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Who Will Win: Induced Pluripotent Stem Cells Versus Embryonic Stem Cells for β Cell Replacement and Diabetes Disease Modeling?

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Abstract

Purpose of Review Ever since the reprogramming of human fibroblasts to induced pluripotent stem cells (hiPSCs), scientists have been trying to determine if hiPSCs can give rise to progeny akin to native terminally differentiated cells as human embryonic stem cells (hESCs) do. Many different somatic cell types have been successfully reprogrammed via a variety of methods. In this review, we will discuss recent studies comparing hiPSCs and hESCs and their ability to differentiate to desired cell types as well as explore diabetes disease models.

Recent Findings Both somatic cell origin and the reprogramming method are important to the epigenetic state of the hiPSCs; however, genetic background contributes the most to differences seen between hiPSCs and hESCs.

Summary Based on our review of the relevant literature, hiPSCs display differences compared to hESCs, including a higher propensity for specification toward particular cell types based on memory retained from the somatic cell of origin. Moreover, hiPSCs provide a unique opportunity for creating diabetes disease models.

Keywords Human pluripotent stem cells · Diabetes · Reprogramming · Differentiation · Disease models

Introduction

Approximately 9% of the worldwide population suffers from diabetes. Diabetes is characterized by hyperglycemia due to autoimmune destruction of insulin-producing β cells (type 1 diabetes; T1D) or peripheral insulin resistance exacerbating exhaustion and depletion of β cells (type 2 diabetes; T2D). All patients with T1D and many with T2D require exogenous insulin administration, but this typically results in suboptimal glucose control. Whole pancreas or pancreatic islet transplantation has the potential to restore normoglycemia, but this is limited by the shortage of donor tissue and the requirement for immunosuppression to prevent rejection of the transplanted tissue. Following islet transplantation, insulin independence has been achieved for up to 5 years [1], suggesting that the implantation of insulin-producing cells is a viable approach for in vivo control of blood glucose for diabetes.

However, the severe shortage of islets available for transplantation indicates the need for renewable sources of functional β cells. Human pluripotent stem cells (hPSCs), including embryonic (hESCs) and induced pluripotent cells (hiPSCs), can serve as a starting material for diabetes β cell replacement therapy given their capacity for extensive expansion and potential to become pancreatic cells. Recent advances in hPSC directed in vitro pancreatogenic differentiation have produced insulin-positive cells, which in most cases require further maturation in vivo for glucose-stimulated insulin secretion (GSIS). Although hiPSCs have successfully been turned into β-like cells, there are concerns that the retention of somatic cell memory after reprogramming and variation in genetic background make streamlining of their differentiation into β cells challenging.

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A great promise for the use of hiPSCs is their derivation from and use in the same patient, potentially eliminating the requirement for immune suppression after implantation of hiPSC-derived β cells. Nonetheless, the conditions and time frame for reprogramming, expansion, and specification may alter the immunoprofile of the differentiated hiPSC progeny. In addition, hiPSC generation is not associated with the ethical concerns burdening that of hESCs. Here, we will review recent findings on the differences between hESCs and hiPSCs as they pertain to differentiation, particularly to β cells, and the use of hiPSC-derived cells in modeling diabetes and related diseases.

Human Embryonic Stem Cells and Human-Induced Pluripotent Stem Cells

Human embryonic stem cells (hESCs) are derived from the inner cell mass of the blastocyst and are considered the gold standard for pluripotent stem cells, even though ethical concerns surround their use. Almost a decade after the derivation of hESCs [2], Takahashi et al. demonstrated the reprogramming of human dermal fibroblasts with the retroviral transduction of POU5F1 (OCT4), SOX2, KLF4, and MYC [3] and Yu et al. used lentiviral transduction of human fetal fibroblasts with OCT4, SOX2, NANOG, and LIN28 [4] to create cells lines that were called human induced pluripotent cells (hiPSCs).

Unlike retroviruses, lentiviruses can infect not only proliferating but also non-dividing cells, increasing the efficiency of the reprogramming protocol. However, viral transduction entails the genomic integration of transgenes that may be problematic for cell line integrity. This can be remedied by using excisable vectors, for example the Cre-Lox system, or non-integrating viruses, including adenoviruses and Sendai viruses, resulting in nearly or completely footprint-free induction of a pluripotent state. Reprogramming with episomal plasmids does not alter the host genome, but reprogramming efficiency is relatively low even when specific plasmids with prolonged stable expression are used. Given that the relative ratio of transcription factor genes impacts the reprogramming outcome, reprogramming now utilizes a single cassette with all the relevant genes. Generally, reprogramming efficiency varies largely from about .0002–10% and takes 12–30 days depending on the culture conditions, reprograming method, and source of somatic cells. Some examples of somatic cells used for the hiPSC derivation include fibroblasts, hematopoietic stem cells, keratinocytes, and mature T cells [5].

