CLAIMS:

1. A method of differentiating anterior primitive streak cells into definitive endoderm (DE) lineage, comprising contacting said anterior primitive streak cells with:

a. one or more activators of TGFβ/Nodal signaling; and
b. one or more inhibitors of BMP signaling or one or more inhibitors of Wnt signaling,

to produce cells of the definitive endoderm (DE) lineage, wherein said cells of the anterior primitive streak are obtained from pluripotent stem cells by contacting said pluripotent stem cells with:
a. one or more activators of TGFβ/Nodal signaling, and
b. one or more activators of Wnt signaling; and
c. one or more inhibitors of PI3K/mTOR signaling
to produce cells of the anterior primitive streak lineage.

2. The method as claimed in 1, wherein the one or more activators of TGFβ/Nodal signaling is selected from the group consisting of Activin A, TGFβ1, TGFβ2 and Nodal.

3. The method as claimed in 1 or 2, wherein the one or more inhibitors of BMP signaling is selected from the group consisting of DM3189/LDN-193189, noggin, chordin, dorsomorphin and DMH1.

4. The method as claimed in any one of claims 1 to 3, wherein the cells of the APS lineage are contacted with 100ng/ml of Activin A and 250nM of LDN-193189.

5. The method as claimed in any one of claims 1 to 3, wherein the cells of the APS lineage are contacted with 1ng/ml to 10μg/ml of Activin A and 1nM to 100mM of LDN-193189.

6. The method as claimed in claim 1 or 2, wherein the one or more inhibitors of Wnt signaling is selected from the group consisting of Iwp2, Dkk1, C-59, Iwr-1 and XAV-939.

7. The method of claim 6, wherein the cells of the APS lineage are contacted with 1ng/ml to 10μg/ml of Activin A and 1nM to 100mM of Iwp2 or 1nM to 100mM C-59.

8. The method of claim 6, wherein the cells of the APS lineage are contacted with 100ng/ml of Activin A and 4μM of Iwp2 or 1 μM of C-59 or 300ng/ml Dkk1.

9. The method of any one of claims 1 to 8, wherein the cells of the defined endoderm lineage comprise elevated endoderm gene expression and decreased pluripotency gene expression relative to undifferentiated cells.

10. The method of any one of claims 1 to 9, wherein the cells of the defined endoderm lineage comprise significantly reduced mesoderm gene expression.

11. The method as claimed in any one of claims 1 to 10, wherein the differentiation of cells from anterior primitive streak lineage into cells of definitive endoderm (DE) lineage is completed within 24 to 96 hours.

12. The method as claimed in any one of claims 1 to 11, wherein the one or more activators of Wnt signaling are CHIR99201 or Wnt3a or other family members of the Wnt signaling pathway.

13. The method as claimed in any one of claims 1 to 12, wherein the one or more inhibitors of PI3K/mTOR signaling are PI-103, PIK-90, GDC0941, or LY294002.

14. The method as claimed in claim 13, wherein the one or more inhibitors of PI3K/mTOR signaling is PI-103.

15. The method as claimed in any one of claims 1 to 14, wherein the pluripotent stem cells are contacted with Activin A, PI-103 and CHIR99201.

16. The method of claim 15, wherein the pluripotent stem cells are contacted with 1ng/ml to 100μg/ml of Activin A, 1nM to 100mM of PI-103 and 1nM to 100mM of CHIR99201.

17. The method of claim 16, wherein the pluripotent stem cells are contacted with 100ng/ml of Activin A, 50nM of PI-103 and 2μM of CHIR99201.

18. The method of any one of claims 1 to 17, wherein the cells of the anterior primitive streak lineage comprise elevated gene expression of anterior streak or pan-streak markers and decreased expression of posterior streak markers relative to undifferentiated cells.

19. The method as claimed in any one of claims 1 to 18, wherein the differentiation of pluripotent stem cells to cells of the APS lineage is completed within 24 to 60 hours.

