

Claims

1. A population of cells expressing markers characteristic of the pancreatic endoderm lineage, wherein greater than 80% of cells in the population co-express PDX1 and NKX6.1, wherein the population is obtained by differentiating cells expressing markers characteristic of the definitive endoderm lineage in a medium supplemented with a protein kinase C activator and at least one factor from the group consisting of a factor capable of inhibiting BMP, a TGF β receptor signaling inhibitor, and a fibroblast growth factor, and wherein the protein kinase C activator is selected from the group consisting of (2S, 5S)-(E, E)-8-(5-(4-(Trifluoromethyl)phenyl)-2,4-pentadiemoylamino) benzolactam, phorbol-12-myristate-13-acetate, and phorbol-12,13-dibutyrate.
2. The population of cells of claim 1, wherein greater than 90% of the cells in the population co-express PDX1 and NKX6.1 and wherein the protein kinase C activator is (2S, 5S)-(E, E)-8-(5-(4-(Trifluoromethyl)phenyl)-2,4-pentadiemoylamino) benzolactam.
3. An unpurified population of cells expressing markers characteristic of the pancreatic endoderm lineage, wherein greater than 80% of the cells in the population co-express PDX1 and NKX6.1, and wherein the population is obtained by differentiating cells expressing markers characteristic of the definitive endoderm lineage in a medium supplemented with a protein kinase C activator selected from the group consisting of (2S, 5S)-(E, E)-8-(5-(4-(Trifluoromethyl)phenyl)-2,4-pentadiemoylamino) benzolactam, phorbol-12-myristate-13-acetate, and phorbol-12,13-dibutyrate.
4. The unpurified population of cells according to claim 3, wherein the protein kinase C activator is (2S, 5S)-(E, E)-8-(5-(4-(Trifluoromethyl)phenyl)-2,4-pentadiemoylamino) benzolactam.
5. The unpurified population of cells according to claim 3, wherein greater than 90% of the cells in the population co-express PDX1 and NKX6.1.
6. The population of cells according to any one of claims 1 to 3, wherein the cells expressing markers characteristic of the pancreatic endoderm lineage are pancreatic endoderm cells.

7. A method to generate a population of cells expressing markers characteristic of the pancreatic endoderm lineage, wherein greater than 80% of the cells in the population co-express PDX1 and NKX6.1, comprising the steps of:

a. differentiating the population of pluripotent stem cells into a population of cells expressing markers characteristic of the definitive endoderm lineage; and

b. differentiating the population of cells expressing markers characteristic of the definitive endoderm lineage into a population of cells expressing markers characteristic of the pancreatic endoderm lineage, in a medium supplemented with a protein kinase C activator and at least one factor from the group consisting of a factor capable of inhibiting BMP, a TGF β receptor signaling inhibitor, and a fibroblast growth factor, wherein the protein kinase C activator is selected from the group consisting of (2S, 5S)-(E, E)-8-(5-(4 (Trifluoromethyl)phenyl)-2,4-pentadienylamino) benzolactam, phorbol-12-myristate-13-acetate, and phorbol-12,13-dibutyrate.

8. A method to generate a population of cells expressing markers characteristic of the pancreatic endoderm lineage, wherein greater than 80% of the cells in the population co-express PDX1 and NKX6.1 comprising differentiating a population of cells expressing markers characteristic of the definitive endoderm lineage into a population of cells expressing markers characteristic of the pancreatic endoderm lineage in a medium supplemented with a protein kinase C activator and at least one factor from the group consisting of a factor capable of inhibiting BMP, a TGF β receptor signaling inhibitor, and a fibroblast growth factor, wherein the protein kinase C activator is selected from the group consisting of (2S, 5S)-(E, E)-8-(5-(4 (Trifluoromethyl)phenyl)-2,4-pentadienylamino) benzolactam, phorbol-12-myristate-13-acetate, and phorbol-12,13-dibutyrate.

