Developing Therapies with Functional Beta Cells to Treat Diabetes

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Abstract

As the key regulator in maintaining blood sugar, insulin is secreted by beta cells residing in pancreatic islets. However, most functional beta cells are disappeared in type I diabetes, and the total beta cell population is seriously reduced in type II diabetes. Over the last decade, insights into beta cell development, combined with the deeper research of related donor stem cells including ESCs, iPSCs and pancreatic progenitors, have led us to generate functional beta cells. Besides, several alternative approaches have also converted other cell types into insulin positive beta-like cells via lineage reprogramming factors (Pdx1, Nkx6-1, Pax4 and Ngn3). Nowadays, people realized that advances in investigating of beta cell biology at the genomic and post-transcription levels are more useful in deeply understanding the cells source, behavior and function. For research in drug screening or diabetes transplantation therapy, herein, we reviewed the recent information about development, replication of beta cells, and production of insulin-positive cells via lineage reprogramming or small molecule treatment.

1 Introduction

The pancreas was reported to serve two major regulatory functions, including production of digestive enzymes and regulation of blood sugar, which is achieved by endocrine cells of the islets of Langerhans (also called pancreatic...
islets). In adult human beings, there are 60–80% beta cells residing in islets, secreting insulin, an important regulatory hormone of in the regulation of carbohydrate and fat metabolism [1]. Since the discovery of insulin in 1922, the pancreatic beta cell has been a major focus in the study of diabetes. Until present, it is well known that the blood high glucose levels, and a metabolic disorder in diabetes are resulted from insufficient amount of insulin committed by beta cell death and dysfunction [2]. Recent genetic and clinical studies have highlighted the role of beta cell failure in diabetes pathogenesis [3]. Despite the loss of beta cell number in type I diabetes and deficient beta cell function in type II diabetes, few existing drugs are effective at protecting or restoring this cell type in humans [4]. Both Type I and type II diabetes mellitus are characterized by progressive beta cell failure [5].

Currently, most efforts are aiming at looking for other alternative beta cell sources. As the beta cell is a key cell type in advancing the promise of regenerative medicine. Transplantation and regeneration based on cell therapy strategies are considered as potential therapeutic alternative instead of traditional diabetes treatments [6]. Successful islets and whole pancreas transplantations have already proved that the insufficient insulin could be restored by replenishment of functional beta cell [7, 8]. However, there are not enough healthy beta cell sources, current research groups are focusing on suitable strategies promoting the replication of pre-existing beta cell progenitors and naive stem cells or transdifferentiation from adult cells [9, 10]. Hence, the production of functional insulin-positive cells from stem/progenitor cells represents one of the most promising scientific avenues, present research attempted to dig and imitate beta cell development in vitro [11].

Moreover, advances in high-throughput screening have enabled a modern focus on identifying novel small molecules that are capable of inducing beta cell proliferation and insulin secretion, reprogramming from other cell types [12–15]. To gain deeper insight of the molecular regulatory network guiding endocrine cell differentiation during pancreas development and increase our understanding of the related cellular transcriptional factors expression levels and signaling pathways activities will be useful when treating diabetes by regeneration of beta cell both in vitro or in vivo [16, 17].

2 Diabetes

Since complicated genetic and environmental factors are reported to induce the loss of functional beta cell population, strict clinical definitions of diabetes usually obscure different mechanistic subtypes [18]. Nevertheless, diabetes
can be generally divided into two subtypes including type I (T1D) caused by the absence of insulin-producing beta cells and type II (T2D) which is resulted from a failure in insulin production [3, 5]. In type II, it is sometimes similar with type I which can be recognized as loss of beta cells mass due to insulin resistance [19]. Without long-term treatment, hyperglycemia in type I diabetes patient could lead to other irreversible complications, such as blindness, renal failure, foot amputation and serious cardiovascular diseases [8]. The reason of functional beta cell loss are different among these subtypes of the disease [20]. With the related researches development, I believe that detailed classifications of diabetes will be accepted. Even so, both I and II types have already been characterized by progressive beta cell failure. In type I diabetes, beta cell death is typically caused by an autoimmune assault. However, the T2D etiology is more highly variable, beta cell death is due to varying degrees of insulin resistance [13, 21].

