

Insulin-secreting β cells require a post-genomic concept

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highly specialized transcription of insulin gene, of which occupies up to 40% their transcriptome. Deficiency of these cells causes diabetes mellitus, a global public health problem. Although tremendous endeavors have been made to generate insulin-secreting cells from human pluripotent stem cells (*i.e.*, primitive cells capable of giving rise to all cell types in the body), a regenerative therapy to diabetes has not yet been established. Furthermore, the nomenclature of β cells has become inconsistent, confusing and controversial due to the lack of standardized positive controls of developmental stage-matched *in vivo* cells. In order to minimize this negative impact and facilitate critical research in this field, a post-genomic concept of pancreatic β cells might be helpful. In this review article, we will briefly describe how β cells were discovered and islet lineage is developed that may help understand the cause of nomenclatural controversy, suggest a post-genomic definition and finally provide a conclusive remark on future research of this pivotal cell.

Key words: Beta cells; Insulin; Post-genome; Concept

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Core tip: Pancreatic β cells are highly effective and efficient in the production of insulin, and specialized in its regulated secretion. Deficiency of β cells causes diabetes mellitus, the prevalence of which keeps climbing, despite new drugs continuously becoming available to clinics. Thus regenerative therapies to this devastating disease show great promise. Nevertheless, the generation of β cells requires multiple forced fate changes from pluripotent stem cells and the latter derived insulin+ cells expressing selective key β -cell transcription factors may not be the genuine islet counterparts. Hence their post-genomic concept may help the future development of diabetes regenerative therapies.

Abstract

Pancreatic insulin-secreting β cells are essential in maintaining normal glucose homeostasis accomplished by

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INTRODUCTION

Pancreatic insulin-secreting β cells are of pivotal importance to our physiology because they play a central role in maintaining normal glucose homeostasis by their ability to produce and secrete insulin - a life hormone released in a fine-tuned manner as the body requires it. Deficiency of glucose-responsive β cells causes diabetes mellitus, a global public health issue with a progressively increasing prevalence. Absolute deficiency of these β cells due to autoimmune-mediated destruction results in type 1 diabetes mellitus (T1D). Relative deficiency leads to type 2 diabetes mellitus (T2D), caused by multiple issues, such as the failure of peripheral metabolic tissues to respond to insulin action, the liver's inability to control the production of glucose and the demise of islet β cells^[1]. Diabetes mellitus currently affects over 387 million people worldwide. Despite a variety of treatments being continuously brought to the clinic, the incidence of this disorder is progressively climbing and is projected to reach 592 million by 2035^[2]. Thus, there is an urgent need for novel treatments, such as regenerative medicine, a field established by the creation of human embryonic stem cells (ESCs) in 1998^[3]. A regenerative therapy would provide a cure of T1D (should autoimmunity to β cells be controlled) and also for a subset of T2D, either by transplantation of donated hormone-secreting islets^[4] or of *in vitro* generated genuine β cells from ESCs or induced pluripotent stem cells (iPSCs), or ultimately by regeneration *in situ* of endogenous β cells.

For diabetes regenerative medicine, tremendous focus has been applied to generate insulin-secreting β cells *in vitro*. However, over the years the nomenclature of β cells has unfortunately become inconsistent, confused and controversial, which in turn has apparently hampered the progress of the field. In order to minimize the negative impact of this confusion and to facilitate critical research, we suggest a post-genomic concept of pancreatic β cells. We will briefly describe how β cells were discovered and the islet lineage developed; how this controversy arose; suggest a post-genomic definition and finally provide concluding remarks on this vital research.

BRIEF HISTORICAL ACCOUNT

The "islet of Langerhans" was named after Paul Langerhans, a German medical student, who in 1869 observed small clusters of "clear cells" within the pancreas that were obviously different from the surrounding pancreatic tissue. Subsequently, Edouard Laguesse termed these clusters as islets of Langerhans (Figure 1). Approximately 30 years later, in 1907, Falkmer *et al.*^[5] found the islet cells harbored distinct granules that were different from the zymogen granules in the acinar cells. For example, one type of islet cells was basophilic (type B) stained by

certain histochemical methods and another was not (type A). A more detailed description of how different types of endocrine cells in the pancreas may be distinguished is documented elsewhere.

In the year 1922, the B cells were discovered to produce the hormone insulin (Figure 1) by Banting and Best^[6] who were awarded the Nobel prize for Medicine in 1923. The presence of insulin in the B cells (now known as β cells) was first confirmed immunohistochemically in 1957^[7]. Glucagon was identified in the A cells (now known as α cells) in 1962; this hormone raises blood sugar levels by releasing glucose stored in the liver as glycogen, which is formed in a process called gluconeogenesis. Insulin was the first protein to be fully sequenced (*cf.* Figure 1). This was accomplished by Frederick Sanger's group in 1955^[8] and in 1958 Sanger received the Nobel Prize in Chemistry for this landmark discovery. In 1977, Ullrich *et al.*^[9] successfully cloned the insulin gene and its cDNA using recombinant DNA technology.

