Concise Review: Human Pluripotent Stem Cells for the Modeling of Pancreatic β-Cell Pathology

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ABSTRACT

Pancreatic β-cells are the only source of insulin. Disturbances in β-cell development or function may thus result in insulin deficiency or excess, presenting as hyper- or hypoglycemia. It is increasingly evident that common forms of diabetes (types 1 and 2) are pathogenically heterogeneous. Development of efficient therapies is dependent on reliable disease models. Although animal models are remarkably useful research tools, they present limitations because of species differences. As an alternative, human pluripotent stem cell technologies offer multiple possibilities for the study of human diseases in vitro. In the last decade, advances in the derivation of induced pluripotent stem cells from diabetic patients, combined with β-cell differentiation protocols, have resulted in the generation of useful disease models for diabetes. First disease models have been focusing on monogenic diabetes. The development of genome editing technologies, more advanced differentiation protocols and humanized mouse models based on transplanted cells have opened new horizons for the modeling of more complex forms of β-cell dysfunction. We present here the incremental progress made in the modeling of diabetes using pluripotent stem cells. We discuss the current challenges and opportunities of these approaches to dissect β-cell pathology and devise new pharmacological and cell replacement therapies. STEM CELLS 2019; 37:33–41

SIGNIFICANCE STATEMENT

This concise review summarizes the evolving progress in the modeling of diabetes using human pluripotent stem cells. Recent studies are discussed, focusing on those interrogating β-cell dysfunction using stem cell-based models and highlight the implementation of novel technologies like genome editing to further improve the disease models.

INTRODUCTION

Diabetes is one of the main global health problems, affecting 415 million people by 2015 and predicted to increase to 642 million by 2040 [1]. Diabetes is diagnosed when blood glucose is sustained at abnormally high levels, reflecting the incapacity of pancreatic β-cells to secrete sufficient amounts of insulin to cope with its demand. Type 2 diabetes (T2D) is by far the most common form of diabetes, most often associated with obesity-induced insulin resistance. However, it is evident that T2D is a very heterogeneous condition and failing β-cell function is the primary determinant of diabetes development [2]. Type 1 diabetes (T1D), which is characterized by autoimmunity against pancreatic β-cells, constitutes approximately 10% of the cases in western countries, but its incidence is highly variable between different countries and populations [3]. In addition, several monogenic forms of diabetes have been identified,

presenting in young adults as maturity onset diabetes of the young (MODY) [4]. These diabetes types are caused by dominantly inherited mutations in genes important for normal β-cell function [5]. Permanent neonatal diabetes mellitus (PNDM) is a rare form of monogenic diabetes that is diagnosed before 6 months of age. It is caused by mutations in genes important for β-cell development or function [6–8]. Mutations in genes involved in β-cell insulin secretion can also lead to congenital hyperinsulinism (CHI), characterized by inappropriately high insulin secretion which often presents as severe neonatal hypoglycemia [9]. In many cases, the hyperinsulinism is not responsive to currently available drugs and new therapeutic options are needed.

In order to understand the pathogenic role of diabetes-associated genetic variants, experimental β-cell models are needed. Transgenic mice have provided a lot of important

information [10]. However, mouse models have their limitations because of species differences between mice and men [11, 12]. The only possibility to experimentally study primary human β-cells is based on islets isolated from cadaveric organ donors. This material is associated with several problems, such as scarce availability, high variability, poor maintenance of cell functionality in vitro and limited possibilities to genetically manipulate the cells. As an alternative, human β-cell lines have been developed. The EndoC-βH1 line is a valuable glucose-responsive human β-cell model [13]. However, these are aneuploid immortalized highly proliferative cells and thus not directly equivalent with primary cells. In addition, it is not possible to study developmental aspects with these cells and their low clonal efficiency has limited possibilities of their genetic modification.