20. A cell culture medium for differentiating a pluripotent stem cell into definitive endoderm comprising the following factors: one or more activators of TGFβ/Nodal signaling, one or more activators of Wnt signaling; one or more inhibitors of PI3K/mTOR signaling; one or more activators of TGFβ/Nodal signaling; one or more inhibitors of BMP signaling or one or more inhibitors of Wnt signaling.

21. A kit when used in the method of any one of claims 1 to 19, comprising one or more containers of cell culture medium as claimed in claim 20, together with instructions for use.

CLEAN CLAIMS

CLAIMS:

1. A method of differentiating anterior primitive streak cells into definitive endoderm (DE) lineage, comprising contacting said anterior primitive streak cells with:

a. one or more activators of TGFβ/Nodal signaling; and
b. one or more inhibitors of BMP signaling or one or more inhibitors of Wnt signaling,

to produce cells of the definitive endoderm (DE) lineage.

2. The method as claimed in 1, wherein the one or more activators of TGFβ/Nodal signaling is the one or more activators of TGFβ/Nodal signaling is selected from the group consisting of Activin A, TGFβ1, TGFβ2 and Nodal.

3. The method as claimed in 1 or 2, wherein the one or more inhibitors of BMP signaling is selected from the group consisting of DM3189/LDN-193189, noggin, chordin, dorsomorphin and DMH1.

4. The method as claimed in any one of claims 1 to 3, wherein the cells of the APS lineage are contacted with about 100ng/ml of Activin A and about 250nM of LDN-193189.

5. The method as claimed in any one of claims 1 to 3, wherein the cells of the APS lineage are contacted with

about 1ng/ml to 10μg/ml of Activin A and about 1nM to 100mM of LDN-193189.

6. The method as claimed in claim 1 or 2, wherein the one or more inhibitors of Wnt signaling is selected from the group consisting of Iwp2, Dkk1, C-59, Iwr-1 and XAV-939.

7. The method of claim 6, wherein the cells of the APS lineage are contacted with about 1ng/ml to about 10μg/ml of Activin A and about 1nM to about 100mM of Iwp2 or about 1nM to about 100mM C-59.

8. The method of claim 6, wherein the cells of the APS lineage are contacted with about 100ng/ml of Activin A and about 4μM of Iwp2 or about 1 μM of C-59 or about 300ng/ml Dkk1.

9. The method of any one of claims 1 to 8, wherein the cells of the defined endoderm lineage comprise elevated endoderm gene expression and decreased pluripotency gene expression relative to undifferentiated cells.

10. The method of any one of claims 1 to 9, wherein the cells of the defined endoderm lineage comprise significantly reduced mesoderm gene expression.

11. The method as claimed in any one of claims 1 to 10, wherein the differentiation of cells from anterior primitive streak lineage into cells of definitive endoderm (DE) lineage is completed within 24 to 96 hours.

CLEAN CLAIMS

12. The method of any one of claims 1 to 11, wherein said cells of the anterior primitive streak are obtained from stem cells by contacting said stem cells with:

a. one or more activators of TGFβ/Nodal signaling, and

b. one or more activators of Wnt signaling; or

c. one or more inhibitors of PI3K/mTOR signaling,

to produce cells of the anterior primitive streak lineage.

13. The method of claim 12, wherein said cells of the anterior primitive streak are obtained from stem cells by contacting said stem cells with:

a. one or more activators of TGFβ/Nodal signaling, and

b. one or more activators of Wnt signaling; and

c. one or more inhibitors of PI3K/mTOR signaling.

14. The method as claimed in claim 12, wherein the one or more activators of TGFβ/Nodal signaling is Activin A, TGFβ1, TGFβ2 or Nodal.

15. The method as claimed in any one of claims 12 to 14, wherein the one or more activators of Wnt signaling are CHIR99201 or Wnt3a or other family members of the Wnt signaling pathway.

16. The method as claimed in any one of claims 12 to 15, wherein the one or more inhibitors of PI3K/mTOR signaling are PI-103, PIK-90, GDC0941, or LY294002.