Since then, knowledge of this important cell type has increased exponentially. In particular following the creation of human ESCs, numerous academic groups and biotechnological companies have attempted to generate β cells *in vitro* from pluripotent stem cells (PSCs, which include ESCs and iPSCs) with the aim of advancing pancreas developmental biology, providing a renewable cell source for drug screening and, ultimately, establishing a regenerative therapy for diabetes. However, an associated negative effect of this period was the appearance of controversies and confusions on the definition of β cells. This confusion arose from simplistically treating PSC-derived insulin⁺ cells expressing several markers of key β -cell transcription factors as a genuine counterpart of *in vivo* glucose-responding cells. In order to help understand this complex and controversial issue, we will briefly introduce the embryology of pancreas development.

EMBRYOLOGY

The pancreas is an endocrine as well as exocrine organ. It is derived from the primitive germ cell layer known as endoderm (the other two layers are the ectoderm and mesoderm) that originates from the inner cell mass from which ESCs were also originally derived. After gastrulation, the thickened endodermal epithelium along the dorsal and ventral surfaces of the posterior foregut gives rise to the primitive pancreas. In mice, this thickening can be identified histologically at embryonic day (E) 9.0-9.5^[10].

The columnar epithelial cells expand into adjacent mesoderm-derived mesenchymal tissue and form the dorsal and ventral buds of the pancreas primordia. These expanding and branching buds fuse together as the developing gut rotates. The fused developing pancreas continues to grow, differentiate and, ultimately, develop into the mature organ. The adult pancreas consists of digestive fluid-transporting ductal tissue, digestive enzyme-secreting acinar tissue and hormone-secreting

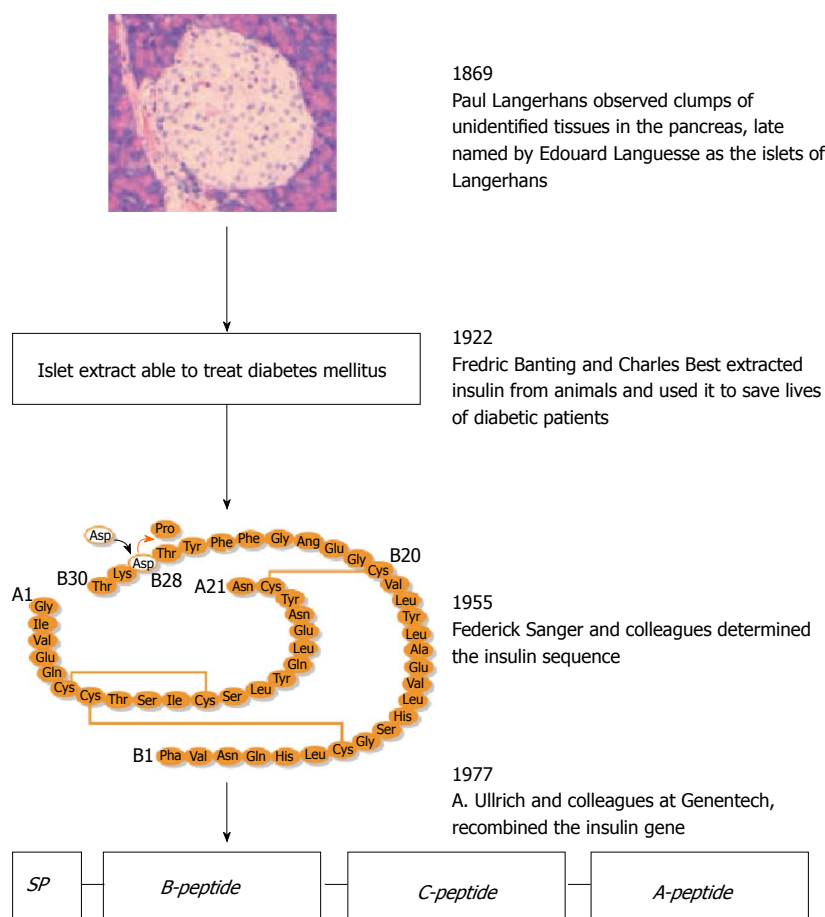


Figure 1 Historical account of the discovery of insulin. Presented four landmark discoveries of the islets of Langerhans, insulin, the sequencing of insulin and insulin gene recombinant technology.

endocrine tissue located in the islets of Langerhans. The latter consist of five types of endocrine cells including in addition to the afore-mentioned β cells and α cells, somatostatin-secreting δ cells, pancreatic polypeptide-secreting PP cells and ghrelin-secreting ϵ cells.

