Adapting Physiology in Functional Human Islet Organogenesis

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Generation of three-dimensional (3D)-structured functional human islets is expected to be an alternative cell source for cadaveric human islet transplantation for the treatment of insulin-dependent diabetes. Human pluripotent stem cells (hPSCs), such as human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs), offer infinite resources for newly synthesized human islets. Recent advancements in hPSCs technology have enabled direct differentiation to human islet-like clusters, which can sense glucose and secrete insulin, and those islet clusters can ameliorate diabetes when transplanted into rodents or non-human primates (NHPs). However, the generated hPSC-derived human islet-like clusters are functionally immature compared with primary human islets. There remains a challenge to establish a technology to create fully functional human islets in vitro, which are functionally and transcriptionally indistinguishable from cadaveric human islets. Understanding the complex differentiation and maturation pathway is necessary to generate fully functional human islets for a tremendous supply of high-quality human islets with less batch-to-batch difference for millions of patients. In this review, I summarized the current progress in the generation of 3D-structured human islets from pluripotent stem cells and discussed the importance of adapting physiology for in vitro functional human islet organogenesis and possible improvements with environmental cues.

Keywords: human islet-like organoids, nuclear receptors, physiology, human pluripotent stem cells, diabetes

INTRODUCTION

Diabetes is a complicated chronic disease that affects more than 450 million people worldwide (Cho et al., 2018; Huang et al., 2018; Rogers et al., 2020; Rogers and Kim, 2020; Shah et al., 2020; Smalls et al., 2020; Tan et al., 2020). Microvasculature defects caused by diabetes lead to stroke, blindness, and kidney failure in some patients (Creager et al., 2003; Luscher et al., 2003; Beckman et al., 2013; Paneni et al., 2013; Cayabyab et al., 2021). Although the discovery of insulin a century ago made significant improvements in diabetic therapeutics, from lethal to treatable, even modern insulin therapy cannot be an alternative to endogenous functional insulin-producing β cells. Insulin treatment requires manual glucose monitoring and adjustment of insulin injection in both amount and timing every day, which also means the high maintenance and economic impact for the patients and expenditure on healthcare in all countries. Continuous, rapid fine-tuning of glucose or other nutrition-sensing insulin secretion is necessary for functional cure of diabetes. Cadaveric islet transplantation provides a functional cure for insulin-dependent type 1 diabetes (T1D) with a success rate of more than 90%, and its efficacy has been sustained for over 20 years in some cases (Lemos et al., 2021). The short supply and requirement of immunosuppressants, which
increase the risk of adverse effects, limit the islet transplantation for the treatment of diabetes. Owing to their pluripotency and self-renewal function, human pluripotent stem cells (hPSCs), such as human induced pluripotent stem cells (hiPSCs) (Takahashi et al., 2007) and human embryonic stem cells (hESCs) (Thomson et al., 1998), offer an alternative cell resource for human cadaveric islets. Step-by-step treatment of small chemicals and recombinant proteins, which induce differentiation from hPSCs, was developed by integrating the knowledge of specific signaling pathways that contribute to the pancreatic lineage specification and islet differentiation. While recent advances in this approach have made it possible to reproducibly generate functional hPSC-derived insulin-producing β-like cells, which are capable of sensing glucose concentration and secreting insulin, there are several major limitations to our knowledge in generating fully functional human β cells or human islets. First, we have not yet clarified the pathway to induce each endocrine cell type separately. Although there are several reports on the production of more biased α cells rather than β cells from hPSCs (Rezania et al., 2011; Peterson et al., 2020), these established protocols do not generate a single-cell type but rather contain other cell types together with differentiation. Second, we have not completely understood the pathway of postnatal functional islet maturation, which typically occurs a few years after birth in humans. hPSC-derived insulin-producing cells are generated either by β-like cells (hPSC-derived β-like cells; monolayer or isolated insulin-producing cells) or human islet-like organoid clusters (hPSC-derived islets; α, β, γ-cells containing 3D-structured cell cluster). Therefore, the current protocol of generating fully functional hPSC-derived β-like cells or islets remains a challenge. Identifying such a pathway is necessary to improve the immaturity of generated islets and reduce the risk of batch-to-batch differences. In this review, I aimed to outline the current progress in generating functional human pancreatic islets from the perspective of 1) pancreatic lineage specification, 2) postnatal functional maturation, 3) differences between primary human islets and hPSC-derived islets, 4) missing factors, and 5) further adaptation of the environmental physiological responses (Figure 1). Additionally, I aimed to discuss future perspectives to improve the current protocol for generating functional human islets from hPSCs.