The remaining option for the modeling of human β-cell biology is based on the use of human pluripotent stem cells (hPSC). Human embryonic stem cells (hESC) are derived from the inner cell mass of human blastocyst stage embryos [14]. Many hESC lines are widely used for research around the world and also for clinical application of stem-cell derived pancreatic cells for diabetes therapy [15]. Human induced pluripotent stem cells (hiPSC) offer an alternative that is not facing many of the ethical problems associated with hESC. These cells are derived from somatic cells by transcription factor-based reprogramming [16–18]. The first iPSC were generated with genome integrating retroviruses, but later several nonintegrative methods have been developed [19]. An obvious benefit of hiPSC is that they can be derived from patient cells, enabling the establishment of cell models with the desired disease-associated genetic background.

Furthermore, iPSC are an ideal material for the biobanking of living cells because of their unlimited expansion and differentiation capacity. Biobanks of iPSC derived from genetically characterized cohorts could be used for the functional validation of diabetes-associated genotypes [20]. Several iPSC biobanks have already been established in Europe (https://www.ebisc.org, www.hipsci.org).

In this concise review, we summarize the progress in utilizing hPSC as a model to study diseases affecting the pancreatic β-cell.

PANCREATIC DIFFERENTIATION OF HPSC

Pancreatic differentiation protocols are based on the knowledge from decades of careful study of pancreatic development in animal models [21, 22]. They have been devised by attempting to replicate in vitro the developmental stages occurring in vivo [23, 24]. This is achieved by using empirically determined cocktails of growth factors and small molecules that create an optimal signaling environment, enabling the progression from the pluripotent stage toward the pancreatic lineage [11].

The human pancreas develops from the endoderm-derived dorsal and ventral pancreatic bud cells of the posterior foregut, which at gestational week 5, start to express PDX1, the master transcription factor for pancreatic development [25]. Pancreatic buds are formed by stratified epithelium that stochastically gets polarized, forming microlumens that then coalesce into a plexus of tubular structures. This plexus eventually gives rise to

the pancreatic ductal tree. Within the developing ductal tree, the peripheral tip domains are specified into the acinar cell lineage, marked by the expression of PTF1A together with SOX9 [12, 26]. The centrally located trunk domain cells will give rise to ductal and endocrine cells. Some of the trunk cells upregulate NEUROG3, triggering endocrinogenesis. This process is controlled by different signaling mechanism in which NOTCH pathway plays a critical role. The NEUROG3 expressing cells commit to the endocrine lineage, delaminating from the pancreatic epithelium to form aggregates representing primitive pancreatic islets. NEUROG3 induces the expression of other transcription factors that establish the endocrine cell gene regulatory network (e.g., NKX2.2, NEUROD1, PAX4, PAX6, ARX, etc.) and it is thus required for the development of all pancreatic endocrine subtypes [27]. The pancreatic islets of Langerhans are complex miniorgans where the interactions between different cell types are functionally important. They consist of five major endocrine cell types: glucagon expressing α-cells, insulin expressing β-cells, somatostatin expressing δ-cells, pancreatic polypeptide expressing PP-cells and ghrelin expressing ε-cells. In mature human islets, approximately 60% of the cells represent β-cells and 30% α-cells, leaving the remaining 10% to the other endocrine cell types. Ghrelin cells are mainly found in embryonic and neonatal islets and are rare in the adult pancreas [28–30]. The different endocrine cell types establish paracrine and autocrine regulatory interactions that control hormone secretion [31, 32]. Therefore, the attempts to generate functional human islets from pluripotent stem cells should reproduce these islet cell proportions, intraislet interactions and cytoarchitecture, which are likely critical to achieve proper physiological function.

First protocols to differentiate ES cells to islet cells were based on embryoid body differentiation [33, 34]. Although some insulin positive cells were derived, these protocols did not produce true pancreatic endocrine cells. In some cases it was not clear whether the cells actually were capable of synthesizing insulin or it was an artifact resulting from insulin media uptake [35].

