JACOBS PUBLISHERS

OPEN ACCESS

Jacobs Journal of Bone Marrow and Stem Cell Research

Commentary

Directed Differentiation of Late Stage islet Lineages Remains a Knowledge Gap in Pancreatic Endocrine Development

Fang-Xu Jiang*[1] , Grant Morahan[2]

[1]*Islet Cell Development Program, Harry Perkins Institute of Medical Research, The University of Western Australia, Perth -6009, Australia*

[2]*Centre for Diabetes Research, Centre for Medical Research, The University of Western Australia, Perth -6009, Australia*

**Corresponding author:* *Dr. Fang-Xu Jiang, Islet Cell Development Program, Harry Perkins Institute of Medical Research,6 Verdun St, Nedlands, WA 6009, Australia, Tel : 61861510758; Fax: 618 61510701; Email: fang-xu.jiang@perkins.uwa.edu.au*

Received: 04-01-2015

Accepted: 04-12-2015

Published: 04-17-2015

Directed differentiation of human embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) into approximately 210 types of functional cells in our body is the ultimate goal of regenerative medicine. The latter is a type of new medicine which is to replace/restore those cells lost through injury or disease with functional new cells. Type 1 and type 2 diabetes mellitus are pandemic metabolic disorders that currently affect approximately 400 million people worldwide, characterized by absolute and relative loss of insulin-secreting β cells and hence are candidate diseases for regenerative medicine.

Over the last decade, intense international efforts have concentrated on differentiation of pluripotent stem cells (PSCs, including ESCs and iPSCs) for replacing/restoring the lost β cell function. Remarkably in such a short timeframe, PSCs have been successfully differentiated following their normal *in vivo* developmental cues into cells of approximately the pancreatic progenitor and/or islet progenitor stages [1-8]. In contrast, due to the lack of knowledge for the late stage pancreatic endocrine lineage [9-10], empirical protocols have been used for their further differentiation. Inevitably the PSC-derived endocrine cells either show a substantial functional variability [11] or respond to glucose poorly and require further maturation *in vivo* to reverse diabetes [12]. Thus generation of genuine insulin-producing endocrine cells remains a challenge. In this commentary, we will briefly discuss a few outstanding issues that hamper the directed differentiation of authentic glucose-responsive β cells.

Multiple fate commitments may accumulate substantial off-target differentiation

PSCs theoretically have the capacity to give rise to all of the functional cell types in the body, so to induce them to becoming desirable β cells requires forced them to make multiple fate commitments under the guidance of exogenous differentiation factors (Figure 1). These factors are always not 100% effective, resulting in a small proportion of cells undifferentiated or differentiated along unwanted pathways, i.e. off-target differentiation. As the MYC transcription factor and core pluripotency networks (Oct4, Nanog and Sox2) of PSCs are the same as the fundamental gene circuits of cancer [13,14], undifferentiated cells in the end products could form tumors after transplantation and a variety of off-target cells, especially those of highly proliferative types, could generate unacceptable biohazards. Directed differentiation of enriched progenitors at various stages of the developmental hierarchy would therefore minimize off-target differentiation.

Empirical protocol is a source of variability in differentiation

The lack of knowledge of differentiation of late stage islet lineages led researchers to develop empirical protocols.