Following their derivation, the pluripotent status of hPSC lines is typically confirmed by examining the (i) expression of markers, such as NANOG, POU5F1 (OCT4), SOX2, TRA-1-60, TRA-1-81, SSEA3, and SSEA4, compared to a reference hPSC line maintained in suitable conditions, (ii) silencing of reprogramming transgenes for hiPSCs, (iii) potential for specification upon directed in vitro differentiation or spontaneous differentiation (e.g., in embryoid body culture) into all three germ layers (endoderm, ectoderm and mesoderm), and (iv) epigenetic state, cell morphology, and proliferation rate. A cell line's capacity for tri-lineage differentiation can also be verified via subcutaneous injection of the cells into immunocompromised mice, resulting in the formation of teratomas. In addition, it is important that the cell lines exhibit a normal karyotype.

Although hESCs are considered the gold standard of pluripotent cells, hiPSCs have distinct benefits (Table 1). Human iPSCs are free of the ethical issues linked to the derivation of hESCs. They can be used as disease models and for candidate drug testing and can be patient-specific, potentially obviating the need for immune suppression after implantation. Furthermore, recent advances in hiPSC generation, such as footprint-free reprogramming and utilization of a single cassette with transcription factors, result in hiPSCs that are more

Table 1 Advantages and disadvantages of hiPSCs and hESCs

	Advantages	Disadvantages
hiPSCs	Autologous cells; potentially no immune rejection	Low reprogramming efficiency
	Can serve as in vitro disease models (derived from corresponding patients)	Clonal cell lines
	Cell source easily accessible	Potential genetic and epigenetic abnormalities
	Multiple cell types have been successfully reprogrammed	Low standardization of cell line derivation
		Risk of teratoma formation
hESCs	Polyclonal	Immunogenic
	Protocols for maintenance and differentiation are better established	Ethical concerns linked to their derivation and use
	Considered the gold standard of pluripotent stem cells	Established lines often derived with xenogenic factors (feeder cells, supplements)
	Suitable for heritable gene defect correction	Risk of teratoma formation

similar to hESCs. However, despite these benefits and advances, there are still concerns that hiPSCs may not function like hESCs as we will discussed below.

Reasons for Differences Between hiPSC and hESCs

Several factors have been proposed to account for the disparities reported between hiPSCs and hESCs. These include differences in culture conditions, passage number, reprogramming method, somatic cell of origin genetic background and epigenetics, and gender between the cell lines used for comparison. These factors also include the typically clonal versus polyclonal nature of hiPSCs and hESCs, respectively.

Many studies have sought to explore the proposed differences between hiPSCs and hESCs. For example, Hussein et al. analyzed 17 hESC lines and 22 hiPSC lines, derived via retroviral or piggyBac reprogramming, and found that significantly more copy number variants (CNVs) are present in early-passage (~passage 5 (P5)) than intermediate passage (~P10–16) hiPSCs. The percent of aberrant cells was 18% in early-passage hiPSCs, while it was 9% in later-passage hiPSCs and 3% in fibroblasts. Most CNVs formed de novo during reprogramming, providing a selective growth or survival disadvantage, so these hiPSCs disappear with passaging, pushing the remaining cells closer to a genetic state resembling that of hESCs. Indeed, CNVs occurred in fragile regions of the genome associated with genes (e.g., *FGF2*, *CTNNB1*, *TP53*, *MYC*, and miRNA *let-7c* and *miR-125b*) that may affect differentiation, pluripotency, or proliferation. Also, early passage hiPSCs exhibited deletions in the subtelomeric region closest to the telomeres, most likely leading to greater genomic instability [6]. This study corroborates the findings of other reports that reprogramming is linked to high rates of mutation underlining the need for a more thorough understanding of pertinent molecular mechanisms and generation of hiPSCs suitable for cell therapies.

Beyond the reprogramming process, observed aberrations in genomic imprints of hiPSCs and hESCs result from in vitro manipulations, including the culture media and surface coatings (e.g., Matrigel® or vitronectin) utilized as well as the presence of feeder cells. For instance, aberrations in *DIRAS3*, *L3MBTL*, and *PEG3* were highly correlated with the use of a specific medium for hPSC maintenance and culture procedures [7]. The impact that culture conditions have on genomic traits of cultured cells speaks to the importance of having consistent cultivation conditions during cell line comparative analysis.

The sex of the cell line also matters since almost all female hPSC lines display X chromosome inactivation, involving X-inactive specific transcript (XIST) long non-coding RNA. Loss of DNA methylation and elevated expression of genes on the inactive X chromosome and loss of XIST expression on X-linked genes in female hPSC lines is linked to prolonged cultivation [8]. These genetic changes, which are seen in both hiPSCs and hESCs, are a source of the disparities reported when hPSC lines are compared. In fact, extended passaging can often lead to genetic aberrations, demonstrating the need for close monitoring of cultured hPSCs [9].