2.1 Subtypes of Diabetes

Nowadays, one million people each year suffering T1D, and it is one of the leading causes of blindness, nephropathy, and heart disease [22]. It is believed that T1D is induced by the autoimmune attack against the islets and most frequently occurs during childhood or adolescence [23]. This form of diabetes is suggested to be induced by a complex combination of genetic and environmental factors. There are about 50% genetic influence happening is HLA class II genes which is believed important for T1D [8]. Not like T1D, T2D is reported determined by genetics according to many family genetic investigations. Data from genome analysis displayed that T2D patient special loci usually contained genes related to beta cell formation and function, but few to do with insulin resistance [18]. Moreover, there is another subtype of diabetes caused by a single gene mutation has been called maturity-onset diabetes of the young (termed MODY) [24].

3 Replication and Function of Beta Cell

Connecting with the duodenum, pancreas plays as a regulator role by producing enzymes to digest foods and hormones to sustain proper blood sugar levels, respectively [2, 25]. The mixed gland is mainly composed consists of two types of glandular tissue: exocrine acini and endocrine islets. [1, 10, 26]. The acinar cells are responsible for producing and secreting digestive enzymes such as lipases, proteases and nucleases. Both acinar and duct cells belong to
pancreatic exocrine part. The endocrine cells are mainly involved in regulating glucose homeostasis and nutrient metabolism. Specifically, the endocrine part of pancreas is usually existed in many small cell clusters of islets, which scattered and detached throughout the pancreatic tissue, and are composed by five cell subtypes such as alpha, beta, delta, eta and PP cells, each of them secretes a particular factor including glucagon, insulin, somatostatin, ghrelin, and pancreatic polypeptide, respectively [27, 28]. Importantly, insulin and glucagon act coordinately to maintain the balance of blood sugar [29, 30]. In this section, we briefly introduce the genetic factors (transcription factors and microRNAs) involved in mass formation, regeneration and insulin secretion of beta cells.

3.1 Pancreatic Development and Transcription Factors

3.1.1 Development of pancreas
Mouse models are usually employed in organ development research. During murine embryonic development, the pancreas develops from the endoderm [1, 31]. The earliest morphological evidences for the pancreas formation can be observed E9.5 [32]. Specifically, there are two outgrowths of the developing pancreas (pancreatic buds) arising along the dorsal and ventral axis. Their appearances are uncoordinated, the dorsal bud can be observed at E9.0, but the ventral bud appears at E9.5 [33]. Then, the latter pancreas morphogenesis consists of two overlapping sections including the primary and secondary transitions [34]. During pancreas development, all exocrine and endocrine lineages are derived from a same population of multipotent progenitors. These pancreatic progenitors proliferation are promoted in primary transition [35]. The secondary transition is responsible for deciding these progenitors towards different lineages [36]. From E13, epithelium expands extensively and mature endocrine cells including beta cells appear after the expression Ngn3, an early marker of pancreatic precursors [37]. And the final formation time point of islets of Langerhans is at shortly after birth (E15) [38, 39].

3.1.2 Key transcription factors in endocrine cells formation
During the early development, programmed expressions of specific transcription factors (TFs) are activated and will determine the differentiation directions of multiple tissues including pancreatic endocrine part [36, 40]. The lineage-tracing studies and TF specific knock-out analyses enhanced our understanding of the molecular mechanisms regulating pancreatic endocrine
development and to confirm the related key TFs at different stages during pancreatic formation [41] (as shown in Figure 1).

Pancreatic and duodenal homeobox 1 (Pdx1), also known as insulin promoter factor 1, is the most famous TF identified for its decisive effect during pancreatic development [42]. However, recent study reported that Pdx1 are mainly responsible for the pancreatic buds formation but not affect the following endocrine differentiation [43]. In both mice and humans the early expression of Pdx1 arise at E8.5 in the dorsal and ventral pancreatic endoderm and continues until E12.5 [46], absence of Pdx1 causes pancreas agenesis at birth [44, 45]. In addition, expression of Pdx1 persistes in mature endocrine cells, it is necessary for the maturation and survival of beta cell, its disappearance promotes a significant decrease in beta cell numbers [47, 48].