It is now evident that the key to successful differentiation protocols is to mimic normal pancreatic development, based on the inductive signals which guide development in vivo [23, 36, 37]. The essential basis was established by the group of investigators led by Emmanuel Baetge in the laboratory of CyThera (later Novocell) Inc., San Diego, CA, USA. They were the first to describe a robust protocol for the derivation of definitive endoderm cells from hESC [21]. Induction of definitive endoderm has become a routine in many laboratories and depending on the fine-tuning of the subsequent differentiation stages, this can give rise to progenitors of several endodermal organs, such as thyroid, lung [38], liver [39], pancreas, and intestine [40].

The Novocell group published the first differentiation protocol mimicking pancreatic development signals [22]. It was based on a monolayer cell culture following a sequential five-stage path through definitive endoderm, gut-tube endoderm, pancreatic endoderm, endocrine precursor to finally yield endocrine hormone expressing cells. The group further showed that these stem cell derived β-like cells can rescue streptozotocin-induced diabetes in mice. After transplantation to immunocompromised mice, the cells were able to develop into single-hormone expressing functional endocrine cells [41]. Using an INS-GFP hES reporter cell line and a monolayer differentiation protocol,

Basford et al. were able to purify INS expressing cells from the differentiation cultures and characterize their transcriptome and functionality and compare with human islets. They concluded that these cells were often polyhormonal, with defective glucose-stimulated insulin secretion (GSIS), and with a transcriptomic profile resembling immature endocrine cells. Also, they observed that the INS+ cells were a heterogeneous population, which could result in important functional differences [42].

Characterization at the transcriptomic and epigenetic levels of the different stages of hPSC-differentiation to β-cells in vitro and after in vivo maturation showed how chromatin architecture is remodeled upon differentiation [43]. These results indicated that polycomb group-mediated repression is an important mechanism to control transcription of developmental regulators. Interestingly, aberrant histone modifications were identified in genes that were not properly induced during in vitro differentiation, suggesting that the protocol used to differentiate the cells was not able to faithfully replicate all the signals necessary for proper islet cell differentiation.

The poor functionality of hPSC-derived β-cells, together with polyhormonality, has been a common problem of the first monolayer differentiation protocols. Bruin et al. characterized the endocrine cells after 1 month of monolayer differentiation, concluding that they do not represent functional mature β-cells because of the lack of GSIS in perifusion assay. In comparison with human islets, these cells lacked protein expression of glucose transporter GLUT1, together with imbalance expression of the potassium channel subunits SUR1 and KIR6.2, which could explain their poor functionality [44].

RNA-sequencing of sorted hPSC-derived β-cells, human fetal and adult β-cells enabled their transcriptomic profile comparison [45]. These analyses showed that hPSC-derived β-cells resemble more closely human fetal β-cells at the transcriptomic level, with the consequent functional differences in terms of poorer GSIS for both cell types.

The derivation of pancreatic progenitor cells that express not only PDX1, but also the β-cell programming factor NKX6.1, is crucial for the successful differentiation into monohormonal β-cells [46, 47]. This can be significantly enhanced by the introduction of EGF and nicotinamide into the pancreatic progenitor specification stage [48].

In 2014 to 2015, three groups published "next generation" differentiation protocols where several stages had been significantly modified achieving more efficient monohormonal β-cell differentiation with functionality in vitro [49–51]. Consisting of 6 to 7 differentiation stages, the protocols were optimized to generate homogeneous populations of pancreatic progenitors (PDX1+NKX6.1+) with low NEUROG3 and controlled induction into the endocrine lineage. β-Cell lineage differentiation was induced by adding the EGFR ligand betacellulin and simultaneous ALK5 and NOTCH signaling inhibition [52]. In the later stages, cells were cultured in air–liquid interphase or as aggregates in suspension in order to increase oxygen transfer and reproduce better the cytoarchitecture of pancreatic islets. The transcription factor MAFA, critical for fetal β-cell maturation, was induced by stimulating thyroid receptor signaling using triiodothyronine (T3) [53].

The transcriptome of the β-like cells derived through these differentiation protocols closely resembled human β-cells [50]. In static incubation, the stem cell derived islet-like cells also demonstrated moderate GSIS that was not significantly different from the responses of isolated human islets. However, dynamic GSIS in perifusion assays revealed minimal responses, whereas suboptimal Ca2+ fluxes further highlighted the immaturity of the β-like cells [49].