**Pancreatic Lineage Specification**

Since the establishment of hESCs (Thomson et al., 1998), insulin-producing β cells have been identified in spontaneously differentiated cells from hESCs (Lumelsky et al., 2001; Assady et al., 2001). In addition with the efforts to identify the essential
pathways for pancreatic lineage specification, efficient endodermal differentiation (D’Amour et al., 2005) and pancreatic progenitor (PP) differentiation (D’Amour et al., 2006) protocols have been developed. hESC- or hiPSC-derived PPs are capable to differentiate into mature functional β cells in vitro within a couple of months of transplantation (Kroon et al., 2008), suggesting that these protocols represent the right track for generating functional β cells in vitro. The key features of stage-specific differentiation are controlled by stage-specific transcriptional factors (TFs) and key identity genes (Tahbaz and Yoshihara, 2021). hPSCs undergo definitive endodermal (DE) differentiation by targeting the Activin A pathway and canonical Wnt (cWnt) pathway, Wnt3A. SRY-box transcription factor 17 (SOX17) and Forkhead box A2 (FOXA2) are prominent DE markers. Fibroblast growth factor 7 (FGF7/KGF) induces a primitive gut tube (PGT). Hepatocyte nuclear factor 1α (HNF1α) and hepatocyte nuclear factor-4α (HNF4α) are well-known PGT markers. Retinoic acid (RA) signaling activation, combined with sonic hedgehog (SSH) inhibition, bone morphogenetic protein (BMP) signal inhibition, and protein kinase C (PKC) activation, leads to PPs. PPs are pancreatic and duodenal homeobox 1 (PDX-1) and NK6 homeobox 1 (NKX6-1) double-positive cells, which can further generate mono-hormonal β cells, α cells (glucagon producing), and δ cells (somatostatin producing). RaAs activation, SSH inhibition, BMP signal inhibition, transforming growth factor β (TGFβ) signal inhibition, and thyroid hormone signaling activation (THR) lead to endocrine progenitor (EP) generation. NEUROG3 (NGN3) is the key transcriptional factor of EP. It has been recently observed that targeting the cytoskeleton by inhibiting actin polymerization enhances NGN3 expression in the endocrine stage (Mamidi et al., 2018; Hogrebe et al., 2020). γ-secretase inhibition, BMP signal inhibition, TGFβ signal inhibition, and THR activation lead to the generation of endocrine hormone-producing cells (α, β, and γ cells). Premature glucose-response β-like cells are generated (Pagliuca et al., 2014; Rezania et al., 2014; Russ et al., 2015). Although these previous studies established reproducible GSIS, impaired glucose-stimulated Ca2+ flux, reduced production of insulin, and many transcriptional differences have been observed in hPSC-derived β-like cells compared to those of primary β cells or islets (Veres et al., 2019; Augsornworawat et al., 2020; Yoshihara et al., 2020; Balboa et al., 2022). These results suggested that hPSC-derived β-like cells may remain a feature of the juvenile/neonatal stage of β cells (Hrvatin et al., 2014).

Postnatal Functional Maturation

Increasing evidence suggests that multiple functional maturation pathways play an important role in the postnatal acquisition of robust GSIS function (Alvarez-Dominguez and Melton, 2021). Therefore, the current focus on improving the protocol for generating functional islets relies on the identification of the new pathways for functional maturation. Islet functional maturation is accelerated after birth to adapt to oxygen- and nutrition-rich environments. There are a number of transcriptional factors and key β-cell marker gene expressions such as INSULIN (INS), MAF bZIP transcription factor A (MAFA), SIX homeobox 2 (SIX2), glucose-6-phosphatase catalytic subunit 2 (G6P2), and urocortin 3 (UCN3), which are lower in hPSC-derived β-like cells generated in vitro (Balboa et al., 2022). Chromatin remodeling and miRNA expression have been suggested to control the gene expression for functional β-cell maturation (Xie et al., 2013; Dhawan et al., 2015; Jacovitti et al., 2015). MAFA expression, regulated by T3 and other unknown signals, is important for INS and G6P2C2 transcription (Martin et al., 2008; Artner et al., 2010; Aguayo-Mazzucato et al., 2011; Hang and Stein, 2011). G6P2C2 expression suppresses low-glucose concentration-stimulated insulin secretion, leading to amplification of the GSIS function. MAF BZIP transcription factor B (MAFB) alternatively regulates INS gene expression in human β cells (Russell et al., 2020). Metabolic shift triggered by weaning enhances GSIS (Jacovitti et al., 2015; Stolovich-Rain et al., 2015). SIX2 is important for sustaining key β-cell lineage and metabolic genes (Arda et al., 2016; Velazco-Cruz et al., 2020a; Bevacqua et al., 2021). ATP and other nutrients, including glucose, have been reported to enhance the maturity of juvenile/neonate β cells in rodents (Rorsman et al., 1989; Hellerstrom and Swenne, 1991). We have previously demonstrated that estrogen-related receptor-γ (ERRγ) and related metabolic genes are upregulated during postnatal development in mouse islets (Yoshihara et al., 2016). ERRγ directly regulates gene clusters related to mitochondrial pyruvate metabolism, tricarboxylic acid (TCA) cycle, and oxidative phosphorylation (OxPhos), causing enhanced glucose-stimulated ATP production and insulin secretion (Yoshihara et al., 2016; Ahmadian et al., 2018; Fan et al., 2018). These metabolic reprogramming may be postnatally coordinated by the non-canonical WNT pathway (ncWNT4) (Bader et al., 2016; Yoshihara et al., 2020). Flattop (Fltp), a receptor of Wnt4, is upregulated postnatally in murine β cells, and Wnt4 enhances the maturation of β cells in mice and humans. We demonstrated that Wnt4 expression is significantly lower in murine neonatal islets or hPSC-derived β-like cells than that in murine adult β cells or primary adult human islets (Bader et al., 2016; Yoshihara et al., 2016; Yoshihara et al., 2020). Additionally, Wnt4 treatment enhances oxidative features and GSIS function in hPSC-derived 3D-structured human islet-like organoids (HILOs). These studies indicate that not only lineage commitment but also metabolic adaptation is important for enhancing the functional maturation of hPSC-derived β-like cells or islets. Other lineage-dependent transcriptional factors (LDTFs) and signal-dependent transcriptional factors (SDTFs), which collaboratively modulate functional maturation, have been discussed elsewhere (Wortham and Sander, 2021). The β-cell function is enhanced through aging in rodents, and p16 plays a key role in reducing cell proliferation and enhancing GSIS function. Circadian rhythms acquired during development also play an important role in β-cell maturation (Alvarez-Dominguez et al., 2020). Recent advances in single-cell technologies have enabled recapturing of the gene clusters involved in the differentiation and in vivo maturation of hPSC-derived β-like cells (Veres et al., 2019; Augsornworawat et al., 2021; Balboa et al., 2022). Several key β-cell genes in hPSC-derived β-like cells, including INS, MAFA, G6P2C2, and UCN3,
have been observed to be expressed in equivalent amounts only after in vivo maturation (Veres et al., 2019; Augsornworawat et al., 2021; Balboa et al., 2022). From these studies, the key changes during functional β-cell maturation seem to be advanced lineage commitment, metabolic reprograming to a more oxidative status with efficient glucose utilization, cell cycle reduction, and adaptation to environmental physiological changes in vivo.

