The pancreas and the liver arise at around embryonic day 8.5 to 9.5 from adjacent regions of the developing primitive foregut under the influence of inductive signals which are secreted by the nearby mesoderm [9]. These signals are likely to command the expression of transcription factors necessary for pancreatic specification such as HXLB9, which marks the dorsal foregut prior to the formation of the pancreatic bud [10, 11] and PDX1 which marks regions of the foregut from which ventral and dorsal pancreatic buds arise [12, 13].

The newly specified pancreatic progenitor quickly expresses additional markers including PTF1A, NKX6.1 and SOX9 and these progenitors give rise to both endocrine (islets of Langerhans) and exocrine (acinar and ductal cells) cells of the pancreas. Similar mechanisms control hepatic specification although they involve different set of transcription factors such as HEX, GATA6, PROX1 and HNF4α [14] and signalling pathways such as BMP and FGFs [15]. Despite this broad knowledge, the molecular mechanisms enabling extracellular signalling pathways to orchestrate the transcriptional networks characterising pancreatic or hepatic progenitors remain to be elucidated especially in human and hPSCs could present unique advantages to complete this major task.

Summary

This disclosure relates to a process for the high efficiency *in vitro* differentiation of pluripotent cells into pancreatic progenitor and pancreatic endocrine cells. This may be useful, for example, in producing pancreatic cells for cell-based therapies or disease modelling.

An aspect of the disclosure provides a method for producing a population of pancreatic progenitor cells which comprises:

i) providing a population of pluripotent cells;

ii) culturing the population in a definitive endoderm (DE) induction medium to produce a population of definitive endoderm cells, wherein said DE induction medium comprises a TGFβ ligand, fibroblast growth factor (FGF), bone morphogenetic protein (BMP), a PI3K inhibitor and optionally a GSK3β inhibitor;

Claims

1. A method for producing a population of pancreatic progenitor cells which comprises:

i) providing a population of pluripotent cells;

ii) culturing the population in a definitive endoderm (DE) induction medium to produce a population of definitive endoderm cells, wherein the definitive endoderm (DE) induction medium comprises a TGFβ ligand, fibroblast growth factor (FGF), bone morphogenetic protein (BMP), a PI3K inhibitor and optionally a GSK3β inhibitor;

iii) culturing the population of definitive endoderm cells in a first pancreatic induction medium comprising an activin antagonist; FGF; retinoic acid; and a BMP inhibitor to produce a population of dorsal foregut cells;

iv) culturing the dorsal foregut cells in a second pancreatic induction medium comprising FGF, retinoic acid, a BMP inhibitor, and a hedgehog signalling inhibitor;

v) culturing the endoderm cells in a third pancreatic induction medium comprising FGF;

thereby producing a population of pancreatic progenitor cells.

2. A method according to claim 1 wherein definitive endoderm (DE) induction medium is a chemically defined medium which comprises TGFβ ligand, fibroblast growth factor (FGF), bone morphogenetic protein (BMP) and a PI3K inhibitor.

3. A method according to claim 1 or claim 2 wherein the definitive endoderm (DE) induction medium is a chemically defined medium which consists of a basal medium supplemented with activin, fibroblast growth factor (FGF), bone morphogenetic protein (BMP) and LY294002.

4. A method according to claim 3 wherein the cells are cultured in said definitive endoderm (DE) induction medium for 2 to 4 days to produce the population of DE cells.

5. A method according to claim 1 or claim 2 wherein step (ii) comprises:

(a) culturing the population of pluripotent cells in said DE induction medium, wherein said DE induction medium further comprises a GSK3β inhibitor,

(b) further culturing the population in the definitive endoderm induction medium lacking GSK3β inhibitor; and,

(c) further culturing the population in anterior definitive endoderm (ADE) induction medium which comprises a TGFβ ligand and fibroblast growth factor to produce the population of definitive endoderm (DE) cells.

6. A method according to claim 5 wherein the anterior definitive endoderm (ADE) induction medium is a chemically defined medium which consists of a basal medium supplemented with activin and fibroblast growth factor (FGF).

7. A method according to claim 5 or claim 6 wherein the population is cultured for 24 hours in each of steps a) to c).

8. A method according to any one of claims 1 to 7 wherein the definitive endoderm cells express SOX17, CXCR4 and GSC.

9. A method according to any one of claims 1 to 8 wherein the first pancreatic induction medium is a chemically defined medium which comprises activin/TGFβ antagonist; FGF; retinoic acid; and a BMP antagonist.