Likewise, the somatic origin of hiPSC is a source of variation in the epigenetic state of the resulting pluripotent cells and consequently of differences between hESCs and hiPSCs. Kim et al. reported the generation of hiPSCs from cord blood cell (CB-iPSC) and keratinocyte (K-iPSC) donors, noting that extended culture did not improve their epigenetic similarity to hESCs and that both tissue-specific and reprogramming-induced methylations were present. K-iPSCs displayed 9.4-fold higher expression of keratin 14 (early keratinocyte differentiation marker) and generated 23-fold more keratinocytes than CB-iPSCs. On the other hand, CB-iPSCs generated a greater frequency of hematopoietic colonies compared to K-iPSC and iPSCs from isolated adult $CD34^+$ blood. Nonetheless, CB-iPSCs and K-iPSCs did not exhibit any differences in their differentiation to definitive endoderm, suggesting an increased propensity displayed by hiPSC lines only toward their somatic origin. These differences were further corroborated by five differentially methylated regions being associated with hematopoiesis for CB-iPSCs and four with epithelial cell phenotypes for K-iPSCs [10]. These findings demonstrate that the hiPSC somatic origin is important for the efficiency of differentiation, possibly favoring progeny matching that of the hiPSC cell source. Although this distinguishes hiPSCs from hESCs, these epigenetic differences are potentially beneficial for the production of particular cell types.

Another source of variation for hiPSC lines stems from the genetic background of the donor. In one study, hiPSC lines were derived from primary fibroblasts, keratinocytes, and endothelial progenitor cells from female and male donors. All hiPSCs were cultivated under the same conditions. Interestingly, donor genetic differences accounted for approximately 38% of transcriptional variation between hiPSC lines, while epigenetic memory from somatic cell origin contributed comparatively little (~4%) and < 1% was attributed to differences between hiPSCs and hESCs (percentages were calculated with a correlation of transcriptional variance with the sum not constrained to 100%). Some of the epigenetic differences may be accounted for by the reprogramming method since endothelial progenitor cells were converted to hiPSCs by retroviral transduction, whereas fibroblasts and keratinocytes were reprogrammed with a Sendai virus. Interestingly, transcriptional memory was noted in 0.06, 0.06, and 0.20% of all expressed genes in fibroblasts, keratinocytes, and endothelial progenitor iPSCs, respectively, signifying that hiPSCs from certain somatic origin cells may retain more memory [11].

Consequently, even though somatic cell origin does not appear to affect epigenetics as much as donor genetic differences, the results warrant further studies into the relative contribution of the two factors to the epigenetic signature of hiPSC lines.

To that point, hiPSC lines derived from the same somatic cell type appear to share aberrant methylation patterns supporting the existence of residual methylation from the cell of origin. Analysis of the methylome of 17 hiPSC lines (derived by retroviral or lentiviral reprogramming), 6 somatic origin cell types, and 7 hESC lines revealed differentially methylated CpG sites common among all hiPSCs. Of note, only 1% of the human genome was covered in this assay, but CpG sites were preselected providing higher information content rather than lower resolution sequencing. Aberrantly methylated CpG sites in hiPSCs were categorized as residual methylation retained by the hiPSCs from the cell of origin and de novo methylation (i.e., not present in either the somatic cell of origin or the hESCs). Residual and de novo methylations accounted for 0.32–1.60% and 0.57–2.98%, respectively, with an overall fraction of aberrantly methylated CpG sites between 0.92–3.82%. Interestingly, some cell types, such as astrocyte-derived lines, displayed less irregular methylation than, for example, fibroblast-derived lines. Comparison of the methylation level of CpG sites on nine genes (*PTPRT*, *TMEM132C*, *TMEM132D*, *TCERG1L*, *DPP6*, *FAM19A5*, *RBFOX1*, *CSMD1*, and *C22ORF34*) generally separated the hiPSC and hESC lines tested. Therefore, it is hypothesized that these genes may represent an applicable core set of atypically methylated genes distinguishing hiPSC and hESC lines. Furthermore, about 20–50% of the aberrantly methylated CpG sites persisted following differentiation of hiPSCs to endoderm or trophectoderm [12]. Thus, there are differentially methylated regions when comparing hiPSCs and hESCs, although their role in the differentiation to functional cells was not explored here.

A subset of the above identified genes was also reported as differentially methylated regions (DMRs) in adipose-derived stem cell-iPSCs (ADS-iPSCs) vs. H1 hESCs. In addition to ADS-iPSCs generated via retroviral transduction, hiPSCs were derived from human foreskin fibroblasts by episomal vector reprogramming (FF-iPSC) and IMR90 fibroblasts with lentiviral transduction (IMR90-iPSCs). Examination of these cell lines revealed that de novo DMRs are created with higher probabilities in certain areas and DMRs present in somatic cells are preserved during reprogramming. Almost half of hiPSC CG-DMRs were similar to progenitor cells and different from hESCs. Two hiPSC lines shared around 70% of the DMRs, but only 16% among all five lines. Several of the DMRs (88% of hypermethylated and 46% of hypomethylated for FF-iPSC clone CG-DMRs) remained in the derived trophoblast cells [13]. As in the Ruiz et al. study, this study showed DMRs between hESCs and hiPSCs.