Differences Between Primary Human Islets and hPSC-Derived Islets

Although there has been a better understanding of the pancreatic differentiation, islets maturation, and the technologies mimicking human organogenesis, there are many differences between the primary human islets supplied by the human donors and the generated hPSC-derived islets. Generally, hPSC-derived islets exhibit lower insulin production and GSIS fold changes than adult human primary islets (Pagliuca et al., 2014; Rezania et al., 2014; Velazco-Cruz et al., 2020b; Yoshihara et al., 2020). These functional differences may be insufficiently represented since the primary adult islets functionally differ among individuals, and the donor islets were typically shipped at 4°C overnight or a few days, which makes the condition of primary human islets unideal. This suggests that the functional differences between hPSC-derived islets and primary adult islets maybe even bigger than the ones described or thought of previously. In 2014, Hrvatin et al. (2014) revealed that the hPSC-derived β-like cells resemble human fetal β cells more transcriptionally rather than adult β cells. Remarkable technological advances have been made to create more glucose-responsive hPSC-derived β-like cells, which are capable of secreting insulin in response to high glucose, to produce mono-hormonal insulin-producing cells rather than poly-hormonal insulin-producing cells, and for improved differentiation efficacy to create insulin-producing cells to about ~70%. The insulin production levels are significantly higher than those in previous protocols (Pagliuca et al., 2014; Rezania et al., 2014; Russ et al., 2015). Although considerable progress has been made in creating functional islets from hPSCs, there are still significant differences between hPSC-derived β-like cells and human primary β cells. Ca2+ influx, which is triggered by the closure of the KATP channel in response to ATP amplification typically induced by glucose metabolism in the mitochondria of hPSC-derived β-like cells, is much slower and abnormal compared with that in the primary human islets. This is linked to the slower and fewer insulin secretion responses in hPSC-derived β-like cells (Pagliuca et al., 2014; Rezania et al., 2014; Nair et al., 2019). Patch-clamp recordings revealed heterogeneity and aberrant ion current such as Na+ (Balboa et al., 2022). Aberrant metabolic features in hPSC-derived β-like cells or islets, such as glycolysis, TCA cycle, and OxPhos, were observed and demonstrated as the target pathways to improve GSIS (Yoshihara et al., 2016; Nair et al., 2019; Davis et al., 2020; Balboa et al., 2022). It has been observed that longer culturing time of hPSC-derived islets at the maturation stage in vitro improves some marker gene expression and GSIS; however, it has been identified that in vivo engraftment for 6 months is necessary to promote further maturation (Veres et al., 2019; Augsornworawat et al., 2020; Balboa et al., 2022).

Current Progress and Missing Factors for Generating Fully Functional Human Islets