9. A method to generate a population of cells expressing markers characteristic of the pancreatic endoderm lineage, wherein greater than 90% of the cells in the population co-express PDX1 and NKX6.1, comprising the steps of:

a. differentiating the population of pluripotent stem cells into a population of cells expressing markers characteristic of the definitive endoderm lineage; and

b. differentiating the population of cells expressing markers characteristic of the definitive endoderm lineage into a population of cells expressing markers characteristic of the pancreatic endoderm lineage, in a medium supplemented with a protein kinase C activator,

wherein the protein kinase C activator is selected from the group consisting of (2S, 5S)-(E, E)-8-(5-(4 (Trifluoromethyl)phenyl)-2,4-pentadiemoylamino) benzolactam, phorbol-12-myristate-13-acetate, and phorbol-12,13-dibutyrate.

10. A method to generate a population of cells expressing markers characteristic of the pancreatic endoderm lineage, wherein greater than 90% of the cells in the population co-express PDX1 and NKX6.1 comprising differentiating a population of cells expressing markers characteristic of the definitive endoderm lineage into a population of cells expressing markers characteristic of the pancreatic endoderm lineage in a medium supplemented with a protein kinase C activator, wherein the protein kinase C activator is selected from the group consisting of (2S, 5S)-(E, E)-8-(5-(4 (Trifluoromethyl)phenyl)-2,4-pentadiemoylamino) benzolactam, phorbol-12-myristate-13-acetate, and phorbol-12,13-dibutyrate.

11. The method of any one of claims 7 to 10, wherein the cells expressing markers characteristic of the pancreatic endoderm lineage are pancreatic endoderm cells.

12. The method of any one of claims 7 to 11, wherein the protein kinase C activator is (2S, 5S)-(E, E)-8-(5-(4-(Trifluoromethyl)phenyl)-2,4-pentadiemoylamino) benzolactam.

13. The method of claim 12, wherein the (2S, 5S)-(E, E)-8-(5-(4-(Trifluoromethyl)phenyl)-2,4-pentadiemoylamino) benzolactam is used at a concentration from 20nM to 500nM.

14. The method of claim 7 or claim 8, wherein the factor capable of inhibiting BMP is noggin.

15. The method of claim 14, wherein noggin is used at a concentration from 50ng/ml to 500µg/ml.

16. The method of claim 7 or claim 8, wherein the medium is supplemented with a protein kinase C activator and at least a factor capable of inhibiting BMP and a TGFβ receptor signaling inhibitor.

17. The method of claim 7 or claim 8, wherein the TGFβ receptor signaling inhibitor is an inhibitor of ALK5.

18. The method of claim 17, wherein the inhibitor of ALK5 is ALK5 inhibitor II.

19. The method of claim 18, wherein ALK5 inhibitor II is used at a concentration from 0.1 μ M to 10 μ M.
20. The method of claim 18, wherein ALK5 inhibitor II is used at a concentration of 1 μ M.
21. The method of claim 7 or claim 8, wherein the fibroblast growth factor is FGF7.
22. The method of claim 7 or claim 8, wherein the fibroblast growth factor is FGF10.
23. The method of claim 7 or claim 8, wherein the medium is supplemented with a protein kinase C activator and at least a factor capable of inhibiting BMP.
24. The method of claim 7 or claim 8, wherein the medium is supplemented with a protein kinase C activator and at least a TGF β receptor signaling inhibitor.
25. The method of any one of claims 7, 8, 21, or 22 wherein the fibroblast growth factor may be used at a concentration from 50pg/ml to 50 μ g/ml.
26. A population of cells produced by the method of any one of claims 8 to 25.
27. A pharmaceutical composition comprising the population of cells according to any one of claims 1 to 6 or 26 and a pharmaceutically acceptable medium optionally comprising a pharmaceutically acceptable carrier or excipient.
28. Use of a population of cells according to any one of claims 1 to 7 or 26, or of a pharmaceutical composition of claim 27 in pancreatic cell replacement therapy or prophylaxis.
29. Use of a population of cells according to any one of claims 1 to 7 or 26, or of a pharmaceutical composition of claim 27 for the manufacture of a medicament for the treatment or prophylaxis of a disease or disorder requiring pancreatic cell-replacement.
30. The method of claim 7 or claim 9, wherein the pluripotent stem cells are human pluripotent stem cells.