Despite major progress, it is evident that functionally mature β-cells have not been robustly derived in vitro until now. Importantly, transplantation of the immature human islet-like cells to immunodeficient mice enables significant further maturation of the islet cells. This is evidenced by human C-peptide secretion in glucose tolerance tests and by morphological and ultrastructural studies [49, 50, 54]. It is important to understand what the critical factors in the in vivo milieu that favor functional maturation are. These could be related with local signals provided by the in vivo niche at the transplant site. Particularly interaction with endothelial cells may play an important role [55]. Interestingly, it was recently shown that maturation occurs faster and more efficiently in female recipient mice, pointing to the potential role of estrogen receptor signaling in maturation [56].

Overall, the attempts to produce bona fide functional β-cells in vitro and in vivo need to be characterized with detailed studies of the functional maturity of the cells, and truly evaluate them in comparison with primary high-quality functional β-cells (reviewed in [57]). Detailed comparison of the differentiation protocols have been reviewed elsewhere [37, 58].

HUMAN PANCREATIC DEVELOPMENT STUDIED THROUGH PLURIPOTENT STEM CELL DIFFERENTIATION

A challenge to develop new modifications to improve the differentiation protocols is the lack of understanding of human fetal β-cell development. The study of human pancreatic development has been limited by the scarcity of human fetal tissues donated to research. The studies conducted on this material have shed light on the similarities and differences between human and mouse development in terms of gene expression [25, 59, 60] and functionality [61, 62]. Additional new knowledge about human pancreatic development, together with the extensive available data on human adult islets, can now be compared with the in vitro hPSC-derived β-cells to determine which stage of human pancreatic development they represent. A good example is the characterization by flow cytometry of human fetal pancreatic cells at defined developmental stages that was later correlated with in vitro derived β-cells [63].

With the new wave of single-cell "omics," it is becoming clear that β-cells do not constitute a homogeneous cell population, a fact to observe when comparing with the hPSC-derived β-cells. β-Cell heterogeneity arises already during pancreatic development and has been documented in rodent and human islets [64]. For example, single cell transcriptomic analysis of human islets has revealed different subpopulations within α- and β-cells [65–69]. Heterogeneity was also observed at the protein expression level when using single cell mass cytometry, with at least three different subpopulations of β-cells observed, including a proliferative one [29].

A closer study of the differentiation cultures with novel techniques might result in better understanding of the human pancreatic development and the elucidation of useful markers that could be utilized to generate better β-cells. Using

transcriptomic analysis of hPSC-derived pancreatic progenitors, Ameri et al. identified GP2 as a surface marker of pancreatic progenitors and described that GP2+ isolated progenitors gave rise to functional β-cells in vitro [70]. This was also reported by Cogger et al., who used a different approach based in mass spectrometry analysis of the *N*-glycome of hPSC-derived pancreatic progenitors and further confirmed that GP2+ cells give rise to monohormonal β-cells [71].

By studying single-cell transcripts using qRT-PCR of different stages of hPSC-differentiation toward β-cell, Petersen et al. reported the identification of two different pancreatic progenitor populations giving rise to monohormonal β-cells [72]. They described that NKX6.1 expression in the progenitor cells that will give rise to β-cells can occur before or after NEUROG3 expression commitment to the endocrine lineage. We have similar observations when performing single-cell RNA sequencing using inDROP platform on hPSC-derived islet-like clusters at the end of the differentiation. Using pseudotemporal ordering of the single-cell transcriptomic profiles, we can identify two different pancreatic progenitor populations giving rise to the β-cell lineage (D. Balboa et al., manuscript in publication). Whether these findings represent the heterogeneity of pancreatic development dynamics or an in vitro artifact will require further examination of human fetal pancreas development [60].