Besides, there are several important TFs also involved in the allocation towards the endocrine lineage. The pancreatic buds contain undifferentiated progenitors cells with bipotentiality into endocrine and ductal tissues. It was reported that the endocrine-lineage differentiation of these progenitors requires Ngn3 which belongs to the basic helix-loop-helix (bHLH) transcription factors [37, 49]. Ngn3, expressing from E8.5 to E15.5, is usually

![Figure 1](image-url)  
**Figure 1**  Key transcription factors in endocrine lineages.
considered as a marker for early pancreatic progenitors (termed Ngn3 precursors) [37]. Mice lacking Ngn3 could not form endocrine cells. Ngn3 expression could be induced by Pdx1, then activate the differentiation of pancreatic bud progenitors towards endocrine direction [37]. Indeed, some other important regulatory TFs expression such as Arx, Pax4, NeuroD1, Nkx2.2, and Nkx6.1 require the activation of Ngn3, which enhance the maturity of endocrine precursors [50–54]. Nkx2.2 and Nkx6.1, which both belong to the NK family, were confirmed to play different essential roles in formation of beta cells and insulin secretion [53, 55]. Nkx2.2 expression is unregulated at E9.5 and persists in islet mature cells except delta cells. Knock out of Nkx2.2 reduces in number of mature beta cell mass. Similarly, mice lacking Nkx6.1 was reported to lead to loss of beta cells mass [54].

Except for the classical TFs, it is already shown that some other downstream TFs are required for the specific differentiation towards endocrine lineage, including Arx, Pax4, Pax6, MafA, MafB, and NeuroD1 [36]. Pax4, a member of the paired box (PAX) family of transcription factors, appears at E9.5 and gradually expresses restricted to mature beta cells in mouse [51]. It is involved in islet formation, mouse studies have demonstrated a role for this gene in lineage determination of insulin producing beta cells, thus, neonatal hyperglycemia was found in mice \( \text{Pax4}^{-/-} \). Different from Pax4, the Arx gene is transcribed at E9.5 and restricted to alpha and PP cells [50]. Tissue-specific knocking-down of Arx in islets increased beta cell population by transdifferentiation from alpha cell to beta cell. Deep research has already indicated that Arx and Pax4 form an inhibitory cross regulatory circuit to affect the endocrine precursors fate as their antagonistic activities [50, 56]. Pax6, also known as aniridia type II protein (AN2) or oculorhombin, can be detected in all three islet cell types. Murine knocking-off study showed Pax6 is required for the entire islet cell number [57, 58]. MafA and MafB, both belonging to the basic leucine-zipper TFs, are also required for alpha and beta cell maturation [59, 60]. MafA remains specially in beta cell [61], whereas MafB is critical for both alpha and beta cell formation and maturation [62]. Moreover, NeuroD is an important TF during pancreatic development, it is necessary during the beta cells formation and their regulatory function in sustaining the blood glucose after birth [52, 63].

3.1.3 Transcription factor-based lineage reprogramming

Based on the research targeting genetic network during beta cell development, reprogramming other cells via the forced expression of some key developmental TFs have achieved insulin-producing beta cells by directional
differentiation and transdifferentiation (Figure 2). In theory, embryonic stem cells (ESCs) with pluripotency are able to differentiate to any adult cell types. It has already been shown that human ESCs can be directly differentiated into mature beta cells by a stepwise approach in vitro [64]. However, this process is tedious, easy to pollute and prone to immunological rejection when used [65–67]. Sharing similar characteristics with ESCs, the induced pluripotent stem cells (iPSCs) from patients themselves provide us an ideal source for beta cell transplantation with lower risk [68]. Early studies focused on the conversion of hepatocytes to beta like cells through the overexpression of Pdx1, MafA and NeuroD [69–71]. However, the immature cells in their reports cannot produce insulin since silence of some key functional factors. Accordingly, viral-mediated delivery of Pdx, Ngn3 and MafA reprogrammed the acinar cells of nude mice into insulin-positive cells [72, 73]. Through the ectopic expression of Pax4, pancreatic Pdx1 progenitors cells reprogrammed

**Figure 2** Generation of beta-cell like by reprogramming.
into glucagon-positive alpha cells [74]. Interestingly, PP cell could be transdifferentiated to beta-cell like via forceful expression of a single gene, Pax4. However, in the case of T1D, such generated beta cells cells would be targets for autoimmunity and this approach may also bring tumor risk [14].