Dynamic Metabolic Circulation

Blood glucose levels are elevated postnatally triggered by weaning owing to the alternation of nutritional sources from fat-rich breast milk to a carbohydrate-rich chow diet (Stolovich-Rain et al., 2015). Glucose has been identified as a maturation factor in rodent β cells (Hellerstrom and Swenne, 1991). Therefore, the current protocol for in vitro β-cell generation is intended to increase glucose concentration during differentiation from the PP stage to the β-cell maturation stage (Yoshihara et al., 2020). The bottleneck of glycolysis (Davis et al., 2020) or mitochondrial metabolism (Yoshihara et al., 2016) in hPSC-derived β-like cells may limit the glucose-induced maturation efficacy. Improving β-cell metabolism has been highlighted as the target for improving maturation. We have previously demonstrated that forced expression of estrogen-related receptor γ (ERRγ), a master regulator of mitochondrial gene expression, including pyruvate metabolism, the TCA cycle, and OxPhos improves GSIS in hPSC-derived β-like cells (Yoshihara et al., 2016). Reaggregation (Nair et al., 2019) or the ncWnt4/planar cell polarity (PCP) pathway (Bader et al., 2016; Yoshihara et al., 2020) enhances mitochondrial metabolism, which enhances GSIS function in hPSC-derived β-like cells. ncWnt4/PCP effector Flattop (Fltp) expression increases during postnatal β cell maturation and is heterogeneously expressed in mature β cells. Fltp-positive (Fltp+) β cells are characterized by higher mitochondrial gene expression and activity (Bader et al., 2016). Wnt4, a component of ncWnt pathway increases during postnatal β cell maturation, and Wnt4 expression is lower in hPSC-derived β-like cells than that in the primary human β cells. Wnt4 stimulation enhances expression in mitochondrial gene expression and activity and GSIS function in hPSC-derived human islet-like organoids (HILos). We have reported that ERRγ is a downstream factor of Wnt4 signaling to regulate mitochondrial gene expression and activity (Yoshihara et al., 2020). Declining mammalian target of rapamycin complex 1 (mTORC1) signaling from neonate to adult maturation has been observed (Ni et al., 2017; Jaafar et al., 2019; Helman et al., 2020). The constitutively active mTORC1 pathway in mouse β cells increases β-cell proliferation while reducing β cells’ maturation gene expressions such as Ins1, Ucn3, Mafa, and G6pc2 and mitochondrial metabolic genes (Jaafar et al., 2019). mTORC1 enhances the responses of amino acid-stimulated insulin secretion, which increases insulin secretion at low glucose concentrations and reduces GSIS thresholds (Helman et al., 2020). In addition to mitochondria metabolism, glycolysis is impaired in hPSC-derived β-like cells, which limits the
pyruvate and related fuel mitochondrial metabolite production. The enzymatic activity, but not the expression of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and phosphoglycerate kinase 1 (PGK1), which are important for the enzymatic conversion of glyceraldehyde 3-phosphate to 3-phosphoglycerate in glycolysis in hPSC-derived β-like cells, is defective (Davis et al., 2020). This bottleneck of enzymatic activity slows down the glycolysis activity, limiting the production of phosphoenolpyruvate to fuel ATP generation through the mitochondria. Cell-permeable intermediate metabolites such as methyl-succinate and methyl-pyruvates partially rescue the GSIS defect in hPSC-derived β-like cells. Since most of the hPSC-derived β-like cells generated in vitro present significantly lower maximum respiration of the mitochondria, efficient supply of the mitochondria and improvement of the mitochondria are some of the key features of immature hPSC-derived β-like cells.

Dynamic continuous nutritional exchange is the natural condition for endogenous functional β-like cells; however, it has not been replicated for in vitro-cultured hPSC-derived β-like cells. The current protocol relies on feeding nutrition-rich media during β-like cell differentiation every day or every other day. In this setting, glucose is utilized without refill for more than 24–48 h and waste such as lactate from the cells continues to accumulate in the culture condition. Lactate and pyruvate transporters, such as monocarboxylate transporter 1 (MCT1), are disallowance genes in functional β cells, and their expression has been observed in neonates or hPSC-derived β-like cells (Yoshihara et al., 2016). This notion raises the concern that accumulated lactate not only oxidizes the media but is also re-absorbed in the hPSC-derived β-like cells to disrupt the proper metabolic activity and differentiation in β cells. In addition, a recent study found that hPSC-derived islets are hyper-sensitive to pyruvate-induced insulin secretion, which typically is not observed in primary adult islets (Balboa et al., 2022). Aberrant pyruvate transporter expression may contribute to this abnormal phenotype of hPSC-derived islets. In vivo-mimicking continuous nutritional exchange by techniques such as microfluidic perfusion (Jun et al., 2019) may aid in improving the metabolic disorder in hPSC-derived β-like cells.

**Oxygen Supply**

In addition to nutrition, oxygen is a critical factor responsible for the functionality of β cells. Mature islets are surrounded by microvessels, which provide circulating oxygen tension (approximately 100 mmHg) (Jansson and Hellerstrom, 1983). It has been observed that in vitro-cultured human islet viability, especially hypoxia-induced necrosis in the center of islets, is improved by hyperoxia (approximately 350 mmHg) (Komatsu et al., 2016; Komatsu et al., 2017). Since there is no oxygen supply from microvessel in vitro-cultured hPSC-derived islets, current typical cell culture conditions, 5% CO₂, 20% O₂ (approximately 160 mmHg), may not be ideal for differentiation and maintenance. The inner cell mass of blastocyst is likely to maintain low O₂ condition (approximately 5% O₂) (Rodesch et al., 1992; Clark et al., 2006; Harvey, 2007; Lees et al., 2019). This is reasonable since hPSC metabolism is characterized by being highly dependent on glycolysis and up to 70% of glucose is converted to lactate (Lees et al., 2019). During differentiation and maturation, this metabolism is shifted to a more oxidative mitochondria-dependent process (Varum et al., 2011). High O₂ levels (approximately 35% O₂) promote the growth of ex vivo-cultured mouse pancreatic buds (Fraker et al., 2007). Similarly, rat pancreata cultured with high O₂ (60–80% O₂) promotes β-cell differentiation (Heinis et al., 2010). Mechanistically, hypoxia activates hypoxia-inducible factor 1α (HIF1α), which in turn activates the hairy and enhancer of split (HES1) to prevent β-cell differentiation (Heinis et al., 2010). High O₂ (60% O₂) exposure between the endoderm and EP stage facilitated differentiation of EPs by activating cWnt signaling and suppressing HIF1α and HES1 expression, which leads to the promotion of NGN3 expression in the insulin-producing cell differentiation model of mouse and human ES cells (Cechin et al., 2014; Hakim et al., 2014). In contrast, an excessive amount of O₂ may increase the reactive oxygen species (ROS) and cause oxidative stress which triggers β-cell dysfunction (Robertson et al., 2004; Gerber and Rutter, 2017). Both hypoxia and oxidative stress/ROS induce thioredoxin-interacting protein (Txnip), a major cause of β-cell dysfunction in diabetes (Yoshihara et al., 2010a; Yoshihara et al., 2010b; Yoshihara et al., 2014; Yoshihara, 2020). Therefore, rather than a continuous supply of excessive O₂, more dynamic regulation of O₂ supply should be considered to improve the current protocol for the differentiation of the β cells. Monitoring the dynamic O₂ supply will benefit the better differentiation and function of hPSC-derived β cells.