At the moment, the characteristics of the differentiation protocols allow the interrogation of mechanistic questions that previously could only be studied in mouse models. Löf-Öhlin et al. used this approach to study the control of pancreatic progenitor polarity regulated by EGFR signaling at different stages of mouse development, validating it in hPSC-derived pancreatic progenitors as model of human pancreas development [73]. Furthermore, the current protocols enable to some extent the modeling of monogenic diabetes forms affecting pancreatic development or β-cell function.

IPSC DIFFERENTIATION AND GENOME EDITING TO UNDERSTAND HUMAN β-CELL DYSFUNCTION

Induced pluripotent stem cell technology makes possible the generation of stem cells from diabetic patients. Combined with the differentiation protocols to β-cells, this constitutes an approach to generate patient-specific models of β-cell dysfunction associated with diabetes. Table 1 presents the studies so far reported on the modeling of β-cell dysfunction using hPSC. Shang and colleagues provided the first example of this approach. They derived iPSC from patients with diabetes caused by a mutation in the *WFS1* gene (Wolfram syndrome) and differentiated them in parallel with healthy-donor control iPSC [74]. They observed that patient iPSC presented elevated endoplasmic reticulum (ER) stress and unfolded protein response, and this disease phenotype could be rescued in vitro by overexpressing WFS1 gene or treatment with chemical chaperones.

Other types of monogenic diabetes have been modeled using direct comparison of patient-derived and healthy-donor control iPSC differentiated to β-cells. A particular mutation of the *HNF1B* gene causing MODY was interrogated by Teo and colleagues, showing that it resulted in a compensatory increase in endoderm and pancreatic markers and reduced PAX6 expression [82]. A mutation in *HNF4A* MODY gene was modeled with

a similar approach using iPSC derived from several members of an affected family. The differentiated β-cells were examined by quantitative proteomics, but no differences were found between the control and patient iPSC-derived cells [83]. This may indicate that the mutation-associated pathogenic mechanism is beyond the stage of differentiation that can be reproduced in vitro.

T1D is associated with β-cell targeted autoimmunity. Genome-wide association studies have identified a number of genes associated with T1D risk. However, with the exception of human leukocyte antigen and INS, the relative risk associated with these genes is rather low [89]. Different studies have generated iPSC and nuclear transfer hES cells from diabetic patients and differentiated them into β-cells. Although two of them found no differences between the T1D and control hPSC-derived β-cells [84, 86], a third one showed increased sensitivity of the β-like cells to cytokine treatment using iPSC derived from a patient with a fulminant form of T1D which is more common in Japan [85]. Overall, it is evident that T1D is not an optimal disease for iPSC-based modeling because of its complex autoimmune pathogenesis, which involves a strong environmental component. In order to recapitulate the interactions between the immune system and β-cells, new disease models are required in which this is facilitated. This could be achieved by coculturing immune cells and iPSC-derived β-cells from the same patient. Another alternative would be the generation of humanized mice with immune system reconstituted from T1D-patient hematopoietic stem cells, in which iPSC-derived β-cells are transplanted [90].