Naturally, human pancreas development displays some features not observed in rodents. For example, the dorsal bud can be detected as early as 26 d post conception (dpc), an equivalent stage to E9.5 embryos in mice, but embryonic β cells are not visible until 52 dpc, approximately 2 wk later than the equivalent stage at which they could be detected in mice. The ontogeny of human embryonic β cells precedes that of embryonic α cells at 8-10 wk of development^[11]. Genetic lineage tracing in mice demonstrates that embryonic β cells do not become postnatal functional insulin-secreting β cells^[12]. All islet cells are detectable at the end of the first trimester in humans^[11], but at very later stages (E17.5) in mice^[13]. These data indicate that the sequence of key developmental events in human pancreatic development is distinct from that in mouse^[14], and this is supported by differences in gene expression patterns during both developmental and disease processes in these species^[15]. Further details of human pancreas development can be found in reviews elsewhere^[16-20]. In the following sections, we will discuss several intermediate stages of islet development, in order to help understand how the confusing and controversial terminology concerning

insulin-producing β cells appeared.

DEVELOPMENT OF INSULIN-SECRETING β CELLS

Definitive endoderm

One of three germ layers to appear during embryogenesis, the definitive endoderm gives rise to numerous organs in a process that is summarized in Figure 2. ESCs can be made *in vitro* to recapitulate their *in vivo* developmental pathways, to give rise to definitive endodermal (DE)-like cells by being cultured in the presence of a high concentration of activin A, a member of the transforming growth factor β superfamily. ESC-derived human expandable DE-like cells are termed endodermal progenitors^[21]. Remarkably, they have been shown to self-renew in the presence of a group of growth factors comprised of bone morphogenetic protein 4, fibroblast growth factor 2, vascular endothelial growth factor and epidermal growth factor^[21]. These progenitors can be passaged at least 24 times with a population expansion of five orders of magnitude. Furthermore, reprogrammed fibroblast-derived DE-like cells have been independently demonstrated to be capable of expanding approximately 65000-fold in the presence of activin A and LiCl^[22]. These data suggest that these DE-like cells are highly proliferative. To ensure their correct

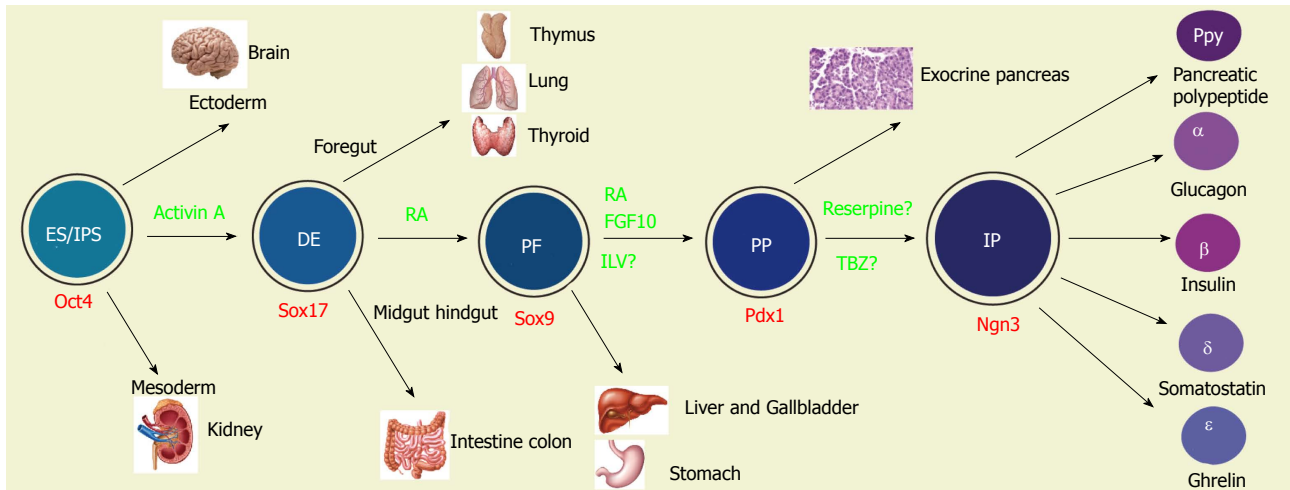


Figure 2 Multiple fate commitments of pluripotent stem cells lead to the development of insulin-secreting β cells. Whereas inner cell mass (ICM) gives rise to three germ layers (the ectoderm, mesoderm and endoderm) during gastrulation, embryonic stem cells (ESC) or induced pluripotent stem cells (iPSC) 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 Sox9 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 (RA) and fibroblast growth factor 10 (FGF10) or indolactam (ILV). Principally towards the exocrine and ductal tissues, the PP also commits to progenitors of the endocrine islet lineages [IP, marked by the expression of a high level of Ngn3, as well as NeuroD1 (neural differentiation 1), IA1 (insulinoma associated 1), Isl1 (Islet 1), Pax6 (paired box factor 6) and Rfx6]. The ES/iPSC-derived Pdx1⁺ cells gave rise to Ngn3⁺ cells in the presence of tetrabenzine (TBZ). The IP then differentiates into five types of islet cells [α , β , δ (somatostatin), PP (pancreatic polypeptide) and ϵ (ghrelin)]. The “?” indicates that the differentiation factors have not yet been completely validated.