3.2 Insulin Production and Secretion
As a common regulator in blood, muscle and fat tissues, insulin is mainly involved in metabolism of carbohydrates and fat. It belongs to a peptide hormone and is produced as part of a larger precursor inactive protein (preproinsulin), which is initially translated [25]. An amino-terminal signal sequence in preproinsulin is needed for passing the endoplasmic reticulum membrane, in which precursor hormone will be post-translational processed [77]. When secreted into the endoplasmic reticulum, the N-terminal signal sequence on preproinsulin will be cut off [75], and disulfide bonds will form to bridge A- and B- chains [76]. Then, the polypeptide is cleaved at two positions to release the intervening chain C [78]. Lastly, the post-translational formation of three vital disulfide bonds occurs, specific peptidases act to cleave proinsulin producing the final functional insulin [16]. At the same time, insulin is mainly stored in secretory granules waiting for release [79].

Normally, secretion of insulin is triggered by sensing changes in ambient glucose levels with two phases, including the first phase occurring in the first few minutes, followed by a more enduring second phase [80]. Briefly, glucose is transported into the cell with specific transporters, where it is phosphorylated by glucokinase and converted into ATP by succedent metabolic reactions. The rise of intracellular ATP levels triggers the closure of K+ channels, membrane depolarization and the opening of Ca2+ channels. The resultant rise in intracellular Ca2+ levels induces the exocytosis of insulin-positive granules and increases insulin levels in blood [81, 82] (Figure 3).

3.3 MicroRNAs Involved in Beta Cell Fate and Insulin Secretion
MicroRNAs (miRNAs) are small noncoding molecules which negatively regulate gene expression by inhibiting their target genes [83]. Emerging studies have shown that miRNAs play diverse roles in diabetes. The present study confirmed the miRNAs that controlled insulin release and production by affecting cell membrane electrical excitability, insulin synthesis, insulin-containing granule exocytosis and beta cell formation [84, 85]. For instance, miR-375 is essential for the formation of pancreatic islets and maintains the
mass of pancreatic alpha and beta cells. miR-15a could repress the uncoupling protein gene Ucp2 directly, which is an important regulator in functional beta cell, it reduces ATP levels, resulting in decrease of ATP/ADP ratio and subsequently decreases glucose-stimulated insulin secretion [86, 87]. miR-29a and miR-29b also negatively control insulin release by reducing the monocarboxylate transporter 1 (Mct1), which acts as a substrate for mitochondrial oxidation to increase cytosolic ATP/ADP ratio and triggers insulin release [88]. miR-124a targets on Foxa2, regulating the KATP channel subunits, Kir6.2 and Sur-1 and pancreatic development [89]. Importantly, in pancreatic cell fate and pancrease formation, miR-21 targets Pdcd4 and induces cell death through the Bax family of apoptoticproteins [90]. miR-146 contributes to the enhancement of free-acid induced beta cell apoptosis [91]. Besides, our research has also confirmed that miR-375 participating in pancreatic progenitors proliferation via targeting Yap1, miR-18a regulating

Figure 3  Glucose-stimulated insulin secretion.
Ptf1a involved in progenitors stemness, and miR-19b which belongs to miR-17-92 cluster could down-regulate insulin 1 by directly repressing NeuroD1 [92–94]. (Figure 4).

### 3.4 Beta Cell Death and Apoptosis in Diabetes

In T1D, beta cell mass is usually reduced by 70–80% when found [6]. Since the obscure insulitis and undetectable beta cell necrosis, it was supposed that the loss of beta cell mass occurs slowly [8]. Initial pathological studies suggested there was a beta cell loss of 25–50% in T2D, while recent reports pointed us that a significant reduction in beta cell mass and an increase in beta cell apoptosis occurs [95]. Although, persuasive data confirmed that both T1D and T2D sharing the same phenotype with appearance of high level inflammatory mediators expression, such as cytokine interleukin IL-1, inducing beta cell apoptosis, mass loss and diabetes [96]. For protection beta cell from cytokine-induced apoptosis, approaches based on both genetic and
small-molecule have been tested [19, 97]. The NF-κB pathway activity has been genetically weakened by ectopic expression of a degradation-resistant NF-κB protein inhibitor, and knockdown of STAT1 in rodent beta cell lines also obviously inhibited apoptosis [98]. Small molecule-based approaches have already tested to weaken the JAK–STAT signalling via silymarin, and HDACs using isoform-selective inhibitors to prevent beta cell dysfunction in human islets [99]. Besides, high levels of glucose and free fatty acids were also reported able to induce endoplasmic reticulum stress and reduce beta cell function and viability [100]. These effects may be suppressed by antioxidants, GS3Kβ inhibitors and HDAC inhibitors mentioned above. (Figure 5).