The aforementioned studies use hiPSCs from different cell sources for a comparison between hESCs and hiPSCs, which hampers the determination of whether the differences are due to hiPSC derivation or other variables, such as genetic background. The best way to control for this is to use genetically matched hESCs and hiPSCs, where the hiPSCs are derived from in vitro-differentiated hESCs. Using this approach and Sendai virus reprogramming, Choi et al. found that genetic background was more important to transcriptional variation than cell origin or reprogramming method. Twice as many promoters (2610) were differentially methylated between unmatched than matched lines (1205), indicating that genetic background is a large determinant of the epigenetic state of hPSCs. Transcriptional variation was significantly higher between genetically unmatched and matched lines and it was similar within groups of genetically matched hiPSC or hESC lines [14••]. In a separate report employing a similar approach, but with lentivirus-mediated reprogramming, there was insignificant differences in gene expression and little variation in methylation for differentiated or undifferentiated cells between hiPSCs and hESCs [15]. These findings point to the genetic background as the most important factor for differences between hiPSCs and hESCs.

Overall, these studies illustrate that there are differences between hiPSCs and hESCs, such as differentially methylated regions and copy number and transcription variations that are dependent on somatic cell origins, reprogramming method, passage number, and culture conditions. However, whether these differences influence the differentiation of hiPSCs or hESCs to functional β cells was not explored.

Differentiation of hESCs vs hiPSCs

From the viewpoint of β cell replacement therapies, the capacity for specification into cells secreting insulin in response to glucose stimulation is of paramount importance for hESCs and hiPSCs. However, the propensity for pancreatogenic differentiation is influenced by the somatic cell origin as recent studies show. In one such report, hiPSCs were generated from human β cells (BiPSCs), non-β cell pancreatic cells (PiPSCs), and fibroblasts (FiPSCs). The reprogramming efficiency of β cells was 0.0001% compared to 0.0025% for fibroblasts, potentially due to the more differentiated state of the β cells. The BiPSCs maintained a partially open chromatin structure (associated with gene transcription) at the INS and PDX1 gene promoters denoted by elevated levels of histone H3 acetylation, while this was not seen in PiPSCs, FiPSCs, or hESCs of similar passage numbers. The hypomethylated state of some genes expressed in β cells was transmitted to BiPSCs, whereas these genes were only methylated in fibroblasts, FiPSCs, and hESCs. These results support the retention of epigenetic memory in BiPSCs. To check whether this memory translates

to a higher proclivity for differentiation to the endodermal lineage, the cells were allowed to differentiate into embryoid body (EB) and teratomas as well as subjected to directed differentiation toward pancreatic endocrine progenitors [16].

INS, *PDX1*, and *FOXA2* mRNA expression in EBs and teratomas was substantially greater for BiPSC than PiPSC, FiPSC, and hESC, yet no significant differences were found in genes for ectodermal, mesodermal, and endodermal tissues. In addition, C-peptide protein was detected in EBs derived from BiPSCs, but not from FiPSCs or hESCs. Transplantation of BiPSC-derived pancreatic endocrine progenitor cells in mice resulted in detectable human C-peptide serum levels at 3 weeks, while hESC-derived pancreatic endocrine progenitors exhibited C-peptide levels that were an order of magnitude lower than for BiPSCs after 6 weeks. Surprisingly, 6–7 weeks post-transplantation BiPSC grafts had INS mRNA expression, whereas the hESC grafts did not. It is possible that the low passage number (P10-20) of the BiPSCs could account for the retention of the epigenetic somatic memory, which is reduced with passaging [16]. Nonetheless, the findings demonstrate the importance of the hiPSC somatic origin (β cells) for enhanced propensity for commitment to a desired cell type, i.e., insulin-producing cells. Therefore, BiPSCs may be better suited than hESCs for differentiation toward insulin-releasing progeny and are shown to mature faster in vivo.

BiPSCs from β cells have also been analyzed by an assay for transposase-accessible chromatin with high-throughput sequencing (ATAC-seq). Adult human islet cells and FiPSCs were also included to find differential open chromatin sites (DOCs) between the cell types that could potentially explain the preferential differentiation of BiPSCs toward endocrine pancreas-like cells. Compared to fibroblast-DOCs, BiPSC-DOCs were enriched in endoderm-development associated weak enhancers, bivalent enhancers/promoters, polycomb-repressed regions, and FOXA2 transcription binding factor sites. While the observed patterns are related to early-stage endodermal development, Bi-DOCS linked to genes in advanced stage of commitment (e.g. *PDX1*, *NKX2–2*, and *INS*) were also reported. Almost all of BiPSC-DOCs-associated genes (99%) were related to open chromatin in human islets. These findings suggest that epigenetic memory does exist, however the BiPSCs were passage number 9–12, while FiPSCs were 18–26, which could account for some of these differences since extended passaging has been shown to lessen epigenetic memory [17••]. Combined with the results reported by Bar-Nur et al. [16] these studies point to the fact that β cell-derived iPSCs exhibit differences compared to other PSCs as they carry traits of the source cells, increasing their proclivity for pancreatic cell specification.