differentiation, the endodermal progenitors should be transcriptomically compared to isolated embryo derived DE cells, at least with mouse cells. Although further studies are required, these endodermal progenitors may provide expandable pre-pancreas progenitors for generation of insulin-secreting β cells.

Sox9-expressing progenitors

Sox genes transcribe members of the Sry (sex determining region Y) box-related high-mobility group transcription factor family and are versatile regulators of the stem/progenitor cell fate^[23] as well as of embryonic development of many organs including the pancreas. Sox9 is a critical transcription factor detectable at E10.5 in the dorsal and ventral pancreatic epithelia^[24]. Importantly, Sox9-expressing embryonic pancreatic epithelia at E13.5 have the capacity to give rise to acinar, ductal and islet lineages in the pancreas^[25]. However, Sox9 expression is gradually confined to pancreatic duct cells by E16.5^[25]. Lineage tracing studies demonstrate that Sox9 is also expressed in other posterior foregut-derived organs including the bile duct, the duodenum and the liver. For example, it is expressed in bile duct cells adjacent to the portal vein from E16.5. Sox9 is also broadly expressed in the intestinal epithelia at E13.5 but become restricted to the crypt from E18.5^[25]. These data indicate that PSC-derived Sox9-expressing cells may commit to multiple endoderm-derived lineages including the pancreas.

Pancreatic progenitors express Pdx1

A group of special cells in the thickened DE epithelium along the dorsal and ventral surfaces of the posterior

mouse foregut at E9.0-9.5 expresses the gene named *Pdx1* (pancreas and duodenal homeobox 1, also known as *IPF1*, insulin promoter factor 1 in humans). Pdx1 is a transcription factor of the paralogous homeobox family and is essential for both the expansion of pancreas primordial populations^[26] and the function of adult β cells^[27,28]. Genetic lineage tracing experiments demonstrated that pancreatic Pdx1-expressing (Pdx1⁺) progenitors give rise to acinar, duct and endocrine tissues in the pancreas^[29]. These progenitors are located at the tip of the branching pancreatic tree marked by Pdx1⁺Ptf1a⁺ (pancreas transcription factor 1a) Cpa1⁺ (carboxypeptidase 1)^[30]. Replacement of most of the homeodomain of PDX-1 with the lacZ reporter, allows visualization of the PDX-1/ β -galactosidase fusion allele, and it was found to be expressed in pancreatic, duodenal and antral stomach lineages^[31]. The non-pancreas endoderm-derived expression of Pdx1 was established with the application of a different labeling strategy^[32]. These studies suggest that PSC-derived Pdx1⁺ cells may commit to any of these lineages. Thus, caution should be taken because all PSC-derived Pdx1⁺ cells may not be the equivalent of the pancreatic Pdx1⁺ progenitors.

In humans, numerous PDX1⁺ progenitors can be detected easily in the developing pancreas between 8 and 21 wk of age^[33,34]. These PDX1⁺ progenitors frequently express SOX9 and are highly proliferative^[35], supporting the notion that PDX1⁺ progenitors are committed from SOX9⁺ multipotent progenitors. The number of PDX1⁺ cells that also express insulin or somatostatin progressively increases during this period of development^[33]. An unanswered fundamental question is the origin of the PDX1⁺

progenitors: Are they generated by self-renewal, or by commitment from their endodermal progenitors, or from both sources?

Following *in vivo* developmental pathways, PSCs can be directed to give rise to Pdx1⁺ cells in the presence of the protein kinase C activator indolactam (ILV)^[36]. These cells are able to proliferate 16-fold in the presence of pancreas-derived mesenchymal cells^[37]. Independent confirmation of these results is essential to verify this capacity of the Pdx1⁺ cells. It is also important to address whether all or only a minor fraction of PSC-derived Pdx1⁺ cells commit along the endocrine pathway. To resolve these issues, identification of a specific marker that allows the purification of the Pdx1⁺ pancreatic progenitor-like cells would be valuable.