CLEAN CLAIMS

17. The method as claimed in claim 16, wherein the one or more inhibitors of PI3K/mTOR signaling is PI-103.

18. The method as claimed in any one of claims 12 to 17, wherein the stem cells are contacted with Activin A, PI-103 and CHIR99201.

19. The method of claim 18, wherein the stem cells are contacted with about 1ng/ml to 100μg/ml of Activin A, about 1nM to 100mM of PI-103 and about 1nM to 100mM of CHIR99201.

20. The method of claim 19, wherein the stem cells are contacted with about 100ng/ml of Activin A, about 50nM of PI-103 and about 2μM of CHIR99201.

21. The method of any one of claims 12 to 20, wherein the cells of the anterior primitive streak lineage comprise elevated gene expression of anterior streak or pan-streak markers and decreased expression of posterior streak markers relative to undifferentiated cells.

22. The method as claimed in any one of claims 12 to 21, wherein the differentiation of stem cells to cells of the APS lineage is completed within 24 to 60 hours.

23. A method of differentiating cells of the DE lineage into cells of the posterior foregut(PFG), by contacting said cells of the DE with retinoic acid, a BMP inhibitor, a Wnt inhibitor and a FGF/MAPK inhibitor.

CLEAN CLAIMS

24. The method of claim 23, wherein the cells of the DE are contacted with about 1nM to 100mM retinoic acid, about 1nM to 100mM of LDN193189, about 1nM to 100mM of IWP2 and about 1nM to 100mM of PD0325901.

25. The method of claim 24, wherein the cells of the DE are contacted with about 2μM retinoic acid, about 250nM of LDN193189, about 4μM of IWP2 and about 0.5μM of PD0325901.

26. The method of any one of claims 23 to 25, wherein the cells of the posterior foregut comprise elevated levels of posterior foregut gene expression without either MHG or AFG gene expression relative to undifferentiated cells.

27. The method of any one of claims 23 to 26, wherein the DE cells are obtained using the method of any one of claims 1 to 21.

28. A method of differentiating cells of the DE lineage into cells of the midgut/hindgut (MHG), by contacting said DE cells with a BMP activator, a Wnt activator and FGF activator.

29. The method of claim 28, wherein the cells of the DE are contacted with about 1ng/ml to 100μg/ml of BMP4, about 1ng/ml to 100μg/ml of FGF2, and about 1nM to 100μM of CHIR99201.

30. The method of claim 29, wherein the cells of the DE are contacted with about 10ng/ml of BMP4, about 100ng/ml of FGF2, and about 3μM of CHIR99201.

CLEAN CLAIMS

31. The method of any one of claims 28 to 30, wherein the cells of the MHG comprise elevated expression levels of MHG genes relative to undifferentiated cells.

32. The method as claimed in any one of claims 28 to 31, wherein the differentiation is completed within 24 to 240 hours.

33. The method of any one of claims 28 to 32, wherein the DE cells are obtained using the method of any one of claims 1 to 21.

34. A method of inducing pancreatic progenitors of the PFG are induced from the DE within three days by contacting said PFG with:
a) one or more FGF/MAPK inhibitors;
b) one or more BMP inhibitors; and
c) retinoic acid (RA).

35. The method of claim 34, further comprising contacting said PFG with one or more Hedgehog inhibitors.

36. The method of claim 34, further comprising contacting said PFG with one or more Wnt inhibitors.

37. The method of claim 34, further comprising contacting said PFG with Activin A.

38. The method of claim 34, further comprising contacting said PFG with one or more Hedgehog inhibitors; one or more WNT inhibitors and Activin A.

CLEAN CLAIMS

39. The method of claim 38, wherein said PFG is contacted with about 1nM to 100mM of PD0325901 or PD173074, about 1nM to 100mM of SANT 1, about 1nM to 100mM of LDN193189, about 1nM to 100mM of IWP2 or C59, about 1nM to 100mM of retinoic acid and about 1ng/ml to 100μg/ml of Activin A.