hiPSCs Disease Modeling

One of the benefits of hiPSCs vs hESCs is the ability to derive hiPSCs from individuals who have diabetes, thus creating a cell line that has the potential to have characteristics of the diseased state once differentiated toward the pancreatic lineage. This is extremely beneficial for testing drugs on a relevant human cell model as well as elucidating disease cellular mechanisms. For example, patients with diabetes have an increased risk for cardiovascular disease, so Drawnel et al. reprogrammed dermal fibroblasts from a patient with fast progression (FP) cardiovascular disease that appeared within 5 years of diabetes diagnosis and one with slow progression (SP) and no cardiovascular disease after 15 years of T2D. FP- and SP-iPSCs were differentiated into cardiomyocytes (FP-CMs and SP-CMs). The cardiomyocyte score (striated pattern of immunofluorescent α-actinin staining quantified by an algorithm) of FP-CMs and SP-CMs was lower than standard cardiomyocytes, representing sarcomeric disarray. In addition, FP-CMs had a decreased calcium transient frequency, increased irregular beat rate, and more intracellular peroxidized lipids, indicating oxidative stress. SP-CMs had a phenotype between FP-CMs and normal CMs with a lower cardiomyocyte score but no significant reduction in calcium transient frequency, irregular beat rate, or increased lipid peroxidation [18]. The hiPSC-derived cardiomyocytes showed a difference between FP and SP cardiovascular disease associated with diabetes, which is very important for understanding the effects of diabetes on cardiovascular health.

Human iPSCs have been derived from patients with T1D and subjected to differentiation into pancreatic cells. The cell lines were analyzed side-by-side with hESCs and hiPSCs from a non-diabetic (ND) subject. The capability to differentiate into $SOX17^+$ and $FOXA2^+$ (definitive endoderm) cells was comparable among all lines, but there were intrapatient clonal differences in HNF4A-/HNF1B- (primitive foregut tube) and INS- and glucagon-expressing (islet) cells. Analysis of differentially expressed genes confirmed that the derived iPSC clones were similar to hESCs. Principal component analysis (PCA) of the transcriptome array data resulted in all iPSC clones from the T1D and ND patients and the hESC line clustering together, but with some variation, most likely due to the hESCs being cultured on feeder cells unlike the hiPSCs. Upon differentiation of ND- and T1D-iPSCs toward pancreatic endoderm, 71–95% were $SOX17^+$ and 80–99% were $CXCR4^+$. Yet, the clones showed variable efficiency of differentiation and although all clones at day 9 expressed *HNF4A* transcripts, only 3 clones could reproducibly be specified to insulin-producing cells. Of note, some of the hiPSC lines did not show significant downregulation of *POU5F1* (OCT4) transcripts after differentiation, *SOX2* expression was not downregulated in advanced stages of differentiation in one ND hiPSC line, and all clones had sustained *c-MYC*

presentation after 26 days of differentiation [19]. These results showcase the significant variability among hiPSC lines even when hiPSC lines are derived with the same method from the same patient and tissue type. This warrants caution, since not all observed differences between hiPSCs and their progeny can be ascribed to the disease of the patient the cells were derived from. In addition, hiPSC lines differentiate into pancreatic cells with varying efficiencies, suggesting that the differentiation regimen requires line-specific optimization as documented for different hESC lines.

Several types of maturity onset diabetes of the young (MODY, hereditary form of diabetes caused by a gene mutation in insulin production) exist. Therefore, studying these mutations may help elucidate genes that play key roles in insulin production and lead to better treatments of the disease. Braverman-Gross et al. generated hiPSCs from two patients with MODY1, a disorder caused by a mutation in the HNF4A transcription factor. The HNF4A-targeted genes with more HNF4A binding sites were most affected by lower HNF4A levels in MODY1 hiPSC-derived cells. Additionally, the effect was more pronounced if the binding sites were closer to the target gene transcription start site and if the target gene had fewer binding sites for other transcription factors in its promoter. However, no significant differentiation capacity differences were found between MODY1 and control hiPSCs. The expression of *PAX6, NEUROD1, NEUROG3*, and *NKX6-1* was only higher with limited significance in MODY1 cells compared to control hiPSCs, which suggests a compensation by MODY1 hiPSCs to overcome the decreased HNF4A levels [20••]. A similar compensation mechanism was reported in another MODY type, MODY5, where upregulation of pancreatic development regulators was suggested to overcome the heterozygosity of HNF1B [21•]. Analysis of the transcriptome difference between MODY1 and control cells showed an increase in the expression of genes associated with lipoproteins, pancreatic and hepatic roles of HNF4A in insulin and glucagon secretion, lipid metabolism, and triglyceride biosynthesis [20••], which correlates with MODY1 patient dyslipidemia and hyperinsulinemia [22].

Another study looked at the pancreatic differentiation of MODY1 derived hiPSCs from both diabetic and non-diabetic subjects and a non-diabetic non-mutation carrying individual in comparison to human islets. All of the hiPSCs cell lines generated insulin-positive cells when differentiated, suggesting that the HNF4A mutation does not prevent expression of insulin genes or the commitment to insulin-producing cells in vitro. However, the differentiated cells exhibited a decreased glucose threshold for insulin release and did not have proper glucose stimulated insulin secretion. Additionally, global proteomic analysis was performed to discover molecular components that distinguish in vitro differentiated cells from human islets. It was found that Urocortin-3 (a marker of functionally mature β cells) and proconvertase 1 (involved in the insulin processing machinery) were increased in human islets and MAFB and KI67 (proliferation marker) were lower in comparison to in vitro-derived insulin-positive cells, indicating that hiPSC-derived β cells are immature [23•]. This is consistent with most protocols for in vitro pancreatogenic specification of hESCs and hiPSCs, which require further (typically in vivo) maturation of the differentiated cells to display proper insulin response to glucose stimulation [24, 25]. While more studies are warranted, hiPSCs derived from patients with MODY pathologies can be useful as disease models.

