

Commentary

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

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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 become 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.

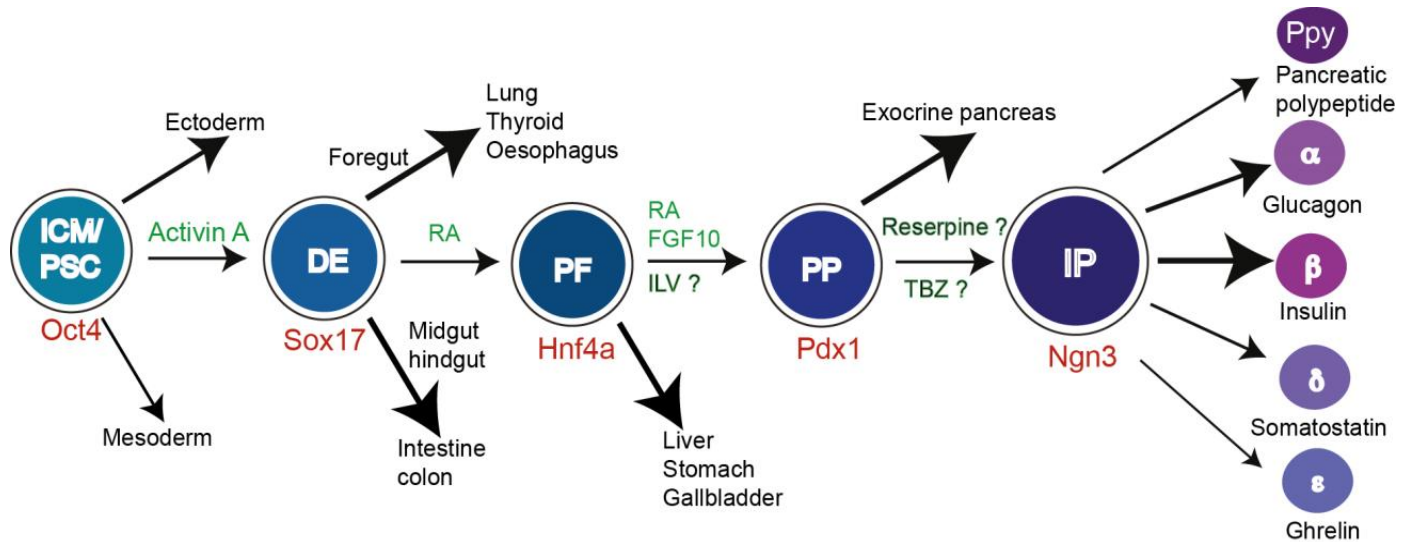


Figure 1. Multiple fate commitments of PSCs lead to the development of the pancreas islet lineages.

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-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.

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