marker is selected from CD49e, CD99, CD340, and combinations thereof. In certain examples~~embodiments~~, the at least one negative surface marker comprises CD49e. In certain examples~~embodiments~~, the at least one positive surface marker comprises CD184 and the at least one negative surface marker comprises CD49e.

In another aspect, the present disclosure provides a composition comprising the cell population disclosed herein. In certain examples~~embodiments~~, the composition disclosed herein is a pharmaceutical composition further comprising a pharmaceutically acceptable carrier. In another aspect, the present disclosure provides kit for inducing differentiation of stem cells to midbrain dopamine neurons or precursors thereof, comprising: (a) at least one inhibitor of SMAD signaling; (b) at least one activator of SHH signaling; (c) at least one activator of Wnt signaling; and (d) at least one activator of FGF signaling.

In certain examples~~embodiments~~, the kit further comprises (f) instructions for inducing differentiation of the stem cells into a population of differentiated cells that express at least one midbrain DA precursor marker.

In another example~~aspect~~, the present disclosure provides a method of preventing and/or treating a neurodegenerative disorder in a subject, comprising administering to the subject an effective amount of one of the followings: (a) the cell population disclosed herein; or (b) the composition disclosed herein.

In certain examples~~embodiments~~, the neurodegenerative disorder is Parkinson's disease, Huntington's disease, Alzheimer's disease, or multiple sclerosis.

In certain examples~~embodiments~~, the cell population disclosed herein or the composition disclosed herein is for use in preventing and/or treating a neurodegenerative disorder in a subject. In certain examples~~embodiments~~, the neurodegenerative disorder is Parkinson's disease, Huntington's disease, Alzheimer's disease, or multiple sclerosis.

In another aspect, the present disclosure refers to a use of an effective amount of one of the followings: (a) the cell population as described herein; or (b) the composition as described herein in the manufacture of a medicament for preventing and/or treating a neurodegenerative disorder in a subject.

In another aspect, the present disclosure refers to the cell population as described herein or the composition as described herein for use in preventing and/or treating a neurodegenerative disorder in a subject.

4. BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows EN1 expression in stem-cell-derived mDAs and precursors from day 3 to day 30 using the Wnt-Boost protocol.

Fig. 2 depicts the protocol using FGF8b in combinations with the Wnt-Boost protocol.

Fig. 3 shows that EN1 protein was maintained in the differentiated cells in a FGF8b contacting duration dependent manner. Cells expressing EN1 also expressed FOXA2 and LMX1A.

WHAT IS CLAIMED IS:

1. An *in vitro* method for inducing differentiation of stem cells, comprising: contacting the stem cells with at least one inhibitor of Small Mothers Against Decapentaplegic (SMAD) signaling, at least one activator of Sonic hedgehog (SHH) signaling, and at least one activator of wingless (Wnt) signaling; and contacting the cells with at least one activator of fibroblast growth factor (FGF) signaling to obtain a population of differentiated cells expressing at least one marker indicating a midbrain dopamine neuron (mDA) or a precursor thereof, wherein the at least one activator of FGF signaling is selected from FGF18, FGF17, FGF8a, and combination thereof.

2. An *in vitro* method for inducing differentiation of stem cells, comprising: contacting the stem cells with at least one inhibitor of Small Mothers Against Decapentaplegic (SMAD) signaling, at least one activator of Sonic hedgehog (SHH) signaling, and at least one activator of wingless (Wnt) signaling; and contacting the cells with at least one activator of fibroblast growth factor (FGF) signaling to obtain a population of differentiated cells expressing at least one marker indicating a midbrain dopamine neuron (mDA) or a precursor thereof, wherein the initial contact of the cells with the at least one activator of FGF signaling is at least about 5 days from the initial contact of the cells with the at least one inhibitor of SMAD signaling.

3. The method of claim 1 or 2, wherein the cells are contacted with the at least one activator of FGF signaling for at least about 1 days.

4. The method of any one of claims 1-3, wherein the cells are contacted with the at least one activator of FGF signaling for up to about 15 days.