Other disease-specific hiPSC line models related to diabetes have been generated, including fulminant T1D [26•], Wolfram Syndrome Types 1 [27] and 2 [28], T1D [29, 30], T2D [30], MODY3 [31, 32], and MODY1, MODY2, MODY5, and MODY8 [32]. When Wolfram Syndrome Type 1 hiPSCs were differentiated into insulin-releasing cells, their hormone production was lower and unfolded protein response was greater than for non-diabetic hiPSCs [27]. Further use of disease-specific hiPSCs and how the genetic differences affect differentiation down the pancreatic lineage may serve to test new drugs as well as elucidate mechanisms that lead to better understanding of disease pathology.

Discussion

Ideally, for comparative analysis between hESCs and hiPSCs, the same culture conditions should be maintained across all lines. Additionally, hiPSCs should be used at an intermediate passage number > 15 for genetic aberrations to be selected out. Footprint-free reprogramming methods are preferable and using isogenic cells is ideal (i.e., reprogramming hESCs to hiPSCs). Reports aligned with these requirements have demonstrated that the main cause of differences between hESCs and hiPSCs was based on genetic background. However, the type of somatic cells used for derivation of iPSCs and the reprogramming method also contribute to the characteristics of the resulting lines, particularly their propensity for specification to a particular fate. Hence, while there are no pronounced differences between undifferentiated hESCs and hiPSCs, significant discrepancies become evident among their committed progeny.

Several studies have presented differentiation of hESCs and hiPSC to β cell implementing protocols with marked similarities. For example, Pagliuca et al. differentiated eight hESC and four hiPSC and did high-glucose challenges. The results showed a range for both hESCs and hiPSCs indicating that the differentiation method exhibits no particular advantage in terms of efficiency for hESCs or hiPSCs [25].

The data [16, 17••] thus far show that β cell-derived iPSCs (BiPSCs) have a higher propensity to differentiate down the

pancreatic lineage to insulin-secreting cells when compared to non-β cell- and fibroblast-derived iPSCs, suggesting that the use of BiPSCs may be advantageous for β cell replacement therapy. A major consideration however is that obtaining β cells from diabetic patients with extensive β cell damage would be very challenging and these patients would most likely require immune suppression after implantation. More importantly for T1D, a vexing issue is whether the derived BiPSCs and their differentiated progeny will retain their immunoprofile and if the patient's body will destroy the derived β cells. This concern can be alleviated through the application of cell encapsulation methods, which are in continuous development.

Variations in the proclivity for efficient pancreatogenic specification among different hESC and hiPSC lines (even when derived from the same patient and tissue [19]), dictate the need for optimization of a differentiation protocol for each cell line. Such optimization can be very time- and resource-intensive. To this end, a rapid screen that allows selection of the best stem cell line(s) with the highest efficiency of functional β cell differentiation given a specific protocol would be helpful. The process can rely on the evaluation of stage-specific markers similar to the common practice of assessing differentiation. For example, Siller et al. determined hPSC endodermal potential based on definitive endoderm morphology [33] and Nishizawa et al. evaluated hiPSC hematopoietic differentiation based on cell line DNA methylation, gene expression, and chromatin structure [34]. Alternatively, an assay for fast evaluation of multiple differentiation regimens in parallel would be highly desirable. Such assays however, should be followed by detailed assessment of the function (e.g., GSIS, metabolic rate, Ca^{2+} transients) of the differentiated cells. Unfortunately, the screen would only be protocol-specific, but there would most likely be overlap of lines that have greater pancreatic lineage differentiation potential.

Another important aspect of diabetes therapy is understanding the mechanisms of pathology. hiPSCs provide a unique opportunity to create hiPSC lines from individuals with diabetes and differentiate them to tissue types affected by the disease. hESCs require genetic manipulation to induce a particular mutation(s). As reviewed above, many hiPSC models for different types of diabetes have been generated and some have been coaxed into insulin-producing cells. These disease hiPSCs are excellent tools for elucidating disease-related faulty mechanisms, testing available drugs, and guiding the development of effective treatments. The shortcomings of hiPSC-based disease models include somatic cell epigenetic memory, reprogramming-induced changes, variation between same patient-derived hiPSCs, lack of cells from diseases that cause embryonic lethality, and variable reprogramming efficiency depending on cell type.