The greatly variable differentiation propensity of iPSC lines is a major problem for their use in disease modeling. In the case of pancreatic differentiation this is clearly illustrated by Sui and colleagues, who compared 12 different iPSC, NT-hESC and hESC lines and showed a range of 8%-75% efficiency in c-peptide+ cell generation using the same protocol [85]. This variation is likely explained by the inherent different capability of the hPSC lines to respond to the differentiation protocols, which only reproduce partially the in vivo milieu of the developing pancreas. A wider understanding of the processes involved in controlling pancreatic development and its implementation into the differentiation protocols will likely reduce the variability. Meanwhile, this obstacle can be solved by the generation of isogenic cell lines, in which the parental cell lines is genome edited to generate or correct a particular mutation of interest. Genome editing technologies (Zinc Finger Nuclease [ZFN], Transcription Activator-Like Effector Nucleases [TALEN], and CRISPR-Cas9) enable the efficient modification of the genome, and they have been used to generate isogenic stem cell lines for disease modeling (reviewed in [91]).
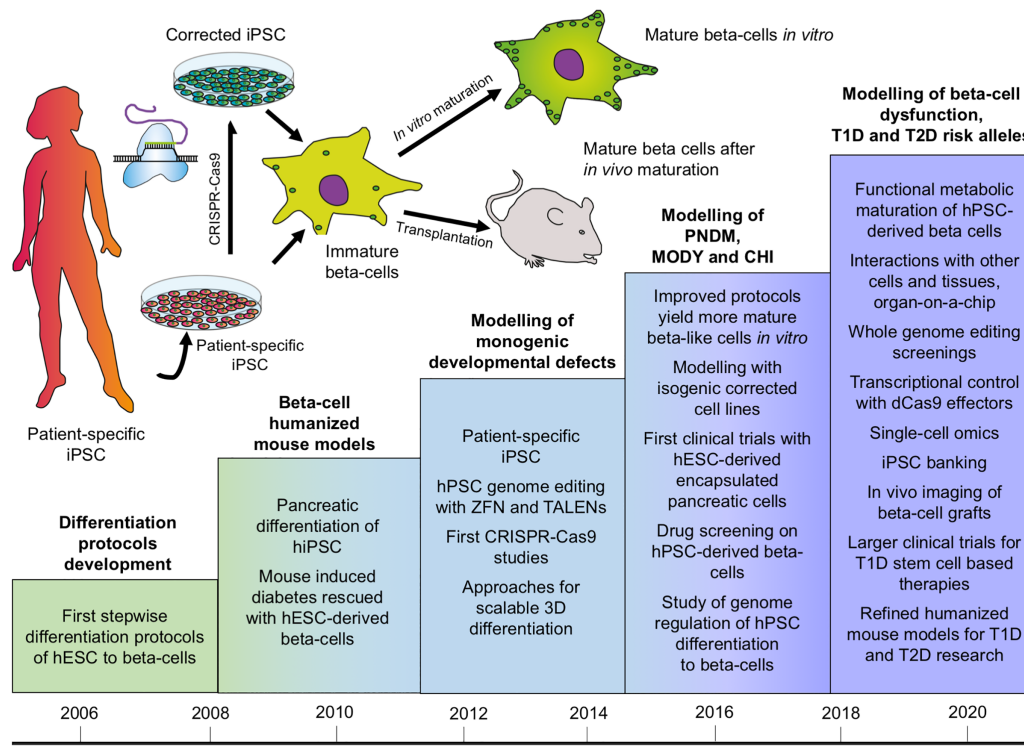
Genome editing of a particular cell line that differentiates efficiently to β-cells can be exploited to generate knock-out models of genes involved in pancreatic development and elucidate their role. Following this approach, *ARX* KO hESC generated with ZFN were shown to have reduced differentiation potential to α-, β- and γ-cells, and increased to δ-cells [88]. Similarly, *NEUROG3* KO hESC generated with CRISPR-Cas9 were used to show that this gene is required for human endocrine differentiation [75], whereas dosage of GATA6 and GATA4 was shown to be crucial for pancreatic development and β-cell