40. The method of claim 39, wherein said PFG is contacted with about 0.5μM of PD0325901 or 100nM of PD173074, about 150nM of SANT 1, about 250nM of LDN193189, about 4μM of IWP2, about 2μM of retinoic acid and about 10ng/ml of Activin A.

41. The method of any one of claims 34 to 40, wherein the cells of the pancreatic progenitors comprise elevated expression levels of pancreatic genes relative to undifferentiated cells and exclude hepatic progenitor gene expression.

42. The method of any one of claims 34 to 41, further comprising contacting said PFG with about 1ng/ml to 100μg/ml of FGF2.

43. The method of claim 41, wherein the PFG is contacted with about 10 to 20 ng/ml of FGF2.

44. The method of any one of claims 34-43, wherein the DE cells are obtained using the method of any one of claims 1 to 21.

45. A method of inducing liver progenitors of the PFG from the DE within four days by contacting said PFG with:

a) one or more TGFβ inhibitors;

b) retinoic acid (RA);

c) one or more BMP activators, and

d) one or more Wnt inhibitors.

46. The method of claim 45, wherein said PFG is contacted with about 1nM to 100mM of A83-01, about 1nM to 100mM of RA, about 1ng/ml to 10μg/ml of BMP4, and about 1nM to 100mM of IWP2 or C59.

47. The method of claim 46, wherein said PFG is contacted with about 1μM of A83-01, about 2μM of RA, about 10ng/ml of BMP4, and about 4μM of IWP2.

48. The method of any one of claims 45 to 47, wherein the cells of the liver progenitors comprise elevated expression levels of hepatic genes relative to undifferentiated cells and exclude pancreatic progenitor gene expression.

49. The method of any one of claims 45 to 48, wherein the DE cells are obtained using the method of any one of claims 1 to 21.

50. A cell culture medium for differentiating a stem cell into definitive endoderm comprising one or more of the following factors: one or more activators of TGFβ/Nodal signaling, one or more activators of Wnt signaling; one or more inhibitors of PI3K/mTOR signaling; one or more activators of TGFβ/Nodal signaling; one or more inhibitors of BMP signaling or one or more inhibitors of Wnt signaling.

CLEAN CLAIMS

51. A cell culture medium for differentiating cells of the DE lineage into cells of the posterior foregut (PFG) comprising one or more of the following factors: retinoic acid, a BMP inhibitor, a Wnt inhibitor or a FGF/MAPK inhibitor.

52. A cell culture medium for differentiating cells of the DE lineage into cells of the midgut/hindgut (MHG), comprising one or more of the following factors: BMP activator, a Wnt activator or FGF activator.

53. A cell culture medium for inducing pancreatic progenitors of the PFG are induced from the DE comprising one or more of the following factors: a) one or more FGF/MAPK inhibitors; b) one or more BMP inhibitors; or c) retinoic acid (RA).

54. A cell culture medium for inducing liver progenitors of the PFG from the DE comprising one or more of the following factors: a) one or more TGFβ inhibitors; b) one or more BMP activators; c) retinoic acid and d) one or more Wnt inhibitors.

55. A cell produced according to the method of any one of claims 1 to 49.

56. A kit when used in the method of any one of claims 1 to 49, comprising one or more containers of cell culture medium as claimed in any one of claims 50 to 54, together with instructions for use.

Claims

1. A method of differentiating stem cells into one or more cell lineages comprising contacting said cells with:
   a. one or more activators of TGFβ/Nodal signaling; and
   b. one or more activators of Wnt signaling.

2. The method as claimed in claim 1, wherein the cell lineage is the anterior primitive streak lineage.

3. The method as claimed in claim 1, further comprising contacting said cells with one or more inhibitors of PI3K/mTOR signaling.

4. The method as claimed in any one of the preceding claims, wherein the one or more activators of TGFβ/Nodal signaling is Activin A, TGFβ1, TGFβ2 or Nodal.

5. The method as claimed in any one of the preceding claims, wherein the one or more activators of Wnt signaling are CHIR99201 or Wnt3a or other family members of the wnt signaling pathway.