Ngn3-expressing islet progenitors

At around E9.5 in mice, a small group of cells in the thickened posterior foregut DE epithelium begins to express the basic helix-loop-helix transcription factor neurogenin 3 (Ngn3, also known as neurog3)^[29,38,39]. These Ngn3⁺ cells are islet progenitors because they can give rise to all islet lineage cells. Whereas mouse *Ngn3* mRNA expression in the developing pancreas peaks around E15.5^[40] (equivalent to week 9 in humans), human *NGN3* expression is low before 9 wk, from which time, its expression increases sharply and remains high until 17 wk^[34].

A number of observations support the importance of Ngn3 in islet development: Islet cells are not observed in *Ngn3* knockout mice^[38]; gene lineage tracing demonstrates that Ngn3⁺ progenitors give rise to all pancreatic endocrine cells^[29]; in adult pancreas, purified Ngn3⁺ cells activated by pancreatic duct ligation (PDL) can, after injection into a fetal pancreas *in vitro*, differentiate into all islet cell types^[39]. In contrast, one group reported that although PDL allows activation of Ngn3 expression, the Ngn3⁺ cells were not able to complete the entire β -cell developmental program^[41] and a more recent study found that β -cell mass and insulin content were totally unchanged by PDL-induced injury^[42]. The reason for these inconsistencies is unknown so future studies are required to resolve this matter.

Interestingly, insulin protein has been detected in islet progenitors in the developing human and mouse pancreas. In a dual fluorescence reporter mouse line, a few Ngn3⁺ cells in the developing pancreas coexpress insulin^[43]. In humans, some NGN3⁺ cells were also detected to coexpress insulin in the fetal pancreas between 10 and 21 wk^[33]. Recently, inhibitors of vesicular monoamine transporter-2 (reserpine and tetrabenzine, TBZ), were shown to mediate differentiation of PSC-derived Pdx1⁺ cells into *Ngn3*-expressing cells^[44]. Again, caution has to be taken regarding the use of genetic lineage tracing in PSC differentiation because successful *in vivo* lineage tracing studies rely on the temporospatial cues (see review^[45]) and Ngn3-expressing cells are present in multiple tissues including the endoderm-derived intestine^[46]. Despite lineage tracing demonstrating

that Ngn3⁺ cells will complete the differentiation process prenatally to all pancreatic endocrine cells including β cells^[29], these only become glucose-responsive postnatally.

INSULIN-SECRETING β CELLS

In adults, there are approximately 1000 endocrine islets in mice and 1×10^6 in humans distributed throughout a healthy pancreas, representing up to 2% of the total mass of the organ^[47]. Each islet varies in size from 100 to 500 μ m in diameter and is made up of 1000-3000 cells^[48]. In rodents, β cells are the major component, accounting for up to 80% of the total number in the islets, with the remainder comprised of α cells (approximately 15%) and the remaining endocrine δ , PP and ϵ cells (approximately 5%). In the human islet, the proportions of δ and PP cells are similar, but β cells are less abundant (48%-59%) and the α -cell population accounts a 33%-46%^[49]. Interestingly, a substantial number of ϵ cells are found in adult islets in humans, but not in other known species^[50].

Insulin orchestrates blood glucose utilization by peripheral metabolic tissues such as the liver, muscle and adipose tissue, while glucagon raises blood glucose concentrations by acting on the liver, brain, adipose tissue and heart^[51]. Thus both hormones are critical in maintaining glucose homeostasis. A close paracrine regulatory loop is present between α and β cells. For example, β cells secrete urocortin 3 to stimulate the release of somatostatin which in turn suppresses secreting glucagon from α cells^[52]; α cells also generate ghrelin, which is normally believed to be produced by ϵ cells, to inhibit insulin secretion but stimulate their own glucagon secretion^[53].

Clearly, the β cell is a highly effective and efficient factory specialized for the production of insulin. For example, on average a rodent β cell contains approximately 10000 insulin granules (Figure 3), corresponding to approximately 10%-20% of the total cell volume. Each granule stores approximately 2×10^5 insulin molecules, thus a β cell could package 2×10^9 insulin molecules^[48]. At least 17 key transcription factors (including FOXA2, FOXO1, HNF1A, INSM1, ISL1, MAFA, MNX1, MYT1, NEUROD1, NKX2.2, NKX6.1, PAX6, PDX1, RFX6 TCF7L2 and RFX3) are required to maintain β -cell function^[54]; some of these are shown in Figure 3. The basic-leucine zipper transcription factor MAFA (musculoaponeurotic fibrosarcoma oncogene family protein A), for example, is an important *INS* transactivator^[55]. PDX1 is well known to activate and maintain *INS* and *GLUT2* (glucose transporter 2) expression in β cells^[56,57]. A gene network controlled by NKX6.1 is essential for maintaining the functional and molecular traits of mature β cells^[58]. Pancreatic β cells require NEUROD1 (neuronal differentiation 1) to achieve and maintain a functional state^[59] by DNA methylation-mediated repression of the lineage determination gene *aristaless*-related homeobox^[60]. In addition to key transcription factors, the fractalkine (also known as CXCL1 or neurotoxin)/CXCL1R (also known as GPR13) system