Table 1. Summary of human pluripotent stem cell-based diabetes disease models

Type of diabetes	Genes mutated	Key findings	Cell lines used	Differentiation	Transplantation	References
PNDM	WFS1	Increased ER-stress, reduced insulin content, processing and GSIS	Patient and control hiPSC	Monolayer	Kidney capsule. NSG mice. 12 weeks	Shang et al. [74]
	NEUROG3	Abrogated endocrine cell differentiation	Genome edited hESC (H1)	Monolayer	Kidney capsule, splenic lobe. NSG mice. 6 weeks	McGrath et al. [75]
	PDX1, RFX6, PTF1A, GLIS3, MNX1, NEUROG3	Impairment of pancreatic progenitor and endocrine cell differentiation	Genome edited hESC (HUES8)	Monolayer + air–liquid interphase	NA	Zhu et al. [76]
	KCNJ11	Impaired insulin secretion in vitro and in vivo	Genome edited hESC (HES3-INS-GFP)	Monolayer + air-liquid interphase	Kidney capsule. SCID-beige mice + STZ. 2 to 10 weeks	Zeng et al. [77]
	STAT3	Premature endocrine cell differentiation via NEUROG3 induction	Mutation corrected patient-hiPSC, control iPSC, hESC	Monolayer + suspension	Kidney capsule. NSG mice. 12 weeks	Saarimäki-Vire et al. [78]
	GATA4, GATA6	GATA6 and GATA4 dosages are critical for pancreatic differentiation	Genome edited hESC (HUES8, H1)	Monolayer + air–liquid interphase	Kidney capsule. SCID-beige mice. 4 to 16 weeks	Shi et al. [79]
	GATA6	Impaired definitive endoderm differentiation and β-cell function in vitro	Mutation corrected patient-hiPSC, genome edited hESC (MEL1-INS-GFP)	Monolayer	NA	Tiyaboonchai et al. [80]
	INS	Increased ER-stress, reduced insulin content, β-cell proliferation, size and mTORC1 signaling	Mutation corrected patient-hiPSC	Monolayer + suspension	Kidney capsule. NSG mice. 4 to 24 weeks	Balboa et al. [81]
MODY	HNF1B (MODY5)	Compensatory increase in endoderm and pancreatic progenitor markers and decrease in PAX6	Patient and control hiPSC	Monolayer	NA	Teo et al. [82]
	HNF4A (MODY1)	No differences on HNF4A mutant differentiated β-cells	Patient and control hiPSC	Monolayer + air–liquid interphase	NA	Vethe et al. [83]
T1D	NA	No differences on T1D differentiated β-cells	Patient and control hiPSC	Suspension	Kidney capsule. SCID-beige mice + Alloxan. 2-20 weeks	Millman et al. [84]
	NA	T1D differentiated showed increased Caspase 3 after cytokines treatment. Differential expression of immune-related genes	Patient and control hiPSC	Monolayer	NA	Hosokawa et al. [85]
	NA	No differences on T1D differentiated β-cells	Nuclear transfer hESC, hESC, and hiPSC	Monolayer + suspension	Subcutaneous and kidney capsule. NSG mice + STZ. 2 to 12 weeks	Sui et al. [86]
T2D	CDKAL1, KCNQ1, KCNJ11	Impaired insulin secretion in vitro and in vivo. CDKAL KO cells sensitive to glucolipotoxicity	Genome edited hESC (HES3-INS-GFP)	Monolayer + air–liquid interphase	Kidney capsule. SCID-beige mice + STZ. 2 to 10 weeks	Zeng et al. [77]
	CDKAL1	CDKAL knockout results in downregulation of metallothionein genes, causing increased ER-stress	Genome edited hESC (HES3-INS-GFP)	Monolayer + air–liquid interphase	Kidney capsule. SCID-beige mice + STZ. 6 weeks	Guo et al. [87]
CHI	ABCC8	Increased insulin secretion in vitro	Genome edited hESC (H1)	Monolayer	NA	Guo et al. [87]
	ABCC8	Increased insulin secretion in vitro and in vivo	Mutation corrected patient-hiPSC and control iPSC	Monolayer + suspension	Kidney capsule. NSG mice. 4 to 24 weeks	Lithovius et al. (manuscript in preparation)
Other	ARX	Reduced β-, α- and γ-cell differentiation and increased delta cell differentiation	Genome edited hESC (CA1)	Monolayer	NA	Gage et al. [88]
	HES1, ARX	HES1 knockout increased endocrine cell differentiation. ARX knockout reduced β-cell and abrogated α-cell differentiation	Genome edited hESC (HUES8)	Monolayer + air–liquid interphase	NA	Zhu et al. [76]

Figure 1. Human pluripotent stem cells for the modeling of pancreatic β-cell pathology. Schematic representation of the achieved and future milestones on the road to establish pluripotent stem cell-based models for β-cell dysfunction.

function [79, 80]. In a remarkable effort, Zhu and colleagues used an inducible Cas9 hESC line to generate KOs for *PDX1, RFX6, PTF1A, GLIS3, MNX1, NEUROG3, HES1,* and *ARX* using CRISPR-Cas9, and studied their role in pancreatic differentiation [76], showing how these genes are essential at different stages of development.