6. The method as claimed in claim 3, wherein the one or more inhibitors of PI3K/mTOR signaling are PI-103, PIK-90, or LY294002.

7. The method as claimed in claim 6, wherein the one or more inhibitors of PI3K/mTOR signaling is PI-103.

8. The method as claimed in any one of claims 2 to 7, wherein the cells are contacted with Activin A, PI-103 and CHIR99201.

9. The method of claim 8, wherein the cells are contacted with about 1ng/ml to 10μg/ml of Activin A, about 1nM to 100mM of PI-103 and about 1nM to 100mM of CHIR99201.

10. The method of claim 9, wherein the cells are contacted with about 100ng/ml of Activin A, about 50nM of PI-103 and about 2μM of CHIR99201.

11. The method of any one of claims 2 to 10, wherein the cells of the anterior primitive streak lineage comprise elevated gene expression of anterior streak or pan-streak markers and decreased expression of posterior streak markers relative to undifferentiated cells.

12. The method as claimed in any one of claims 2 to 11, wherein the differentiation is completed within 24 to 60 hours.

13. The method as claimed in any one of claims 2 to 12, wherein said cells of the anterior primitive streak lineage are further differentiated into cells of definitive endoderm (DE) lineage, by contacting said cells of the anterior primitive streak lineage with one or more activators of TGFβ/Nodal signaling; and one or more inhibitors of BMP signaling or one or more inhibitors of Wnt signaling.

14. The method as claimed in claim 13, wherein the one or more activators of TGFβ/Nodal signaling is Activin A, TGFβ1, TGFβ2 or Nodal.

15. The method as claimed in claim 13 or claim 14, wherein the one or more inhibitors of BMP signaling is DM3189/LDN-193189, noggin, chordin, or dorsomorphin or DMH1.

16. The method of any one of claims 13 to 15, wherein the cells are contacted with about 1ng/ml to 10μg/ml of Activin A and about 1nM to 100mM of LDN-193189.

17. The method of claim 16, wherein the cells are contacted with about 100ng/ml of Activin A and about 250nM of LDN-193189.

18. The method as claimed in claim 13 or claim 14, wherein the one or more inhibitors of Wnt signaling is Iwp2, Dkk1, C-59, or Iwr-1 or XAV-939.

19. The method of claim 18, wherein the cells are contacted with about 1ng/ml to about 10μg/ml of Activin A and about 1nM to about 100mM of Iwp2 or about 1nM to about 100mM C-59.

20. The method of claim 19, wherein the cells are contacted with about 100ng/ml of Activin A and about 4μM of Iwp2 or about 1 μM of C-59.

21. The method of any one of claims 13 to 20, wherein the cells of the defined endoderm lineage comprise elevated

endoderm gene expression and decreased pluripotency gene expression relative to undifferentiated cells.

22. The method of any one of claims 13 to 21, wherein the cells of the defined endoderm lineage comprise significantly reduced mesoderm gene expression.

23. The method as claimed in any one of claims 13 to 22, wherein the differentiation is completed within 24 to 96 hours.

24. The method as claimed in any one of claims 13 to 23, wherein the cells of the DE lineage are further differentiated into cells of the anterior foregut (AFG), by contacting said DE cells with a TGFβ inhibitor and a BMP inhibitor.

25. The method as claimed in claim 24, wherein one or more inhibitors of TGFβ/Nodal signaling is A-83-01, SB431542, Lefty1 or Lefty 2.

26. The method as claimed in claim 24 or claim 25, wherein one or more inhibitors of BMP signaling is DM3189/LDN193189, noggin, chordin or dorsomorphin.

27. The method of any one of claims 24 to 26, wherein the cells of the DE are contacted with about 1nM to 100mM of A-83-01 and about 1nM to 100mM of LDN193189.

28. The method of claim 27, wherein the cells of the DE are contacted with about 1μM of A-83-01 and about 250nM of LDN193189.