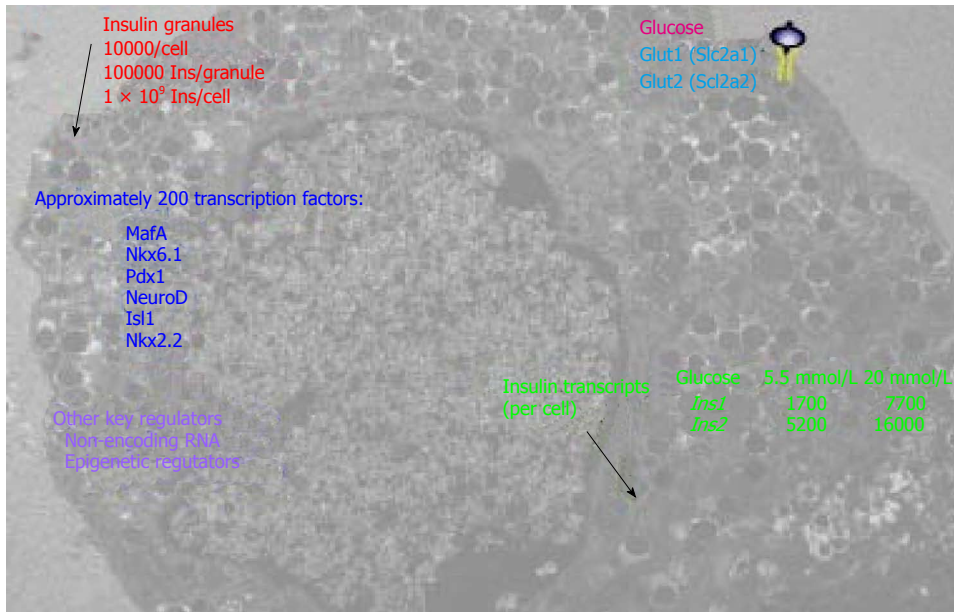


Figure 3 An integral view of insulin-secreting β cells. The highly specialized cells have a powerful function that is regulated by multiple layers of signaling.

also regulates β -cell function and insulin secretion^[61]. Furthermore, β cells develop a highly sophisticated electrophysiology^[62] and glucose sensing system for blood glucose concentrations for fine-tuned secretion of insulin granules to maintain normal glucose homeostasis, which is critical for normal physiology of many pivotal organs.

Recently, the application of high throughput RNA and DNA sequencing technologies has given us a more integral view of insulin-secreting β cells. Deep RNA sequencing of purified human β cells demonstrated *INS* is the most abundantly transcribed gene, representing approximately 38% of the β -cell transcriptome^[63], within which also contains transcripts from over 9900 other genes^[64]. Massively parallel signature sequencing demonstrated that there are over 200 β -cell specific transcription factor genes^[65] that regulate this fine-tuned function. Uniquely, the human *INS* gene is marked by high levels of histone acetylation and H3K4 demethylation at around approximately 80 kb from the transcription start site. These modifications in many other human genes are concentrated around only 1 kb of the start site^[66]. Consistently, high-throughput sequencing of formaldehyde-assisted isolation of regulatory elements (FAIRE-seq) identified approximately 3300 human islet-selective open chromatin sites^[67]. Polyadenylated mRNA sequencing reveals that over 1000 long intergenic noncoding RNA species are transcribed in mouse and human β cells^[68,69]. A review of transcriptomes and other omics of β cells can be found elsewhere^[70].

CONFUSION IN THE CONCEPT OF β CELLS

Reductionist approaches applied over the last two decades have uncovered a complex transcription regulatory net-

work for islet lineage development^[71,72]. Despite the fact that intense international efforts have concentrated on differentiation of PSCs for replacing/restoring the lost β cell function, application of this knowledge for translational research to produce functional β cells *in vitro* has not been straightforward. This is because knowledge generated from *in vivo* studies in rodent models is not necessarily applicable to *in vitro* studies, in particular for human cells. Over this period, at least 11 nomenclatures and definitions have been given to insulin-producing cells (Figure 4) that were generally believed to be the equivalent of *in vivo* β cells.

As the pancreatic islet population and neural cells share a large number of markers and perhaps mechanisms of differentiation^[73], mouse ESCs were early reported to give rise to insulin-positive cells in culture conditions that were used for neural cell differentiation^[74]. Although the differentiated cells were stained positive for insulin, it was subsequently shown this was due to the uptake of insulin from the culture medium rather than the activation of robust insulin transcription^[75]. Additionally, there were several reports of generating pancreatic endocrine cells or functional β cells from PSCs^[76-78]. Later these cells were however demonstrated to be similar to fetal β cells^[79] and to lack the transcriptomic and epigenetic profiles of adult islet cells^[80].