5. The method of any one of claims 1-4, wherein the cells are contacted with the at least one activator of FGF signaling for about 5 days.

6. The method of any one of claims 1-5, wherein the initial contact of the cells with the at least one activator of FGF signaling at least about 5 days from the initial contact of the cells with the at least one inhibitor of SMAD signaling.

7. The method of any one of claims 1-6, wherein the initial contact of the cells with the at least one activator of FGF signaling is about 10 days from the initial contact of the cells with the at least one inhibitor of SMAD signaling.

8. The method of any one of claims 1-7, wherein the initial contact of the cells with the at least one activator of FGF signaling is 12 days from the initial contact of the cells with the at least one inhibitor of SMAD signaling.

9. The method of any one of claims 1-8, wherein the cells are contacted with the at least one inhibitor of SMAD signaling for about 5 days.

10. The method of any one of claims 1-9, wherein the cells are contacted with the at least one inhibitor of SMAD signaling for 7 days.

11. The method of any one of claims 1-10, wherein the cells are contacted with the at least one activator of SHH signaling for about 5 days.

12. The method of any one of claims 1-11, wherein the cells are contacted with the at least one activator of SHH signaling for 7 days.

13. The method of any one of claims 1-12, wherein the cells are contacted with the at least one activator of Wnt signaling for about 10 days.

14. The method of any one of claims 1-13, wherein the cells are contacted with the at least one activator of Wnt signaling for 12 days.

15. The method of any one of claims 1-14, wherein the concentration of the at least one activator of Wnt signaling is increased about 4 days from its initial contact with the stem cells.

16. The method of claim 15, wherein the concentration of the at least one activator of Wnt signaling is increased by between about 300 % and about 1000 % from the initial concentration of the at least one activator of Wnt signaling.

17. The method of any one of claims 15-16, wherein the concentration of the at least one activator of Wnt signaling is increased to a concentration of between about 3 μM and 10 μM.

18. The method of any one of claims 15-17, wherein the concentration of the at least one activator of Wnt signaling is increased to a concentration of about 3 μM.

19. The method of any one of claims 15-17, wherein the concentration of the at least one activator of Wnt signaling is increased to a concentration of about 7.5 μM.

20. The method of any one of claims 1-19, wherein at least one activator of FGF signaling comprises FGF18.

21. The method of any one of claims 1-20, wherein the at least one inhibitor of SMAD signaling is selected from inhibitors of TGFβ/Activin-Nodal signaling, inhibitors of bone morphogenetic protein (BMP) signaling, and combinations thereof.

22. The method of claim 21, wherein the at least one inhibitor of TGFβ/Activin-Nodal signaling is comprises an inhibitor of ALK5.

23. The method of claim 21 or 22, wherein the at least one inhibitor of TGFβ/Activin-Nodal signaling comprises SB431542, or a derivative, or a mixture thereof.

24. The method of claim 23, wherein the derivative of SB431542 is A83-01.

25. The method of any one of claims 217-24, wherein the at least one inhibitor of TGFβ/Activin-Nodal signaling comprises SB431542.

26. The method of claim 21, wherein the at least one inhibitor of BMP signaling comprises LDN193189, Noggin, dorsomorphin, a derivative thereof, or a mixture thereof.

27. The method of claim 21 or 26, wherein the at least one inhibitor of BMP comprises LDN-193189.

28. The method of any one of claims 1-27, wherein the at least one activator of Wnt signaling comprises an inhibitor of glycogen synthase kinase 3β (GSK3β) signaling.

29. The method of claim 28, wherein the at least one activator of Wnt signaling is selected from CHIR99021, Wnt3A, Wnt1, derivatives thereof, and mixtures thereof.

30. The method of any one of claims 1-29, wherein the at least one activator of SHH signaling is selected from SHH protein, Smoothened agonists (SAG), derivatives thereof, and mixtures thereof.

31. The method of claim 30, wherein the SHH protein comprises a recombinant SHH, a purified SHH, or a combination of the foregoing.