**Fibroblasts, Pancreatic Exocrine, and Immune Cell Interactions**

Islet microenvironments consist of pancreatic fibroblasts, acinar cells, vascular cells, and immune cells such as macrophages. Increasing evidence suggests that exocrine acinar cells play a critical role in maintaining islet function and defects in exocrine link to the pathogenesis of some types of diabetes (Rickels et al., 2020; Chiou et al., 2021). The pancreatic organ-matched mesenchyme promotes self-renewal of the hPSC-derived PP's without further direct differentiation (Sneddon et al., 2012). These studies suggest that the islet microenvironment created by non-endocrine cells contributes to islet development and functional regulation. Further investigation of the spatiotemporal interaction between the endocrine cells and non-endocrine cells in islets is required to identify the key pathway that facilitates islet differentiation and physiological activity during fetal and postnatal development.

**Peripheral Neuro-Vasculature System**

It has been widely recognized that central and peripheral neurons play an important role in regulating the exocrine and endocrine pancreas (Buijs et al., 2001; Chandra and Liddle, 2013). Neural projections from the dorsal motor neuron (DMN) in the brain stem innervate the pancreas and regulate enzymatic and hormonal secretions in the exocrine and endocrine systems, respectively. Thyrosine hydroxylase (TH)-positive sympathetic nerves have been detected in the fetal pancreas starting from the early stages, such as 8 weeks post-conception (w.p.c.) (Krivova et al., 2021). The sympathetic nerve innervation of mouse islets is
| Common name                    | Symbol | Abbreviation | Ligands                           | Function                                      | Human islets (FPKM) | Mouse islets (FPKM) | References                                      |
|-------------------------------|--------|--------------|-----------------------------------|-----------------------------------------------|---------------------|---------------------|------------------------------------------------|
| Thyroid hormone receptor-α    | NR1A1  | TRα          | Thyroid hormone                   | Upregulation MAFA, GCK gene expression.      | >10                 | >20                 | Aguayo-Mazzucato et al. (2013)                 |
| Thyroid hormone receptor-β    | NR1A1  | TRβ          | Thyroid hormone                   | Upregulation MAFA, GCK gene expression.      | >1                  | 2                   | Aguayo-Mazzucato et al. (2013)                 |
| Retinoic acid receptor-α       | NR1B1  | RARα         | Vitamin A, retinoic acids          | Endocrine differentiation.                   | >5                  | >5                  |                                                 |
| Retinoic acid receptor-β       | NR1B2  | RARβ         | Endocrine differentiation          | >0.5                                          | >0.4               |                     |                                                 |
| Retinoic acid receptor-γ       | NR1B3  | RARγ         | Endocrine differentiation          | >1                                            | 10                 |                     |                                                 |
| Peroxisome proliferator-activated receptor-α | NR1C1 | PPARα | Fatty acids, prostaglandins | Enhances fatty acid oxidation and insulin secretion | >3                  | >0.3               | Brun et al. (2015), Matthews et al. (2004), Stafford and Prince, (2002) |
| Peroxisome proliferator-activated receptor-β | NR1C2 | PPARβ | Enhances fatty acid oxidation and insulin secretion | >6                                            | >12                |                     | Perez et al. (2013), Fedullo et al. (2021), Stafford and Prince, (2002) |
| Peroxisome proliferator-activated receptor-γ | NR1C3 | PPARγ | Enhances fatty acid oxidation and insulin secretion | >0.3                                          | >5                 |                     | Laloyer et al. (2006), Zhou et al. (1998)     |
| Rev-ErbAα                      | NR1D1  | Rev-ErbAα    | Heme                              | Circadian oscillation                        | >14                 | >30                 | Tang et al. (2013)                             |
| Rev-ErbAβ                      | NR1D2  | Rev-ErbAβ    | Heme                              | Circadian oscillation                        | >20                 | >30                 |                                                 |
| RAR-related orphan receptor-α  | NR1F1  | RORa         | Cholesterol, retinoic acids        | Suppresses GSIS. Circadian oscillation        | >3                  | 1                   |                                                 |
| RAR-related orphan receptor-β  | NR1F2  | ROβ         | Cholesterol, retinoic acids        | Suppresses GSIS. Circadian oscillation        | >1                  | >0.01              |                                                 |
| RAR-related orphan receptor-γ  | NR1F3  | ROγ         | Cholesterol, retinoic acids        | Suppresses GSIS. Circadian oscillation        | >7                  | >10                 |                                                 |
| Liver X receptor-β             | NR1H2  | LXRβ        | Cholesterol                       | Enhances glycerol/fatty acids cycling         | >13                 | >30                 |                                                 |
| Liver X receptor-α             | NR1H3  | LXRα        | Cholesterol                       | Enhances glycerol/fatty acids cycling         | >2                  | >5                  |                                                 |
| Farnesoid X receptor-β         | NR1H4  | FXRβ        | Bile acids                        | Upregulation of INS, GLP1R gene expression    | 1                   | 9                   |                                                 |
| Farnesoid X receptor-β         | NR1H5  | FXRα        | Bile acids                        | -                                              |                      | >0.01              | Renga et al. (2010), Kong et al. (2019a), Kong et al. (2019b), Dufer et al. (2012), Kong et al. (2021) |
| Vitamin D receptor             | NR1I1  | VDR         | Vitamin D                         | -                                              | >3                  | >40                 | Wei et al. (2018)                              |
| Pregnane X receptor            | NR1I2  | PXR         | Xenobiotics                       | Unclear                                       | >0.01              | >0.05              |                                                 |
| Constitutive androstanone      | NR1I3  | CAR         | Androstanone                      | Unclear                                       | >0.1               | >0.03              |                                                 |
| Hepatocyte nuclear factor-4-α  | NR2A1  | HNF4α       | Fatty acids                        | Responsible gene for MODY1                    | >3                  | >10                 | Yamagata et al. (1996)                         |
| Hepatocyte nuclear factor-4-γ  | NR2A2  | HNF4γ       | Pancreatic differentiation         | >0.2                                          | 2                   |                     | Boj et al. (2001)                              |
| Retinoid X receptor-α          | NR2B1  | RXRα        | Retinoic acids                     | Pancreatic differentiation                    | >10                 | 12                  | Kane et al. (2010), Kadison et al. (2001)     |
| Retinoid X receptor-β          | NR2B2  | RXRβ        | Pancreatic differentiation         | >18                                            | >18                 |                     | Kane et al. (2010), Kadison et al. (2001)     |
| Retinoid X receptor-γ          | NR2B3  | RXRγ        | Pancreatic differentiation         | >5                                             | >0.1               |                     | Kane et al. (2010), Kadison et al. (2001)     |
| Testicular receptor 2          | NR2C1  | TR2         | -                                 | Unclear                                       | >3                  | >3                  |                                                 |
| Testicular receptor 4          | NR2C2  | TR4         | -                                 | Unclear                                       | >6                  | >6                  |                                                 |