Nevertheless in such a short timeframe, PSCs have been convincingly differentiated following their normal *in vivo* developmental mechanisms into cells of approximately at the pancreatic progenitor and/or islet progenitor stages^[21,36,37,80-84]. In contrast, due to a lack of knowledge of the late stage pancreatic endocrine lineage^[85,86], empirical protocols have been used for their further differentiation. Inevitably the PSC-derived endocrine populations may only contain a small fraction of genuine insulin-secreting cells or are immature, as reversal of diabetes in mice requires

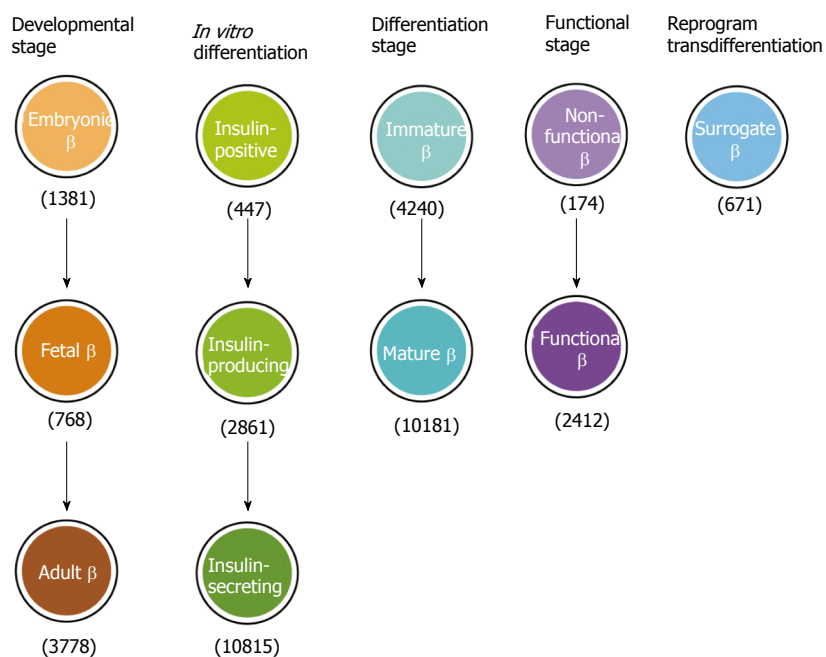


Figure 4 Conceptual confusions in insulin-secreting β cells. Insulin-secreting cells have been given a variety of nomenclature depending on the developmental stages, *in vitro* differentiation, functional states or reprogram/transdifferentiation. The number of used nomenclature in parenthesis was from a Pubmed search in May 2015.

five million SC- β cells^[87] or further maturation *in vivo*^[88]. Readers are referred to recent fine reviews regarding the current state and problems on PSC differentiation towards pancreatic endocrine cells^[20,86,89-91]. Perhaps the problems and confusions on the concept of insulin-secreting β cells seem to have produced negative impacts in the academic community while generating unhelpful excitement and expectations on the reality of future diabetes regenerative medicine to the general public. Furthermore, the confusion and controversy has hampered the progress of not only the field of islet developmental biology but also the establishment of a regenerative therapy to diabetes *per se*. The following section exemplifies several potential, but not exclusive, causes of the confusion and controversy.

The presence of extrapancreas insulin-producing cells

Making the issues more complicated, multiple sites in the body can produce insulin. The thymus, another foregut-derived organ (Figure 2), for example, normally produces insulin, in order to induce self-tolerance and protection of the body from the autoimmune destruction of pancreatic insulin-secreting β cells^[92] as thymus-specific deletion of insulin results in both autoimmune destruction of these cells and diabetes^[93]. Certain areas of the brain also express the insulin gene and produce insulin protein^[94] and these share several transcription factors of the islet lineage^[73]. In different diabetic models, including streptozotocin-treated mice and rats, ob/ob mice, and mice fed high-fat diets, insulin mRNA and protein expression have been detected in the liver, adipose tissue, spleen, bone marrow as well as thymus^[95]. An interesting question is whether these extrapancreatic insulin-producing cells are able to give rise *in vitro* to functional insulin-secreting cells. Otherwise, such extrapancreatic insulin-producing cells are simply non-functional cells.

Taken together, these data suggest that PSC-derived insulin-producing cells might consist of physiologically irrelevant insulin-producing cells.

