exacerbated when such protocols are applied to genetically diverse embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs). Accordingly, there is a need for the development of alternatives to pluripotent cells as a source for pancreatic cells that overcomes, or at least ameliorates, one or more of the disadvantages described above. There is also a need for the development of methods and culture conditions to support long-term self-renewal of these alternatives.

SUMMARY

[0005] In one aspect, there is provided a method of culturing a pancreatic progenitor cell comprising contacting said cell with: a. epidermal growth factor (EGF); b. retinoic acid (RA); c. an inhibitor of transforming growth factor-β (TGF- β) signaling; and d. 3T3-J2 fibroblast feeder cells.

[0005a] In one aspect, there is provided a method of method of culturing a pancreatic progenitor cell comprising contacting said cell with: a. epidermal growth factor (EGF); b. retinoic acid (RA); c. an inhibitor of transforming growth factor-β (TGF- β) signaling; d. an inhibitor of Notch signaling; e. N2 suuplement; f. B27 supplement; g. dexamethasone; h. fibroblast growth factor 10 (FGF10); and i. 3T3-J2 fibroblast feeder cells.

[0006] In one aspect, there is provided a cell produced according to the method of as described herein.

[0007] In one aspect, there is provided a kit when used in the method as described herein, comprising one or more containers of cell culture medium, together with instructions for use.

[0007a] In one aspect, there is provided a kit when used in the method as described herein, comprising one or more containers of cell culture medium , together with instructions for use, wherein the one or more containers of cell culture medium comprises: a. epidermal growth factor (EGF); b. retinoic acid (RA); c. an inhibitor of transforming growth factor-β (TGF- β) signaling; d. an inhibitor of Notch signaling; e. N2 supplement; f. B27 supplement; g. dexamethasone; and h. fibroblast growth factor 10 (FGF10).

DEFINITIONS

[0008] As used herein, the term "progenitor cell" refers to cells that have greater developmental potential, i.e., a cellular phenotype that is more primitive (e.g., is at an earlier step along a developmental pathway or progression) relative to a cell which it can give rise to by differentiation. Often, progenitor cells have significant or very high proliferative potential. Progenitor cells can give rise to multiple distinct cells having lower developmental potential,

i.e., differentiated cell types, or to a single differentiated cell type, depending on the developmental pathway and on the environment in which the cells develop and differentiate. The term “pancreatic progenitor cell” refers to a cell that can give rise to multiple distinct cells of the pancreatic lineage. It is to be understood that a pancreatic progenitor cell is developmentally more proximal to specialized cells of the pancreas as compared to pluripotent stem cells. It will generally be understood that pancreatic progenitors may be derived using a variety of methods known in the art. In one example, pancreatic progenitors may be generated according to the method described in the Experimental Procedures section.

Claims

1. A method of culturing a pancreatic progenitor cell comprising contacting said cell with:
   a. epidermal growth factor (EGF);
   b. retinoic acid (RA);
   c. an inhibitor of transforming growth factor-β (TGF- β) signaling;
   d. an inhibitor of Notch signaling;
   e. N2 suuplement;
   f. B27 supplement;
   g. dexamethasone;
   h. fibroblast growth factor 10 (FGF10); and
   i. 3T3-J2 fibroblast feeder cells.

2. The method of claim 1, wherein the inhibitor of transforming growth factor-β (TGF-β) signaling is an inhibitor of activin receptor-like kinase (ALK) receptor.

3. The method of claim 2, wherein the inhibitor of activin receptor-like kinase (ALK) receptor is SB431542.

4. The method of any one of claims 1 to 3, wherein the inhibitor of Notch signaling is a γ-secretase inhibitor.

5. The method of claim 4, wherein the γ-secretase inhibitor is DAPT.

6. The method of any one of claims 1 to 5, wherein the pancreatic progenitor cell is contacted with:
   a. about 1 ng/ml to about 100 ng/ml of EGF;
   b. about 100 nM to about 10μM of RA; and
   c. about 1μM to about 100μM of SB431542.

7. The method of any one of claims 1 to 6, wherein the pancreatic progenitor cell is contacted with:
   a. about 1 ng/ml to about 100 ng/ml of EGF;
   b. about 1 ng/ml to about 100 ng/ml of FGF10;
   c. about 100 nM to about 10μM of RA;
   d. about 1nM to about 100nM of dexamethasone;
   e. about 100nM to about 10μM DAPT;
   f. about 1μM to about 100μM of SB431542;
   g. about 1x B27 supplement; and
   h. about 1x N2 supplement.

8. The method of claim 7, wherein the pancreatic progenitor cell is contacted with:
   a. about 50 ng/mL EGF;
   b. about 50 ng/ml FGF10;

c. about 3μM RA;
d. about 30nM dexamethasone;
e. about 1μM DAPT;
f. about 10μM SB431542;
g. about 1x B27 supplement; and
h. about 1x N2 supplement.

9. The method of any one of claims 1 to 8, wherein the pancreatic progenitor cell is a pancreatic progenitor cell population.

10. The method of claim 9, wherein the pancreatic progenitor cell population is substantially homogenous.

11. The method of claim 10, wherein the pancreatic progenitor cell population is at least 60%homogenous.

12. The method of claim 11, wherein the pancreatic progenitor cell population is at least 99% homogenous.

13. The method of any one of claims 1 to 12, wherein the pancreatic progenitor cell is cultured for at least 5 passages, at least 10 passages, at least 15 passages or at least 20 passages.

14. The method of any one of claims 1 to 13, wherein the pancreatic progenitor cell is derived from a stem cell.

15. The method of claim 14, wherein the stem cell is a human embryonic stem cell (hESC).

16. The method of claim 14, wherein the stem cell is an induced pluripotent stem cell (iPSC).

17. The method of any one of claims 1 to 16, wherein the pancreatic progenitor cell expresses PDX1, SOX9, HNF6, FOXA2 and GATA6.

18. The method of claim 17, wherein the pancreatic progenitor cell does not express SOX2.

19. A kit when used in the method of any one of claims 1 to 18, comprising one or more containers of cell culture medium , together with instructions for use, wherein the one or more containers of cell culture medium comprises:

a. epidermal growth factor (EGF);
b. retinoic acid (RA);

c. an inhibitor of transforming growth factor-β (TGF- β) signaling;
d. an inhibitor of Notch signaling;
e. N2 supplement;
f. B27 supplement;
g. dexamethasone; and
h. fibroblast growth factor 10 (FGF10).

20. The kit according to claim 19, wherein the kit further comprises 3T3-J2 feeder cells.