Challenge Remains for Directed Differentiation of Late Stage Pancreatic Islet Lineage

Fang-Xu Jiang* and Grant Morahan

1Islet Cell Development Program, Harry Perkins Institute of Medical Research, the University of Western Australia, Australia
2Centre for Diabetes Research, Harry Perkins Institute of Medical Research, the University of Western Australia, Australia

Generation of functional somatic cells is the ultimate goal of regenerative medicine for replacing/restoring those lost through injury or disease. Absolute or relative loss of insulin-secreting β cells is characteristic of type 1 or type 2 diabetes mellitus, a major public health problem that currently affects approximately 400 million people worldwide. To replace/restore their lost function, intense international efforts have for over a decade concentrated on the differentiation of β cells from embryonic stem cells (ESCs) since their creation in 1998, at which the regenerative medicine era began. Remarkably in such a short timeframe, pluripotent stem cells (PSCs, including induced pluripotent stem cells, iPSCs) have been successfully differentiated following their normal in vivo developmental pathway into approximately pancreatic progenitor and/or islet progenitor stage [1-8]. In contrast, due to the lack of knowledge for late stage pancreatic islet lineage [9,10], empirical protocols have been used for their further differentiation but not achieved a major breakthrough. This is because the PSC-derived endocrine cells either showed a substantial functional variability [11] or responded to glucose poorly and required in vivo maturation to reverse diabetes [12]. Thus challenges remain for generation of genuine insulin-producing endocrine cells. In this commentary, we will briefly discuss a few prominent issues that hamper the directed differentiation of functional glucose-responsive β cells.

Multiple fate commitments may accumulate substantial off-target differentiation

As they theoretically have the capacity to give rise to all over 200 functional cell types in the body, PSCs are forced to make multiple fate commitments under the guidance of exogenous differentiation factors prior to becoming a desirable β cell (Figure 1). These factors are always not 100% effective, resulting in a small proportion of cells undifferentiated or differentiated along unwanted pathways, namely 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 would form tumours and a variety of off-target cells, especially those of highly proliferative could generate unacceptable biohazards. Directed differentiation of enriched progenitors at various stages of the developmental hierarchy would minimise off-target differentiation.

Empirical protocol is a source of variability

The lack of knowledge on differentiation of late stage islet lineage led researchers to develop empirical protocols. Development of such protocols depends heavily on the experience of research workers, which is a cause of, in addition to a high variability, its low reproducibility. Better understanding their differentiation and its underlying mechanisms would therefore allow the establishment of a standardised directed differentiation protocol, which is not normally affected by researcher’s experience and the use of which might thus minimise 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 [15,16]. Although having been the focus of tremendous 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 [18,20]. In addition, caution has to be taken for the use of genetic lineage tracing in PSC differentiation because tempospatial 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.

In summary, the establishment of protocols for directed differentiation into functional β cells from purified islet progenitors present in developing pancreas will be essential for
generation of new knowledge for a late-stage differentiation of PSCs. This information may accelerate the production of genuine β cells for a regenerative therapy to diabetes.

REFERENCES


18. Sugiyama T, Rodriguez RT, McLean GW, Kim SK. Conserved markers