Multiple fate commitments may accumulate non-functional insulin-producing cells

PSCs theoretically have the capacity to give rise to all of the functionally-defined 210 cell types in the body, so to induce them to becoming desirable β cells requires forcing them to make multiple fate commitments under the guidance of exogenous differentiation factors (Figure 2). Treatment with these factors of course is not always 100% effective, resulting in some cells differentiating along unwanted pathways, even giving rise to non-functional insulin-producing cells especially in suboptimal or abnormal differentiation conditions. Currently, there is no documentation on whether any PSC-derived insulin-producing cells in the differentiated product are similar to those of extrapancreas-derived ones.

Empirical protocol may generate non-functional insulin-producing cells

The lack of knowledge of differentiation of late stage islet lineages^[85,86] led researchers to develop cocktail protocols containing factors that have not been well-characterized. Development of such protocols depends heavily on the experience of researchers and poorly characterized combinations of factors may promote generation of non-functional insulin-producing cells. A better understanding of the β -cell differentiation pathway and its underlying mechanisms would therefore allow the establishment of a standardized directed differentiation protocol and stage-specific differentiation strategies, so that generation of non-functional insulin-producing cells could be minimized or avoided.

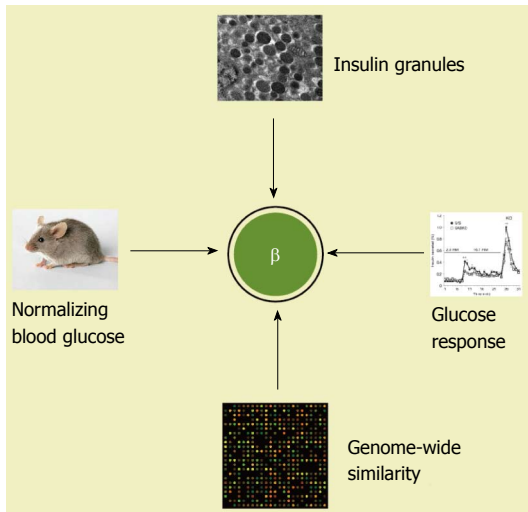


Figure 5 A post-genomic concept of insulin-secreting β cells. Whereas four criteria are proposed, a highly similar transcriptomic profile to adult β cells is essential to establish pluripotent stem cells-derived β cells.

β CELLS REQUIRE A POST-GENOMIC CONCEPT

A major obstacle/challenge in defining PSC-derived insulin-secreting β cells is that the temporospatial cues that help identify these *in vivo* are absent in differentiation *in vitro*. As insulin is only a member of an insulin-related family^[96,97], it is critical to absolutely exclude whether any of the insulin antibodies (especially polyclonals) that have been used to characterize “insulin-producing cells” do not cross-react with other members of this family or even with other polypeptides. This is because “antibodies often recognize extra proteins in addition to the ones they are told to detect” and their reproducibility needs to be dramatically improved^[98,99].

We propose at least four essential criteria for insulin-secreting β cells for further discussions and considerations. Compared to adult β cells, the *in vitro* PSC-derived cells must have: (1) An equivalent number of insulin granules under electron microscopy; (2) a similar dynamic glucose stimulated insulin secretion; (3) a highly similar transcriptomic profile (not a similarity in a selected gene profile of transcriptomic datasets), and (4) the capability to normalize hyperglycemia within a few weeks after transplantation as an equivalent number of functional β cells do (Figure 5).

Definition of functional insulin-secreting β cells at the transcriptomic level is an essential requirement. Alternatively, single-cell transcriptomic and epigenomic analyses of PSC-derived insulin-producing cells could help establish this concept.

CONCLUSION

Currently the sophisticated insulin pump also known as the “Closed Loop Therapy” or “Artificial Pancreas” can deliver insulin in a precise manner, resulting in a significant

improvement in the blood glucose control and the quality of life for people with diabetes^[100,101]. Perhaps we should exercise extra caution for stem cell therapies to diabetes, due to the concern of tumorigenesis^[102], off-target differentiation^[89], biosafety and reliability having not yet been convincingly addressed. The application of genomic, epigenomic, transcriptomic, and/or proteomic approaches to characterize differentiated products will not only verify their safety profile and differentiated state but also shed light on their transcription regulation and molecular mechanisms. The pharmaceutical and biotechnological sectors should work together with the academic community to strengthen fundamental research, identify ways to purify/enrich PSC-derived progenitors at specific stages and develop directed differentiation protocols for the development of the stage-specific progenitors towards genuine insulin-secreting β cells. The progenitors at different stages and differentiated insulin-secreting cells would also be useful for fundamental research and drug screening. Thus, the ability to generate the highly specialized functional β cells *in vitro* will not only generate new knowledge of pancreatic endocrine lineages, but also provide a critical cell source for a diabetes regenerative therapy, a potentially robust and better medicine. In doing so, safe, stable, reliable and functional cellular products will ultimately be available to people with T1D and those with some forms of T2D.

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