29. The method of any one of claims 24 to 28, wherein the cells of the anterior foregut comprise elevated anterior foregut markers (including but not limited to *OTX2*, *IRX3*, *TBX1*, *PAX9*, *SOX2* without either posterior foregut (PFG) or midgut/hindgut (MHG) transcription factors relative to undifferentiated cells.

30. The method as claimed in any one of claims 13 to 23, wherein the cells of the DE lineage are further differentiated into cells of the PFG, by contacting said cells of the DE with retinoic acid, a BMP inhibitor, a Wnt inhibitor and a FGF/MAPK inhibitor.

31. The method of claim 30, wherein the cells of the DE are contacted with about 1nM to 100mM retinoic acid, about 1nM to 100mM of LDN193189, about 1nM to 100mM of IWP2 and about 1nM to 100mM of PD0325901.

32. The method of claim 31, wherein the cells of the DE are contacted with about 2μM retinoic acid, about 250nM of LDN193189, about 4μM of IWP2 and about 0.5μM of PD0325901.

33. The method of any one of claims 30 to 32, wherein the cells of the posterior foregut comprise elevated levels of posterior foregut gene expression without either MHG or AFG gene expression relative to undifferentiated cells.

34. The method as claimed in any one of claims 13 to 23, wherein the cells of the DE lineage are further differentiated into cells of the MHG, by contacting

said DE cells with a BMP activator, a Wnt activator and FGF activator.

35. The method of claim 34, wherein the cells of the DE are contacted with about 1ng/ml to 10µg/ml of BMP4, about 1ng/ml to 10µg/ml of FGF2, and about 1nM to 10µM of CHIR99201.

36. The method of claim 35, wherein the cells of the DE are contacted with about 10ng/ml of BMP4, about 100ng/ml of FGF2, and about 3µM of CHIR99201.

37. The method of any one of claims 34 to 36, wherein the cells of the MHG comprise elevated expression levels of MHG genes relative to undifferentiated cells.

38. The method as claimed in any one of claims 24 to 37, wherein the differentiation is completed within 24 to 240 hours.

39. The method as claimed in any one of claims 30 to 33, wherein pancreatic progenitors of the PFG are induced from the DE within three days by contacting said PFG with:

a) one or more FGF/MAPK inhibitors;
b) one or more BMP inhibitors; and
c) retinoic acid (RA).

40. The method of claim 39, further comprising contacting said PFG with one or more Hedgehog inhibitors.

41. The method of claim 39, further comprising contacting said PFG with one or more Wnt inhibitors.

42. The method of claim 39, further comprising contacting said PFG with Activin A.

43. The method of claim 39, further comprising contacting said PFG with one or more Hedgehog inhibitors; one or more WNT inhibitors and Activin A.

44. The method of claim 43, wherein said PFG is contacted with about 1nM to 100mM of PD0325901 or PD173074, about 1nM to 100mM of SANT 1, about 1nM to 100mM of LDN193189, about 1nM to 100mM of IWP2 or C59, about 1nM to 100mM of retinoic acid and about 1ng/ml to 10μg/ml of Activin A.

45. The method of claim 44, wherein said PFG is contacted with about 0.5μM of PD0325901 or 100nM of PD173074, about 150nM of SANT 1, about 250nM of LDN193189, about 4μM of IWP2, about 2μM of retinoic acid and about 10ng/ml of Activin A.

46. The method of any one of claims 39 to 45, wherein the cells of the pancreatic progenitors comprise elevated expression levels of pancreatic genes relative to undifferentiated cells and exclude hepatic progenitor gene expression.

47. The method of any one of claims 39 to 46, further comprising contacting said PFG with about 1ng/ml to 10μg/ml of FGF2.

48. The method of claim 47, wherein the PFG is contacted with about 10 to 20 ng/ml of FGF2.

49. The method as claimed in any one of claims 30 to 33, wherein liver progenitors of the PFG are induced from the DE within four days by contacting said PFG with:

a) one or more TGFβ inhibitors;

b) retinoic acid (RA);

c) one or more BMP activators, and

d) one or more Wnt inhibitors.