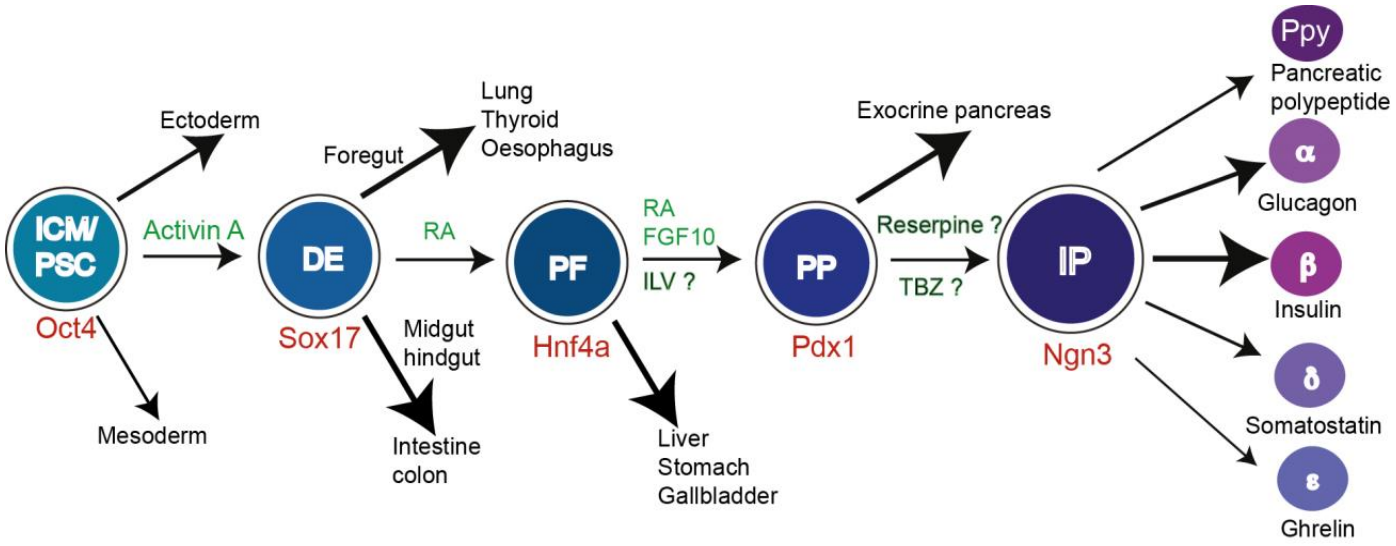

Figure 1. Multiple fate commitments of PSCs lead to the development of the pancreas islet lineages.
Whereas inner cell mass (ICM) gives rise to three germ layers (the ectoderm, mesoderm and endoderm) during gastrulation, pluripotent stem cells [PSC, including embryonic stem cells (ESC) or induced PSC (iPSC)] preferentially differentiate into definitive endodermal cells [DE, marked by the expression of Sox17 (the Sry-related HMG box transcription factor 17) and Foxa2 (foxhead homeobox 2a)] in the presence of activin A. Along the anterior-posterior axis the DE is divided into foregut (giving rise to the lung, thyroid and oesophagus), posterior foregut [PF, marked by the expression of the transcription factor Hnf4a (hepatocyte nuclear factor 4a) and hindgut (committing the intestine and colon). *In vitro*, retinoid acid would direct the DE cells to PF cells. Rather than to the stomach, liver and gallbladder, the PF cells preferentially give rise to pancreatic progenitors (PP, marked by the expression of the transcription factor Pdx1) in the presence of retinoid acid and fibroblast growth factor 10. Principally towards the exocrine and ductal tissues, the PP also commits to progenitors of the endocrine islet lineages [IP, marked by the expression of high level Ngn3, as well as NeuroD (neural differentiation 1), IA1 (insulinoma associated 1), Isl1 (Islet 1), Pax6 (paired box factor 6) and Rfx6]. The IP then differentiates into five types of islet cells [α,β,γ(somatostatin), PP (pancreatic polypeptide) and ε (ghrelin)]. Thick arrows indicate major lineage commitment directions. The "?" indicates that the differentiation factors have not yet completely validated.

Development of such protocols depends heavily on the experience of researchers, which contribute to high variability and low reproducibility between different research groups. A better understanding of the differentiation pathway and its underlying mechanisms would therefore allow the establishment of a standardized directed differentiation protocol, the use of which would thus minimize the high batch-to-batch variability observed in the latest PSC-derived insulin-producing cells [11].

The ability to directly differentiate islet progenitors is critical

As crucial progenitors of functional β cells and other pancreatic endocrine cells [15-17], the islet progenitors (Figure 1) are developed from pancreatic progenitors and express a high level of the key fate determinant neurogenin 3 (Ngn3, also known as neurog3), a helix-loop-helix transcription factor [15,17]. Although having been the focus of many studies over a dozen years, including characterization of their development, gene function and transcriptomic analyses [18-24], $Ngn3^+$ progenitors have not been directly differentiated *in vitro* into functional endocrine cells [22,24]. In addition, caution has to be taken for the use of genetic lineage tracing in PSC differentiation because temporospatial cues are critical for the success of *in vivo* lineage tracing studies. Owing to being developmentally expressed in multiple endoderm-derived tissues including the intestine [25], the PSC-derived $NGN3\text{-}GFP^+$ cells [26,27] in culture should therefore not be treated simply as the equivalent of islet progenitors. Thus, efforts should be supported to establish protocols for directed differentiation into functional β cells from purified islet progenitors present in developing pancreas as the ability of such differentiation would fill the knowledge gap of late stage islet lineages.

References

1. Basford CL, Prentice KJ, Hardy AB, SarangiF, Micallef SJ et al. The functional and molecular characterisation of human embryonic stem cell-derived insulin-positive cells compared with adult pancreatic beta cells. Diabetologia. 2012, 55(2): 358-371.

2. Chen S, Borowiak M, Fox JL, Maehr R, Osafune K et al. A small molecule that directs differentiation of human ESCs into the pancreatic lineage. Nat Chem Biol. 2009, 5(4): 258-265.

3. Cheng X, Ying L, Lu L, Galvao AM, Mills JA et al. Self-renewing endodermal progenitor lines generated from human pluripotent stem cells. Cell Stem Cell. 2012, 10(4): 371-384.

4. Chetty S, Pagliuca FW, Honore C, Kweudjeu A, Rezania, A et al. A simple tool to improve pluripotent stem cell differentiation. Nat Methods. 2013, 10(6): 553-556.

