Claims

1. A method for producing cells expressing markers characteristic of pancreatic endocrine cells from pluripotent stem cells, comprising the steps of:

    a. culturing pluripotent stem cells;

    b. differentiating the pluripotent stem cells into cells expressing markers characteristic of foregut endoderm cells; and

    c. differentiating the cells expressing markers characteristic of foregut endoderm cells into cells expressing markers characteristic of pancreatic endocrine cells by treatment with a medium supplemented with a thyroid hormone selected from triiodothyronine, thyroxine, analogues of triiodothyronine, analogues of thyroxine or mixtures thereof, or an ALK5 inhibitor, or both thyroid hormone and ALK5 inhibitor.

2. The method of claim 1, wherein at least ten percent of the resulting cells are positive for NKX6.1, PDX1, and HB9.

3. The method of claim 1, wherein the method enhances the expression of HB9 in NKX6.1 positive pancreatic endoderm precursor cells.

4. The method of claim 1, wherein the method decreases expression of NKX2.2.

5. The method of claim 1, wherein the method suppresses SOX2 and albumin expression.

6. The method of claim 1, wherein the thyroid hormone is triiodothyronine.

7. The method of claim 1, wherein step (c) comprises culturing in a medium supplemented with triiodothyronine and an ALK5 inhibitor.

8. The method of claim 7, wherein the method enhances HB9 expression relative to cells that are not cultured with a medium supplemented with triiodothyronine and an ALK5 inhibitor.

9. The method of claim 1, wherein the medium of step (c) is further supplemented with SANT-1, retinoic acid, and ascorbic acid.

10. The method of claim 1, wherein step (c) further comprises formation of cells expressing markers characteristic of pancreatic endocrine cells by culturing cells expressing markers characteristic of pancreatic endoderm/endocrine precursor cells in a medium supplemented with triiodothyronine.

11. The method of claim 10, wherein the medium is further supplemented with an ALK5 inhibitor.

12. A method of producing cells expressing markers characteristic of pancreatic endocrine cells, comprising differentiating cells expressing markers characteristic of foregut endoderm cells into cells expressing markers characteristic of pancreatic endocrine cells by treatment with a medium supplemented with a thyroid hormone selected from triiodothyronine, thyroxine, analogues of triiodothyronine, analogues of thyroxine or mixtures thereof, or an ALK5 inhibitor, or both thyroid hormone and ALK5 inhibitor.

13. The method of claim 12, wherein at least ten percent of the resulting cells are positive for NKX6.1, PDX1, and HB9.

14. The method of claim 12, wherein the method enhances the expression of HB9 in NKX6.1 positive pancreatic endoderm precursor cells.

15. The method of claim 12, wherein the method decreases expression of NKX2.2.

16. The method of claim 12, wherein the method suppresses SOX2 and albumin expression.

17. The method of claim 12, wherein the thyroid hormone is triiodothyronine.

18. The method of claim 12, wherein the method comprises culturing in a medium supplemented with triiodothyronine and an ALK5 inhibitor.

19. The method of claim 18, wherein the method enhances HB9 expression when compared to cells that are not cultured with a medium supplemented with triiodothyronine and an ALK5 inhibitor.

20. The method of claim 12, wherein the method further comprises formation of cells expressing markers characteristic of pancreatic endocrine cells by culturing cells expressing markers characteristic of pancreatic endoderm/endocrine precursor cells in a medium supplemented with triiodothyronine.

21. The method of claim 20, wherein the medium is further supplemented with an ALK5 inhibitor.

22. The method of claim 12, wherein the medium is further supplemented with a BMP receptor inhibitor and a PKC activator.

23. A method of (a) increasing HB9 expression and (b) suppressing SOX2 and albumin expression, comprising culturing cells expressing markers characteristic of pancreatic foregut precursor cells in a medium supplemented with triiodothyronine and an ALK5 inhibitor.

24. A method of down-regulating glucagon, somatostatin and ghrelin in cells expressing markers characteristic of pancreatic foregut precursor cells, cells expressing markers characteristic of pancreatic endoderm/endocrine precursor cells or cells expressing markers characteristic of pancreatic endocrine cells, comprising culturing the cells in a medium supplemented with triiodothyronine and an ALK5 inhibitor.

25. The method of claim 24, wherein the medium is further supplemented with SANT-1, retinoic acid, and ascorbic acid.

26. The method of claim 25, wherein the cells are cells expressing markers characteristic of pancreatic foregut precursor cells, and wherein the medium is further supplemented with FGF7.

27. The method of claim 8, wherein said ALK5 inhibitor is selected from the group consisting of ALK5 inhibitor II, ALK5i, SD208, TGF-B inhibitor SB431542, ITD-1, LY2109761, A83-01, LY2157299, TGF-β receptor inh V, TGF-β receptor inh I, TGF-β receptor inh IV, TGF-β receptor inh VII, TGF-β receptor inh VIII, TGF-β receptor inh II, TGF-β receptor inh VI, TGF-β receptor inh III.