50. The method of claim 49, wherein said PFG is contacted with about 1nM to 100mM of A83-01, about 1nM to 100mM of RA, about 1ng/ml to 10μg/ml of BMP4, and about 1nM to 100mM of IWP2 or C59.

51. The method of claim 50, wherein said PFG is contacted with about 1μM of A83-01, about 2μM of RA, about 10ng/ml of BMP4, and about 4μM of IWP2.

52. The method of any one of claims 49 to 51, wherein the cells of the liver progenitors comprise elevated expression levels of hepatic genes relative to undifferentiated cells and exclude pancreatic progenitor gene expression.

53. A method of differentiating anterior primitive streak cells into cells of definitive endoderm (DE) lineage, by contacting said cells of the anterior primitive streak lineage with one or more activators of TGFβ/Nodal signaling, and one or more inhibitors of BMP signaling, or one or more inhibitors of Wnt signaling.

54. A method of differentiating cells of the DE into cells of the AFG, by contacting said DE cells with a TGFβ inhibitor and a BMP inhibitor.

55. A method of differentiating cells of the DE lineage into cells of the PFG, by contacting said cells of the DE with retinoic acid, a BMP inhibitor, a Wnt inhibitor and a FGF/MAPK inhibitor.

56. A method of differentiating cells of the DE lineage into cells of the MHG, by contacting said DE cells with a BMP activator, a Wnt activator and an FGF activator.

57. A method of inducing pancreatic progenitors of the PFG from the DE within three days by contacting said PFG with one or more FGF/MAPK inhibitors; one or more Hedgehog inhibitors; one or more BMP inhibitors; one or more Wnt inhibitors; retinoic acid (RA), and Activin A.

58. A method of inducing liver progenitors of the PFG from the DE within four days by contacting said PFG with one or more TGFβ inhibitors; one or more BMP activators, retinoic acid and one or more Wnt inhibitors.

59. A cell culture medium for differentiating a stem cell into one or more cell lineages comprising one or more of the following factors: one or more FGF/MAPK inhibitors; one or more Hedgehog inhibitors; one or more BMP inhibitors, one or more Wnt inhibitors, retinoic acid, Activin A and one or more inhibitors of PI3K/mTOR signaling.

60. A cell culture medium for differentiating a stem cell into one or more cell lineages comprising one or more of the following factors: a matrix component, one or more activators of TGFβ/Nodal signaling, one or more activators of Wnt/β-catenin signaling, and one or more inhibitors of PI3K/mTOR signaling.

61. A cell culture medium for differentiating a stem cell into one or more cell lineages comprising one or more of the following factors: one or more activators of TGFβ/Nodal signaling, and one or more inhibitors of BMP signaling.

62. A cell culture medium for differentiating a stem cell into one or more cell lineages comprising a TGFβ inhibitor and a BMP inhibitor.

63. A cell culture medium for differentiating a stem cell into one or more cell lineages comprising one or more of the following factors: retinoic acid, a BMP inhibitor, a Wnt inhibitor and a FGF/MAPK inhibitor.

64. A cell culture medium for differentiating a stem cell into one or more cell lineages comprising one or more of the following factors: BMP4, a Wnt activator and an FGF activator.

65. A cell culture medium for differentiating a stem cell into one or more cell lineages comprising one or more of the following factors: one or more FGF/MAPK inhibitors; one or more Hedgehog inhibitors; one or more BMP inhibitors; one or more Wnt inhibitors; retinoic acid (RA), and Activin A.

66. A cell culture medium for differentiating a stem cell into one or more cell lineages comprising one or more of the following factors: one or more TGFβ inhibitors; one or more BMP activators, retinoic acid, and one or more Wnt inhibitors.

67. A cell produced according to the method of any one of claims 1 to 58.

68. A kit when used in the method of any one of claims 1 to 58, comprising one or more containers of cell culture medium as claimed in any one of claims 59 to 66, together with instructions for use.