5. Kelly OG, Chan MY, Martinson LA, Kadoya K, Ostertag TM et al. Cell-surface markers for the isolation of pancreatic cell types derived from human embryonic stem cells. Nat Biotechnol. 2011, 29(8): 750-756.

6. Kroon E, Martinson LA, Kadoya K, Bang AG, Kelly OG et al. Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells *in vivo*. Nat Biotechnol. 2008, 26(4): 443-452.

7. Sneddon JB, Borowiak M, Melton DA. Self-renewal of embryonic-stem-cell-derived progenitors by organ-matched mesenchyme. Nature. 2012, 491(7426): 765-768.

8. Xie R, Everett LJ, Lim HW, Patel NA, Schug J et al. Dynamic chromatin remodeling mediated by polycomb proteins orchestrates pancreatic differentiation of human embryonic stem cells. Cell Stem Cell. 2013, 12(2): 224-237.

9. Melton DA. Using stem cells to study and possibly treat type 1 diabetes. Philos Trans R Soc Lond B Biol Sci. 2011, 366(1575): 2307-2311.

10. Pagliuca FW, Melton DA. How to make a functional beta-cell. Development. 2013, 140(2): 2472-2483.

11. Pagliuca FW, Millman JR, Gurtler M, Segel M, Van Dervort A et al. Generation of Functional Human Pancreatic beta Cells *In Vitro*. Cell. 2014, 159(2): 428-439.

12. Rezania A, Bruin JE, Arora P, Rubin A, Batushansky I et al. Reversal of diabetes with insulin-producing cells derived in vitro from human pluripotent stem cells. Nat Biotechnol. 2014, 32: 1121-1133.

13. Ben-Porath I, Thomson MW, Carey VJ, Ge R, Bell GW et al. An embryonic stem cell-like gene expression signature in poorly differentiated aggressive human tumors. Nat Genet. 2008, 40(5): 499-507.

14. Kim J, Woo AJ, Chu J, Snow JW, Fujiwara Y et al. A Myc network accounts for similarities between embryonic stem and cancer cell transcription programs. Cell. 2010, 143(2): 313-324.

15. Gradwohl G, Dierich A, LeMeur M, Guillemot F. neurogenin3 is required for the development of the four endocrine cell lineages of the pancreas. Proc Natl Acad Sci U S A. 2000, 97(4): 1607-1611.

16. Gu G, Dubauskaite J, Melton DA. Direct evidence for the pancreatic lineage: NGN3$^+$ cells are islet progenitors and are distinct from duct progenitors. Development 2002. 129(10): 2447-2457.

17. Schwitzgebel VM, Scheel DW, Conners JR, Kalamaras J, Lee JE et al. Expression of neurogenin3 reveals an islet cell precursor population in the pancreas. Development. 2000, 127(16): 3533-3542.

18. Desgraz R, Herrera PL. Pancreatic neurogenin 3-expressing cells are unipotent islet precursors. Development. 2009, 136(21): 3567-3574.

19. Johansson KA, Dursun U, Jordan N, Gu G, Beermann F et al. Temporal control of neurogenin3 activity in pancreas progenitors reveals competence windows for the generation of different endocrine cell types. Dev Cell. 2007, 12(3): 457-465.

20. Miyatsuka T, Kosaka Y, Kim H, German MS. Neurogenin3 inhibits proliferation in endocrine progenitors by inducing Cdkn1a. Proc Natl Acad Sci U S A. 2011, 108(1): 185-190.

21. Miyatsuka T, Li Z, German MS. Chronology of islet differentiation revealed by temporal cell labeling. Diabetes. 2009, 58(8): 1863-1868.

22. Sugiyama T, Rodriguez RT, McLean GW, Kim SK. Conserved markers of fetal pancreatic epithelium permit prospective isolation of islet progenitor cells by FACS. Proc Natl Acad Sci U S A. 2007, 104(1): 175-180.

23. White P, May CL, Lamounier RN, Brestelli, JE, Kaestner KH. Defining pancreatic endocrine precursors and their descendants. Diabetes. 2008, 57(3): 654-668.

24. Xu X, D'Hoker J, Stange G, Bonne S, De Leu N et al. Beta cells can be generated from endogenous progenitors in injured adult mouse pancreas. Cell. 2008, 132(2): 197-207.

25. Jenny M, Uhl C, Roche C, Duluc I, Guillermin V et al. Neurogenin3 is differentially required for endocrine cell fate specification in the intestinal and gastric epithelium. EMBO J. 2002, 21(23): 6338-6347.

26. Cai Q, Bonfanti P, Sambathkumar R, Vanuytsel K, Vanhove J et al. Prospectively isolated NGN3-expressing progenitors from human embryonic stem cells give rise to pancreatic en-

docrine cells. Stem Cells Transl Med. 2014, 3(4): 489-499.

27. Sakano D, Shiraki N, Kikawa K, Yamazoe T, Kataoka M et al. VMAT2 identified as a regulator of late-stage beta-cell differentiation. Nat Chem Biol. 2014, 10(2): 141-148.