Conclusion

Overall, there appear to be no major differences between hiPSCs and hESCs that make a particular hPSC type unsuitable for β cell replacement therapy. Epigenetic memory seems to help β cell-derived hiPSCs to have increased pancreatic differentiation potential compared to fibroblast- or non-β cell-pancreatic cell-derived hiPSCs and hESCs. It has yet to be seen if these β cell-derived hiPSCs are fully functional β cells with physiological GSIS, matching hESC-derived β cells that have been matured in vivo. Furthermore, hiPSCs are effective genetic disease models for studying diabetes and can serve to test drugs and help discover novel diabetes cell mechanisms. Based on the studies so far, it appears that hiPSCs are equally as good as hESCs for β cell replacement if not better and are the best for creating diabetes disease models.

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Compliance with Ethical Standards

Conflict of Interest Elena F. Jacobson and Emmanuel S. Tzanakakis declare that they have no conflict of interest.

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References

Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

1. Jin SM, Kim KW. Is islet transplantation a realistic approach to curing diabetes? Korean J Intern Med. 2017;32(1):62–6.
2. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, et al. Embryonic stem cell lines derived from human blastocysts. Science. 1998;282(5391):1145–7.
3. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell. 2007;131(5):861–72.
4. Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, et al. Induced pluripotent stem cell lines derived from human somatic cells. Science. 2007;318:1917–20.
5. Malik N, Rao MS. A review of the methods for human iPSC derivation. Methods Mol Biol. 2013;997:23–33.
6. Hussein SM, Batada NN, Vuoristo S, Ching RW, Autio R, Narva E, et al. Copy number variation and selection during reprogramming to pluripotency. Nature. 2011;471(7336):58–62.
7. Nazor KL, Altun G, Lynch C, Tran H, Harness JV, Slavin I, et al. Recurrent variations in DNA methylation in human pluripotent stem cells and their differentiated derivatives. Cell Stem Cell. 2012;10(5):620–34.