32. The method of claim 31, wherein the recombinant SHH comprises a recombinant protein that is at least about 80% identical to a mouse Sonic Hedgehog N-terminal fragment.

33. The method of claim 31 or 32, wherein recombinant SHH comprises SHH C25II.

34. The method of claim 30, wherein the SAG comprises purmorphamine.

35. The method of any one of claims 1-34, wherein the at least one marker indicating a midbrain dopamine neuron or a precursor thereof is selected from EN1, OTX2, TH, NURR1, FOXA2, PITX3, LMX1A, LMO3, SNCA, ADCAP1, CHRNA4, GIRK2, and combinations thereof.

36. The method of any one of claims 1-35, wherein the differentiated cells have a detectable level of expression of the at least one marker indicating a midbrain dopamine neuron or a precursor thereof at least about 10 days from the initial contact of the stem cells with the at least one inhibitor of SMAD signaling.

37. The method of any one of claims 1-36, wherein the differentiated cells have a detectable level of expression of EN1 about 30 days from the initial contact of the stem cells with the at least one inhibitor of SMAD signaling.

38. The method of any one of claims 1-36, wherein the differentiated cells have a detectable level of expression of EN1 about 40 days from the initial contact of the stem cells with the at least one inhibitor of SMAD signaling.

39. The method of any one of claims 1-38, wherein the differentiated cells do no express at least one marker selected from PAX6, EMX2, LHX2, SMA, SIX1, PITX2, SIM1, POU4F1, PHOX2A, BARHL1, BARHL2, GBX2, HOXA2, HOXB2, POU5F1, NANOG, and combinations thereof.

40. The method of any one of claims 1-39, further comprising subjecting the population of differentiated cells to conditions favoring differentiation of midbrain dopamine neuron precursors to midbrain dopamine neurons.

41. The method of claim 40, wherein the conditions comprise exposing the cells to at least one of brain-derived neurotrophic factor (BDNF), glial cell-derived neurotrophic factor (GDNF), Cyclic adenosine monophosphate (cAMP), Transforming growth factor beta 3 (TGFP3), ascorbic acid (AA), and DAPT.

42. The method of any one of claims 1-41, wherein the stem cells are selected from human, nonhuman primate or rodent nonembryonic stem cells; human, nonhuman primate or rodent embryonic stem cells; human, nonhuman primate or rodent induced pluripotent stem cells; and human, nonhuman primate or rodent recombinant pluripotent cells.

43. The method of any one of claims 1-42, wherein stem cells are human stem cells.

44. The method of any one of claims 1-43, wherein the stem cells are pluripotent or multipotent stem cells.

45. The method of any one of claims 1-44, wherein the stem cells are pluripotent stem cells.

46. The method of any one of claims 1-45, wherein the pluripotent stem cells are selected from embryonic stem cells, induced pluripotent stem cells, and combinations thereof.

47. A cell population of *in vitro* differentiated cells, wherein said *in vitro* differentiated cells are obtained by a method of any one of claims 1-46.

48. A cell population of *in vitro* differentiated cells, wherein at least about 50% of the cells express at least one marker indicating a midbrain dopamine neuron or a precursor thereof, and less than about 50% of the differentiated cells express at least one marker selected from PAX6, EMX2, LHX2, SMA, SIX1, PITX2, SIM1, POU4F1, PHOX2A, BARHL1, BARHL2, GBX2, HOXA2, HOXB2, POU5F1, NANOG, and combinations thereof.

49. The cell population of claim 48, wherein the at least one marker indicating a midbrain dopamine neuron or a precursor thereof is selected from EN1, OTX2, TH, NURR1, FOXA2, LMX1A, PITX3, LMO3, SNCA, ADCAP1, CHRNA4, GIRK2, and combinations thereof.

50. A composition comprising the cell population of claim 48 or 49.

51. The composition of claim 50, which is a pharmaceutical composition further comprising a pharmaceutically acceptable carrier.

52. A method for isolating midbrain dopamine neurons and precursors thereof from a population of cells, comprising isolating cells that do not express a detectable level of at least

one negative surface marker and express a detectable level of at least one positive surface marker.