References (Continued on following page)
### TABLE 1 | (Continued) Physiological role of nuclear receptors in functional β cells.

| Common name                                                                 | Symbol     | Abbreviation | Ligands                | Function                                                                 | Human islets (FPKM) | Mouse islets (FPKM) | References                                                                 |
|----------------------------------------------------------------------------|------------|--------------|------------------------|-------------------------------------------------------------------------|---------------------|---------------------|---------------------------------------------------------------------------|
| Homolog of the Drosophila tailless gene                                      | NR2E1      | TLX          | -                      | Enhances β-cell proliferation                                           | >0.01               | -                   | Shi et al. (2019), Shi et al. (2015)                                       |
| Photoreceptor cell-specific nuclear receptor                                 | NR2E3      | PNR          | -                      | Unclear                                                                 | >0.01               | >0.01               | -                                                                         |
| Chicken ovalbumin upstream promoter-α                                        | NR2F1      | COUP-TFα     | -                      | Negatively regulates the mouse INS2 gene                               | >0.5                | >0.3                | Zhang et al. (2017), Boutant et al. (2012), Qin et al. (2010), Perilhou et al. (2008), Bardoux et al. (2005) |
| Chicken ovalbumin upstream promoter-β                                        | NR2F2      | COUP-TFβ     | -                      | Positively regulates β-cell proliferation, islet tumorigenesis         | >6                  | >1                  | Qin et al. (2010), Boutant et al. (2012)                                   |
| Chicken ovalbumin upstream promoter-γ                                        | NR2F6      | COUP-TFγ     | -                      | Unclear                                                                 | >25                 | >23                 | -                                                                         |
| Estrogen receptor-α                                                          | NR3A1      | ERα          | Estrogens              | Regulates insulin synthesis. Suppresses lipid synthesis                | >1                  | >0.03               | Alonso-Magdalena et al. (2008), Wong et al. (2010), Tiao et al. (2011)   |
| Estrogen receptor-β                                                          | NR3A2      | ERβ          | -                      | Regulates insulin synthesis. Suppresses lipid synthesis                | >0.01               | >0.02               | Tiao et al. (2011), Wong et al. (2010), Alonso-Magdalena et al. (2008)   |
| Estrogen-related receptor-α                                                  | NR3B1      | ERRα         | -                      | Unclear/possibly upregulates mitochondrial gene expression and enhances oxidative metabolism | >23                 | >10                 | -                                                                         |
| Estrogen-related receptor-β                                                  | NR3B2      | ERRβ         | -                      | Unclear                                                                | >0.1                | >0.1                | -                                                                         |
| Estrogen-related receptor-γ                                                  | NR3B3      | ERRγ         | -                      | Upregulates mitochondrial gene expression and enhances oxidative metabolism and postnatal maturation | >2                  | >2                  | Yoshihara et al. (2016), Yoshihara et al. (2020)                          |
| Glucocorticoid receptor                                                      | NR3C1      | GR           | Cortisol               | Promotes pancreatic differentiation, Induces apoptosis                | >14                 | >17                 | Aylward et al. (2021), Ghazalli et al. (2018), Reich et al. (2012), Matthews and Hanley, (2011), Ranta et al. (2006), Gesina et al. (2006), Shapiro et al. (2000) |
| Mineralocorticoid receptor                                                   | NR3C2      | MR           | Aldosterone            | Enhances insulin secretion through a-cell GLP1 secretion              | >5                  | >2.5                | Goto et al. (2019)                                                        |
| Progesterone receptor                                                        | NR3C3      | PR           | Progesterone           | Negatively regulates β-cell proliferation                             | >2                  | >0.05               | Picard et al. (2002)                                                       |
| Androgen receptor                                                            | NR3C4      | AR           | Testosterone           | Enhances GSIS                                                          | >0.2                | >0.1                | Navarro et al. (2016)                                                      |
| Nerve growth factor 1                                                        | NR4A1      | NGF          | -                      | Positively and negatively regulates β-cell proliferation              | >2                  | >15                 | Yu et al. (2015), Tessem et al. (2014), Briand et al. (2012)               |
| Nuclear receptor related 1                                                   | NR4A2      | NURR1        | -                      | Positively regulates β-cell proliferation                             | >1                  | >1                  | Close et al. (2019)                                                        |
| Neuron-derived orphan receptor 1                                             | NR4A3      | NOR1         | -                      | Positively and negatively regulates β-cell proliferation              | >0.5                | >0.05               | Tessem et al. (2014), Briand et al. (2012), Close et al. (2019)            |
| Steroidogenic factor 1                                                       | NR5A1      | SF-1         | Phosphatidylinositols  | Unclear                                                               | -                   | -                   | Hale et al. (2014), Baque et al. (2018), Cobo-Vulleumier et al. (2018)    |
| Liver receptor homolog-1                                                     | NR5A2      | LRH-1        | -                      | Pancreas organogenesis. Protects from stress-induced β-cell apoptosis  | >2                  | >0.4                | -                                                                         |
| Germ cell nuclear factor                                                     | NR6A1      | GONF         | -                      | Unclear                                                               | >0.2                | >2.5                | -                                                                         |
| Dosage-sensitive sex reversal, adrenal hypoplasia critical region, on chromosome X gene 1 | NR0B1     | DAX1         | -                      | Unclear                                                               | >17                 | -                   | -                                                                         |
| Small heterodimer partner                                                    | NR0B2      | SHP          | -                      | Negatively regulates β-cell survival                                 | >7                  | >0.01               | Noh et al. (2013), Seo et al. (2008), Park et al. (2007), Suh et al. (2004) |
reported to be a critical factor for proper islet architecture and function in a β-adrenergic dependent manner (Borden et al., 2013). Although how the innervation of peripheral nerves in human islets is important for organizing islet architecture, development, and function is still controversial or remains unknown, many receptors for neurotransmitters are expressed in human islets and regulate hormone secretion (Ahren, 2000; Burns et al., 2015; Rosario et al., 2016; Lin et al., 2021).