10. A method according to any one of claims 1 to 9 wherein the first pancreatic induction medium is a chemically defined medium which consists of a basal medium supplemented with SB-431542; FGF; retinoic acid; and noggin.

11. A method according to any one of claims 1 to 10 wherein the dorsal foregut cells express RFX6, FOXA2, HNF1b, SOX2, HNF4a, and HLXB9.

12. A method according to any one of claims 1 to 11 wherein the second pancreatic induction medium is a chemically defined medium which comprises FGF, a BMP antagonist, retinoic acid, and a hedgehog signalling inhibitor.

13. A method according to any one of claims 1 to 12 wherein second pancreatic induction medium is a chemically defined medium which consists of a basal medium supplemented with FGF; retinoic acid; noggin; and KAAD-cyclopamine.

14. A method according to any one of claims 1 to 13 wherein the third pancreatic induction medium is a chemically defined medium which comprises FGF.

15. A method according to any one of claims 1 to 14 wherein third pancreatic induction medium is a chemically defined medium which consists of a basal medium supplemented with FGF.

16. A method according to claim 14 wherein the third pancreatic induction medium further comprises retinoic acid.

17. A method according to any one of claims 1 to 16 wherein the pancreatic progenitor cells express PDX1, SOX9, HNF6, NKX6.1 and PTF1a.

18. A method according to any one of claims 1 to 17 comprising maturing the pancreatic progenitor cells to produce a population of pancreatic endocrine cells.

19. A method according to claim 18 wherein the pancreatic progenitor cells are matured by i) culturing in a first endocrine induction medium and ii) culturing in a second endocrine induction medium to produce the population of pancreatic endocrine cells,

wherein the first endocrine induction medium is a chemically defined medium comprising a Notch signalling inhibitor; and the second endocrine induction medium is a chemically defined medium devoid of differentiation factors.

20. A method according to claim 19 wherein the first endocrine induction medium is a chemically defined medium consisting of a supplemented basal medium and N-[N-(3,5-difluorophenacetyl)-1-alanyl]-S-phenylglycine t-butyl ester (DAPT); and the second endocrine induction medium is a chemically defined medium consisting of a supplemented basal medium.

21. A method according to claim 18 wherein the pancreatic progenitor cells are matured by i) culturing in a first endocrine induction medium and ii) culturing in a second endocrine induction medium to produce the population of pancreatic endocrine cells,

wherein the first endocrine induction medium is a chemically defined medium comprising a Notch signalling inhibitor and retinoic acid; and the second endocrine induction medium is a chemically defined medium devoid of differentiation factors other than retinoic acid.

22. A method according to claim 21 wherein the first endocrine induction medium is a chemically defined medium consisting of a supplemented basal medium, N-[N-(3,5-difluorophenacetyl)-1-alanyl]-S-phenylglycine t-butyl ester (DAPT) and retinoic acid; and the second endocrine induction medium is a chemically defined medium consisting of a supplemented basal medium and retinoic acid.

23. A method according to any one of claims 18 to 22 wherein the pancreatic endocrine cells express NGN3, INS, SST and GLU.

24. A method according to any one of claims 1 to 23 comprising monitoring and/or detecting the expression of one or more cell markers in the population of differentiating cells.

25. A method according to any one of claims 1 to 24 comprising expanding the population of pancreatic progenitor cells or pancreatic endocrine cells.

26. A method according to any one of claims 1 to 25 comprising culturing or maintaining the population of pancreatic progenitor cells or pancreatic endocrine cells.

27. A method according to any one of claims 1 to 26 comprising storing the population of pancreatic progenitor cells, or pancreatic endocrine cells.

28. A method according to any one of claims 1 to 27 comprising admixing the population of pancreatic progenitor cells, pancreatic endocrine cells or pancreatic endocrine cells with a therapeutically acceptable excipient.

29. A method according to any one of claims 1 to 28 wherein the pluripotent cells are human pluripotent cells.

30. A method according to any one of claims 1 to 29 wherein the pluripotent cells are ESCs or iPSCs.

31. A method according to any one of claims 1 to 30 wherein the pluripotent cells are iPS cells derived from an individual with a genetic background associated with a pancreatic condition.

32. A method according to any one of claims 1 to 31 wherein the pluripotent cells are iPS cells derived from an individual with a genetic disorder associated with a pancreatic condition.