53. A method for isolating midbrain dopamine neurons and precursors thereof from a population of cells, comprising isolating cells that (a) do not express a detectable level or express a reduced level of at least one negative surface marker as compared to the mean expression level of the at least one negative surface marker in the population of cells; and (b) an increased level of at least one positive surface marker as compared to the mean expression level of the at least one positive marker in the population of cells.

54. The method of claim 52 or 53, wherein the at least one negative surface marker is selected from CD171, CD184, and combinations thereof.

55. The method of any one of claims 52-54, wherein the at least one negative surface marker comprises CD184.

56. The method of any one of claims 52-55, wherein the at least one negative surface marker is selected from CD49e, CD99, CD340, and combinations thereof.

57. The method of any one of claims 52-56, wherein the at least one negative surface marker comprises CD49e.

58. The method of any one of claims 52-57, wherein the method comprises isolating cells that do not express a detectable level of CD49e and express a detectable level of CD184.

59. The method of any one of claims 52-58, wherein the method comprises isolating cells that do not express a detectable level or express a reduced level of CD49e as compared the mean expression level of CD49e in the population of cells; and express an increased level of CD184 as compared to the mean expression level of CD184 in the population of cells.

60. A cell population of *in vitro* differentiated cells, wherein at least about 50% of the cells express a detectable level of at least one positive surface marker and do not express a detectable level of at least one negative surface marker.

61. A cell population of *in vitro* differentiated cells, wherein at least about 50% of the cells express an increased level of at least one positive surface marker as compared to the mean expression level of the at least one positive marker in the population of cells; and do

not express a detectable level or express a reduced level of at least one negative surface marker as compared to the mean expression level of the at least one negative surface marker in the population of cells.

62. The cell population of claim 60 or 61, wherein the at least one negative surface marker is selected from CD171, CD184, and combinations thereof.

63. The cell population of any one of claims 60-62, wherein the at least one negative surface marker comprises CD184.

64. The cell population of any one of claims 60-63, wherein the at least one negative surface marker is selected from CD49e, CD99, CD340, and combinations thereof.

65. The cell population of any one of claims 60-64, wherein the at least one negative surface marker comprises CD49e.

66. The cell population of any one of claims 60-65, wherein the at least one negative surface marker comprises CD184 and the at least one negative surface marker comprises CD49e.

67. A composition comprising the cell population of any one of claims 60-66.

68. The composition of claim 67, which is a pharmaceutical composition further comprising a pharmaceutically acceptable carrier.

69. A kit for inducing differentiation of stem cells to midbrain dopamine neurons or precursors thereof, comprising:

(a) at least one inhibitor of SMAD signaling;

(b) at least one activator of SHH signaling;

(c) at least one activator of Wnt signaling; and

(d) at least one activator of FGF signaling.

70. The kit of claim 69, further comprising (f) instructions for inducing differentiation of the stem cells into a population of differentiated cells that express at least one midbrain DA precursor marker.

71. Use of an effective amount of one of the followings: (a) the cell population of any one of claims 47-49 and 60-66; or (b) the composition of any one of claims 50, 51, 67 and 68 in

the manufacture of a medicament for~~A method of~~ preventing and/or treating a neurodegenerative disorder in a subject~~, comprising administering to the subject an effective amount of one of the followings:~~

~~(a) the cell population of any one of claims 47-49 and 60-66; or~~

~~(b) the composition of any one of claims 50, 51, 67 and 68~~.

72. The ~~method~~use of claim 71~~68~~, wherein the neurodegenerative disorder is Parkinson's disease, Huntington's disease, Alzheimer's disease, or multiple sclerosis.

73. The cell population of any one of claims 47-49 and 60-66 or the composition of any one of claims 50, 51, 67 and 68 for use in preventing and/or treating a neurodegenerative disorder in a subject.

74. The cell population or composition for use of claim 73, wherein the neurodegenerative disorder is Parkinson's disease, Huntington's disease, Alzheimer's disease, or multiple sclerosis.