Paracrine Regulation
Islets are multicellular mini-organs consisting of differential hormone-expressing cells with paracrine hormone regulation (Huising, 2020). A cells secrete glucagon and glucagon-like peptide-1 (GLP-1), which enhance insulin secretion in β cells. δ cells secrete somatostatin, which suppresses insulin secretion in β cells and glucagon secretion from α cells (Strowski et al., 2000). Insulin inhibits glucagon secretion by stimulating somatostatin secretion by sodium-glucose co-transporter-2 (SGLT2) (Vergari et al., 2019). UCN3, co-secreted with insulin, also stimulates somatostatin secretion from δ cells to enhance paracrine feedback regulation of β cells (van der Meulen et al., 2015). Although this paracrine hormone regulation plays an important role in proper insulin secretion in islets, the effect of paracrine signaling on islet differentiation and maturation has not yet been elucidated.

Organ–Organ Interaction
Islets are embedded in the exocrine pancreas while through the nervous innervation and vasculature network receiving the signals from outside the pancreas. The intestine plays a central role in regulating β-cell function through the enteroincretory hormone secretion directly or via the vagus nerve–brain–pancreas axis. Adipokines and hepatokines also play an important role in β-cell function (El Ouaamari et al., 2013; Prentice et al., 2021). These signals from outside the pancreas influence the differentiation and maturation of islets remain poorly understood. Since the organs are simultaneously differentiated and mature during fetal and postnatal development, it is possible that organ–organ interactions also play an important role in islet differentiation and maturation.