3.5 Pancreatic Beta Cell Regeneration

After birth, most beta cells mass expansion considerably slows down, however, it has been shown that the beta cell mass can expand under some physiologic or pathologic stimulations including pregnancy and obesity [101–103]. In murine
models, there is also evidence showing the pancreas preserves the ability to regenerate its beta cell population in response to several non-physiological injuries, for example, low-dose injection of streptozotocin (a chemical drug for specifically killing beta cell) will promote beta cell progenitors reproduction [104, 105]. As reported, several progenitors residing in ductal and non-ductal tissues have been shown as potential sources for beta cell renewal [106, 107]. In addition, progenitors from other organs, such as liver, bone marrow and adipose have been considered as either potential sources of islets [108–110]. However, persuasive evidences regarding neogenesis and self-renew for maintenance the normal of beta cells mass still need to be supported.

Besides genetic reprogramming strategies for production of insulin-positive beta cells, small molecules treatment without genome alteration attract more attentions. Different kinds of small molecules could activate or inactivate certain factors, studies of chemical screening confirmed some small molecules could promote the programming of pluripotent stem cells and reprogramming somatic cells into functional beta cells [14]. Recently, our partner group Deng et al. showed that it is possible to generate iPSCs from mouse somatic cells with compound small molecules with no gene modification [111]. The versatile differentiating agent retinoic acid enhanced the generation of Pdx1-positive progenitors from human ESCs and also enhanced further differentiation into insulin-producing beta cells [112]. A small molecule Indolactam V could direct the differentiation of human ESCs into Pdx1-expressing cells [113]. Addition of BRD7552 can also upregulate expression of Pdx1 by epigenetically altering Pdx1 promoter area in human cells [114]. In mice, the TGF-beta signalling pathway activating small molecules (IDE-1 and IDE2) are able to induce pancreatic progenitors from mouse ESCs when used along with Indolactam V [15]. Swerstin has ability to reprogram murine NIH3T3 cells into islet-like clusters [115]. Another small molecular called WS6 is capable to promote beta cell proliferation in human [116]. Thus, when treated with four small molecules together including selenite, 5-AZA, RA and TSA, liver stem-like WB cells turned directly into beta cells [117].

4 Clinical Islets Transplantation for Diabetes

Until present, there’re mainly two types of transplantation successfully treating diabetes including allo-transplantation and auto-transplantation. The allo-type is a procedure in which islets from deceased organ donor are purified and transferred into patients with T1D [118]. However, patients usually have
to take two or more transplants to obtain enough functioning islets to bring blood sugar levels back to normal. The second type is performed for patients after whole pancreatectomy. The patient’s own islets are infused to their livers [27]. This type is not suitable in T1D [8]. The transplantation of islets can alleviate insulin dependence for T1D, however, due to the limitation of immune suppression therapies and the shortage of islet donors, this approach remains in little popularity [6, 119]. Interestingly, there is another sub-type of allo-transplantation called whole pancreas transplantation which has been well established for reversing severe T1DM [120].

5 Conclusions

Aiming to establish new therapeutic applications for diabetes, many studies are targeting at the mechanisms of beta cell function, proliferation and differentiation. This present comprehensive review describes the advances in the field of recent potential therapy approaches based on beta cell transplantation and small molecule treatment, thus, the background information is also summarized including beta cell development, replication and regeneration. Recently, a spurt of progress has been made towards both understanding how beta cells form and proliferate during development and how functional beta cells might be generated. Advances in our understanding of beta cell behavior at the genomic and post-transcription levels will be useful for defeating diabetes mellitus.

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Biography

Y. Zhao is a lecturer of Biology at the Northeast University, China. He received his Ph.D. from Jilin University, and eventually earned a joint training degree from Peking University. After his five-year career as postgraduate on transgenic animals and murine stem cell projects, Dr. Yicheng decided it was time for a change of research area and moved to Harbin, a beautiful city in North China, where he was offered tenure at Northeast Forestry University. In addition to teaching, Dr. Yicheng is focusing on non-coding RNA and pancreatic precursor/stem cell.