8. Pick M, Stelzer Y, Bar-Nur O, Mayshar Y, Eden A, Benvenisty N. Clone- and gene-specific aberrations of parental imprinting in human induced pluripotent stem cells. Stem Cells. 2009;27(11):2686–90.
9. Mayshar Y, Ben-David U, Lavon N, Biancotti JC, Yakir B, Clark AT, et al. Identification and classification of chromosomal aberrations in human induced pluripotent stem cells. Cell Stem Cell. 2010;7(4):521–31.
10. Kim K, Zhao R, Doi A, Ng K, Unternaehrer J, Cahan P, et al. Donor cell type can influence the epigenome and differentiation potential of human induced pluripotent stem cells. Nat Biotechnol. 2011;29(12):1117–9.
11. Rouhani F, Kumasaka N, de Brito MC, Bradley A, Vallier L, Gaffney D. Genetic background drives transcriptional variation in human induced pluripotent stem cells. PLoS Genet. 2014;10(6): e1004432.
12. Ruiz S, Diep D, Gore A, Panopoulos AD, Montserrat N, Plongthongkum N, et al. Identification of a specific reprogramming-associated epigenetic signature in human induced pluripotent stem cells. PNAS. 2012;109(40):16196–201.
13. Lister R, Pelizzola M, Kida YS, Hawkins RD, Nery JR, Hon G, et al. Hotspots of aberrant epigenomic reprogramming in human induced pluripotent stem cells. Nature. 2011;471(7336):68–73.
14.•• Choi J, Lee S, Mallard W, Clement K, Tagliazucchi GM, Lim H, et al. A comparison of genetically matched cell lines reveals the equivalence of human iPSCs and ESCs. Nat Biotechnol. 2015;33(11):1173–81 **Study used isogenic hESCs and hiPSCs for comparison to determine the role of genetic background on epigenetic differences between hESCs and hiPSCs.**
15. Mallon BS, Hamilton RS, Kozhich OA, Johnson KR, Fann YC, Rao MS, et al. Comparison of the molecular profiles of hESCs and hiPSCs of isogenic origin. Stem Cell Res. 2014;12(2):376–86.
16. Bar-Nur O, Russ HA, Efrat S, Benvenisty N. Epigenetic memory and preferential lineage-specific differentiation in induced pluripotent stem cells derived from human pancreatic islet beta cells. Cell Stem Cell. 2011;9(1):17–23.
17.•• Thurner M, Shenhav L, Wesolowska-Andersen A, Bennett AJ, Barrett A, Gloyn AL, et al. Genes Associated with Pancreas Development and Function Maintain Open Chromatin in iPSCs Generated from Human Pancreatic Beta Cells. Stem Cell Rep. 2017;9(5):1395–405 **The open chromatin structure (associated with active gene transcription) of β cell-derived hiPSCs compared to fibroblast-originating hiPSCs as they relate to open chromatin structures in human islets was studied**.
18. Drawnel FM, Boccardo S, Prummer M, Delobel F, Graff A, Weber M, et al. Disease modeling and phenotypic drug screening for diabetic cardiomyopathy using human induced pluripotent stem cells. Cell Rep. 2014;9(3):810–21.
19. Thatava T, Kudva YC, Edukulla R, Squillace K, De Lamo JG, Khan YK, et al. Intrapatient variations in type 1 diabetes-specific iPS cell differentiation into insulin-producing cells. Mol Ther. 2013;21(1):228–39.
20.•• Braverman-Gross C, Nudel N, Ronen D, Beer NL, McCarty MI, Benvenisty N. Derivation and molecular characterization of pancreatic differentiated MODY1-iPSCs. Stem Cell Res. 2018;31:16–26 **hiPSCs were derived from a MODY1 (HNF4A transcription factor mutation) patient and differentiated to the pancreatic lineage. It was reported that the HNF4A target gene's differential expression depends on the number of HNF4A binding sites. The effect is more pronounced if the binding sites are closer to the target gene transcription start site and the target gene has fewer binding sites for other transcription factors in its promoter**.
21.• Teo AK, Lau HH, Valdez IA, Dirice E, Tjora E, Raeder H, et al. Early developmental perturbations in a human stem cell model of MODY5/HNF1B pancreatic hypoplasia. Stem Cell Rep. 2016;6(3): 357–67 **Human iPSCs were generated from a MODY5 (HFN1B mutation) patient and differentiated to pancreatic cell progenitors. The resulting cells show increased expression of several pancreatic cell transcription factors (*PDX1*, *PTF1A*, *GATA4*, and *GATA6*) and a decrease in *PAX6* expression in comparison to the control**.
22. Stanescu DE, Hughes N, Kaplan B, Stanley CA, De Leon DD. Novel presentations of congenital hyperinsulinism due to mutations in the MODY genes: HNF1A and HNF4A. J Clin Endocrinol Metab. 2012;97(10):E2026–30.
23.• Vethe H, Bjorlykke Y, Ghila LM, Paulo JA, Scholz H, Gygi SP, et al. Probing the missing mature beta-cell proteomic landscape in differentiating patient iPSC-derived cells. Sci Rep. 2017;7(1):4780 **Human iPSCs were produced from a MODY1 patient and successfully differentiated into insulin-positive cells. This suggests that a HNF4A mutation does not prevent expression of insulin genes. The differentiated cells were shown to be immature in comparison to human islets.**
24. Rezania A, Bruin JE, Arora P, Rubin A, Batushansky I, Asadi A, et al. Reversal of diabetes with insulin-producing cells derived in vitro from human pluripotent stem cells. Nat Biotechnol. 2014;32(11):1121–33.
25. Pagliuca FW, Millman JR, Gurtler M, Segel M, Van Dervort A, Ryu JH, et al. Generation of functional human pancreatic beta cells in vitro. Cell. 2014;159(2):428–39.
26.• Hosokawa Y, Toyoda T, Fukui K, Baden MY, Funato M, Kondo Y, et al. Insulin-producing cells derived from 'induced pluripotent stem cells' of patients with fulminant type 1 diabetes: vulnerability to cytokine insults and increased expression of apoptosis-related genes. J Diabetes Investig. 2017. https://doi.org/10.1111/jdi.12727. **Human iPSCs were derived from a patient with fulminant type 1 diabetes to better understand β-cell destruction. Differences were observed vs. control hiPSCs in response to cytokines.**
27. Shang L, Hua H, Foo K, Martinez H, Watanabe K, Zimmer M, et al. β-cell dysfunction due to increased ER stress in a stem cell model of Wolfram syndrome. Diabetes. 2014;63:923–33.
28. La Spada A, Ntai A, Genovese S, Rondinelli M, De Blasio P, Biunno I. Generation of human-induced pluripotent stem cells from Wolfram syndrome type 2 patients bearing the c.103 + 1G>a CISD2 mutation for disease modeling. Stem Cells Dev. 2018;27(4):287–95.
29. Maehr R, Chen S, Snitow M, Ludwig T, Yagasaki L, Goland R, et al. Generation of pluripotent stem cells from patients with type 1 diabetes. Proc Natl Acad Sci U S A. 2009;106(37):15768–73.
30. Kudva YC, Ohmine S, Greder LV, Dutton JR, Armstrong A, De Lamo JG, et al. Transgene-free disease-specific induced pluripotent stem cells from patients with type 1 and type 2 diabetes. Stem Cells Transl Med. 2012;1(6):451–61.
31. Griscelli F, Ezanno H, Soubeyrand M, Feraud O, Oudrhiri N, Bonnefond A, et al. Generation of an induced pluripotent stem cell (iPSC) line from a patient with maturity-onset diabetes of the young type 3 (MODY3) carrying a hepatocyte nuclear factor 1-alpha (HNF1A) mutation. Stem Cell Res. 2018;29:56–9.
32. Teo AK, Windmueller R, Johansson BB, Dirice E, Njolstad PR, Tjora E, et al. Derivation of human induced pluripotent stem cells from patients with maturity onset diabetes of the young. J Biol Chem. 2013;288(8):5353–6.
33. Siller R, Naumovska E, Mathapati S, Lycke M, Greenhough S, Sullivan GJ. Development of a rapid screen for the endodermal differentiation potential of human pluripotent stem cell lines. Sci Rep. 2016;6:37178.
34. Nishizawa M, Chonabayashi K, Nomura M, Tanaka A, Nakamura M, Inagaki A, et al. Epigenetic variation between human induced pluripotent stem cell lines is an indicator of differentiation capacity. Cell Stem Cell. 2016;19(3):341–54.