28. The method of claim 27, wherein said ALK5 inhibitor is ALK5 inhibitor II.

29. The method of claim 18, wherein said ALK5 inhibitor is selected from the group consisting of ALK5 inhibitor II, ALK5i, SD208, TGF-B inhibitor SB431542, ITD-1, LY2109761, A83-01, LY2157299, TGF-β receptor inh V, TGF-β receptor inh I, TGF-β receptor inh IV, TGF-β receptor inh VII, TGF-β receptor inh VIII, TGF-β receptor inh II, TGF-β receptor inh VI, TGF-β receptor inh III.

30. The method of claim 29, wherein said ALK5 inhibitor is ALK5 inhibitor II.

31. The method of claim 22, wherein said BMP receptor inhibitor is selected from LDN-193189, Noggin and Chordin, and said PKC activator is selected from TPB, PDBu, PMA and ILV.

32. An *in vitro* cell culture for differentiating cells derived from pluripotent stem cells comprising:

    a. a culture vessel;

    b. a volume of differentiation medium; and

    c. a population of differentiated cells derived from pluripotent stem cells wherein at least ten percent of said differentiated cells co-express PDX1, NKX6.1 and HB9.

33. The cell culture of claim 32, wherein said differentiation medium comprises a growth medium supplemented with a thyroid hormone selected from triiodothyronine, thyroxine, analogues of triiodothyronine, analogues of thyroxine and mixtures thereof, or an ALK5 inhibitor or both thyroid hormone and ALK5 inhibitor.

34. The cell culture of claim 33, wherein said growth medium is MCDB131.

35. The cell culture of claim 32, wherein said differentiated cells comprise cells expressing markers characteristic of pancreatic endocrine cells.

36. The cell culture of claim 33, wherein said growth medium is further supplemented with one or more of:

    a. a smoothened receptor inhibitor selected from MRT10 or cyclopamine;

    b. a SHH signaling pathway antagonist selected from SANT-1 or HPI-1;

    c. a BMP Receptor Inhibitor selected from LDN-193189, Noggin or Chordin;

    d. a PKC activator selected from TPB, PDBu, PMA, and ILV;

    e. a fibroblast growth factor selected from FGF7 or FGF10;

    f. retinoic acid;

    g. ascorbic acid;

    h. heparin; and

    i. zinc sulfate.

37. The cell culture of claim 36, wherein said growth medium is further supplemented with SANT-1, retinoic acid and ascorbic acid.

38. An *in vitro* cell culture comprising a population of differentiated pluripotent stem cells expressing markers characteristic of pancreatic endocrine cells wherein at least ten percent of said cells express HB9, PDX1 and NKX6.1.

39. The cell culture of claim 38, wherein at least thirty percent of the cells that express both NKX6.1 and PDX1 also express HB9.

40. The cell culture of claim 38, wherein at least fifty percent of the cells that express both NKX6.1 and PDX1 also express HB9.

41. The cell culture of claim 38, wherein at least eighty percent of the cells that express both NKX6.1 and PDX1 also express HB9.

42. The method of claim 1, wherein said cells expressing markers characteristic of pancreatic endocrine cells produce insulin.

43. The method of claim 12, wherein said expressing markers characteristic of pancreatic endocrine cells produce insulin.

44. The cell culture of claim 35, wherein said differentiated cells comprise cells expressing markers characteristic of β cells.

45. The cell culture of claim 44, wherein said differentiated cells produce insulin.

46. A method for generating cells expressing markers characteristic of pancreatic endocrine cells, comprising culturing cells derived from pluripotent stem cells in growth media supplemented with a thyroid hormone selected from triiodothyronine, thyroxine, analogues of triiodothyronine, analogues of thyroxine or mixtures thereof, or an ALK5 inhibitor, or both thyroid hormone and ALK5 inhibitor, wherein cells derived from pluripotent stem cells are cultured in said growth media for a period of from about three to about nine days.

47. A method for generating cells expressing markers characteristic of pancreatic endocrine cells, comprising culturing cells derived from pluripotent stem cells in growth media supplemented with a thyroid hormone selected from triiodothyronine, thyroxine, analogues of triiodothyronine, analogues of thyroxine or mixtures thereof, or an ALK5 inhibitor, or both thyroid hormone and ALK5 inhibitor, wherein said cells are cultured in a first growth medium supplemented with thyroid hormone but not ALK5 inhibitor for a period of from about two to three days, followed by culturing the resulting cell population in additional growth media supplemented with both thyroid hormone and ALK5 inhibitor for about three to six days.

48. The method of claim 47, wherein said cells are further cultured for a period of about three days in a medium containing said thyroid hormone but not ALK5 inhibitor.

49. The method of claim 47, wherein said thyroid hormone is triiodothyronine.

50. A method for increasing the yield of cells expressing markers characteristic of ß cells via the differentiation of pluripotent stem cells, or cells derived therefrom, comprising culturing said cells in a growth media supplemented with a thyroid hormone selected from triiodothyronine, thyroxine, analogues of triiodothyronine, analogues of thyroxine or mixtures thereof, or an ALK5 inhibitor, or both thyroid hormone and ALK5 inhibitor.

51. The method of claim 50 wherein said thyroid hormone is triiodothyronine.

52. The method of claim 51 wherein said cells expressing markers characteristic of ß cells express insulin.