In a pioneer attempt of modeling the role of T2D risk genes *KCNJ11, KCNQ1* and *CDKAL1*, Zeng and colleagues generated knockout hESC-lines, differentiated them to β-cells and generated humanized mouse models. Although inactivation of *KCNJ11* and *KCNQ1* resulted in impaired in vitro and in vivo GSIS, *CDKAL1* knockout β-cells were more sensitive to glucolipotoxicity [77]. A follow-up study by the same group showed that *CDKAL1* knockout results in metallothionein gene downregulation and increased sensitivity to ER-stress [87]. However, it is obvious that efficient in vitro modeling of β-cell failure in T2D will require achieving full functional maturation, as well as ability to reproduce β-cell responses to long-term exposure to metabolic stress agents. Currently, these remain major challenges.

Another approach is to correct the diabetes causative point mutation in patient-derived iPSC. The generation of these mutation-correction isogenic cell lines preserves the genetic background of the diabetic patient and enables faithfully interrogation of the mutation role in the disease. This approach was utilized to investigate the mechanism of STAT3 mutation in a rare case of neonatal diabetes, revealing an unexpected mechanism based on premature differentiation of multipotent pancreatic progenitors [78]. Genome editing was also used to correct the *GATA6* mutation in iPSC from a patient with pancreatic agenesis to demonstrate the essential function of GATA6 in pancreatic development and β-cell function [80].

Mutation corrected patient iPSC have also been used to dissect the impact of *INS* mutations for β-cell development (D. Balboa et al., manuscript in publication).

Finally, the stem-cell based approaches can also be used to study β-cell defects behind CHI. There is an obvious need for a relevant model to study the defects in insulin secretion in this disease, because there are no effective drugs for many of the patients who suffer from severe neonatal hypoglycemia caused by mutations in genes encoding ATP-sensitive potassium channels. The disease phenotype can be recapitulated in β-like cells differentiated from patient iPSC, providing a platform for drug screening [92].

CONCLUSION

The establishment of an in vitro method for the differentiation of pluripotent stem cells into a highly specialized endocrine tissue such as pancreatic islets is a formidable challenge. Looking back, it is evident that progress in this area has been remarkable (Fig. 1). The essential steps of the process; commitment into definitive endoderm, then into pancreatic progenitors and further into immature islet-like cells, have been firmly established in multiple laboratories. This has already allowed the use of this method to reveal mechanisms of monogenic diabetes and even of T2D susceptibility genes that act at the level of β-cell differentiation. The remaining challenge is to achieve metabolic maturation of the endocrine cells, required for the β-cells to fine-tune their insulin secretion to nutrient stimuli. This is essential for the detection of phenotypes associated with genetic defects impairing metabolic regulation of insulin

secretion. During the next few years we can expect to see the realization of many of the promises introduced by the combination of the pluripotent stem cells and genome editing also in the diabetes research field. This will include the functional analysis of many risk alleles associated with common forms of diabetes using physiological studies in isogenic pairs of stem-cell derived β-like cells discordant just for the variant of interest. Importantly, this will not be limited to coding sequences because functional validation of the noncoding genome is equally feasible. At the same time, large-scale drug screens will become possible using β-like cells with diabetes patients genotypes. It is also likely that the rapidly developing organoid technologies will allow more physiological and stable models of pancreatic islet function based on patient-derived cells (Fig. 1). β-Cell replacement therapeutic applications will also finally become clinically efficient, potentially revolutionizing the treatment of T1D. However, even before that, it is evident that major clinical benefits will be gained by using in vitro disease models to increase the understanding of pathogenic mechanisms behind β-cell dysfunction, with the consequent development of more efficient and specific drugs for various forms of diabetes.

AUTHOR CONTRIBUTIONS

D.B.: Literature review, manuscript writing, manuscript revision, table and figures design. J.S-V.: Literature review, manuscript writing and figures design. T.O.: Manuscript writing, manuscript revision and final approval.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The authors indicated no potential conflicts of interest.

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