Adapting Physiology
Adapting to physiological environmental stimuli is a hallmark of mature adult organs, which are broadly regulated by nuclear hormone receptor (NR) signaling (Evans and Mangelsdorf, 2014). NR, a superfamily of 48 transcriptional factors in humans, regulates gene expression in response to various hormones, lipids, and metabolites and plays a master role in development and physiology (Evans and Mangelsdorf, 2014). Many NRs are expressed in rodent and human islets and are related to the β-cell physiology in response to lipid metabolism (Chuang et al., 2008) (Table 1). Free fatty acids, such as palmitate, stimulate insulin secretion for the short term, whereas chronic exposure to high concentrations of free fatty acids induces compensatory β-cell expansion and eventually leads to β-cell growth arrest and apoptosis. Nuclear receptor subfamily 4 group A member 1 (NR4A1) is translocated to the nucleus in response to free fatty acids and plays a key role in lipotoxicity-induced β-cell apoptosis (Susini et al., 1998; Roche et al., 1999; Briand et al., 2012; Yu et al., 2015; Close et al., 2019). Mechanistically, NR4A1 binds to Foxo1 at the MafA promoter region and suppresses its expression to reduce insulin transcription (Briand et al., 2012). NR4A1 is required for Nkx6-1-mediated β-cell mass expansion (Tessem et al., 2014). Cholesterol sensor Liver X receptor (LXR; LXRα and LXRβ) stimulates GSIS and insulin biosynthesis (Efano et al., 2004; Zitzer et al., 2006). LXRβKO mice exhibit a glucose-intolerant phenotype secondary to blunted GSIS in vivo (Gerin et al., 2005; Zitzer et al., 2006). Peroxisome proliferator-activated receptors (PPARs; PPARγ, PPARα, and PPARδ) broadly regulate fatty acid oxidation, thereby enhancing GSIS and reducing lipotoxic effects in β cells. Estrogen receptors (ERs; ERα and ERβ) also protect against lipotoxicity, especially in females. Testosterone-mediated andorrogen receptor (AR) signaling enhances GSIS and glucagon-like peptide-1 (GLP-1)-mediated insulin secretion in males (Navarro et al., 2016). Vitamin D receptor (VDR) regulates SWI/SNF chromatin remodeling together with bromodomain-containing protein 7 (BRD7) and BRD9, and protection from cytokines induces dedifferentiation of β cells in rodents and hPSC-derived β-like cells (Wei et al., 2018). Estrogen-related receptor γ (ERRγ) regulates mitochondrial gene expression related to the TCA cycle and OxPhos (Yoshihara et al., 2016). Farnesoid X receptors (FXRs; FXRa and FXRβ) regulate Ins (Renga et al., 2010), adenylyl cyclase 8 (Adcy8) (Kong et al., 2019a), transient receptor potential ankyrin 1 (Trpa1) (Kong et al., 2019b), and GLP-1 receptor (Glp1r) (Kong et al., 2021) gene expression and protect from lipotoxicity (Popescu et al., 2010) in the rodents. Glucocorticoids and steroids are known to induce hyperglycemia, which may cause diabetes. Glucocorticoid receptor (GR) signaling through dexamethasone was initially believed to have a negative impact on islet physiology by reducing insulin synthesis and insulin secretion and inducing apoptosis in murine β cells (Philippe and Missotten, 1990; Goodman et al., 1996; Ranta et al., 2006; Reich et al., 2012; Alyward et al., 2021). In contrast, GR plays an essential role in human fetal and adult islet development by controlling β-cell transcriptionomes, such as PDX-1, GCK, GLUT2, SIX2, and SIX3 (Gesina et al., 2006; Matthews and Hanley, 2011; Ghazalli et al., 2018). Thyroid hormone receptors (TRs; TRα and TRβ) regulate MafA expression during postnatal rat β-cell maturation (Aguayo-Mazzucato et al., 2013). Hypothyroidism impairs hPSC-PP maturation in mice suggesting the importance of TRs signaling in vivo functional β-cell maturation (Bruin et al., 2016). Circadian rhythms regulate β-cell maturation and physiology (Marcheva et al., 2010; Perelis et al., 2015; Alvarez-Dominguez et al., 2020). The clock protein REV-ERβ regulates the circadian rhythms in β cells, and downregulation of REV-ERβa impairs time-dependent GSIS (Vieira et al., 2012). REV-ERβ may be an important physiological regulator of β cells in time-restricted feeding or preventing pathogenesis of T2D by modulating β-cell rhythmic transcriptional regulation by adapting to environmental changes over time (Petrenko et al., 2020; Brown et al., 2021). Hepatic nuclear receptor 4−α (HNF4α) is responsible for maturity-onset diabetes of the young 1 (MODY1) (Yamagata et al., 1996).
Although NRs integrate gene regulation and cell function to adapt to environmental physiological stimulation, the contribution of NRs to functional maturation to adapt to environmental stimulation in islets is largely unknown. Understanding how these factors cooperatively regulate β-cell differentiation and physiological adaptation may improve generation of a physiological level of functional β cells.

CONCLUSION

I discussed the existing limitations of the technology to generate human islets from pluripotent stem cells. The current protocol is generated on the basis of lineage specification; however, sufficient number of integrated functional maturation pathways are not there. The challenge remains to create cells with protocols within a few weeks of additional maturation, which exhibit equal functionality with primary human islets in terms of 1) amount of insulin secretion, 2) speed and accuracy of response to glucose, 3) transcriptome, and 4) controlled differentiation to individual endocrine cell types by integrating the new pathways adapting to environmental physiological stimulation. The key challenge in the field is that maturation may not only be coordinated by single signaling but corporately regulates by multiple signaling as the environmental sensor switch (Figure 1). Additionally, the required signaling may not be constitutively activated or suppressed; however, it is dynamically regulated by adapting to environmental physiological situations to work flexibly and properly in the spatiotemporal physiological situation. The future challenge of developing new technology should focus on dissecting many differential signaling and transcriptional networks that are involved in postnatal functional maturation and integrating how those signaling pathways synergistically regulate islet maturation. The robustness of scalability, consistency of high quality, and less batch-to-batch generation of different human islets in vitro should be continuously improved. One important notion for adapting to environmental signals is the duration and timing of the stimulation of those signals. The current protocol generates glucose-responsive hPSC-derived β-like cells or islets within 4–10 weeks of culturing in vitro. However, it takes approximately 40 weeks of differentiation and a few years of postnatal functional islet maturation in human body. This significant gap in the developmental time may need to be considered adapting to environmental cues in functional human islet organogenesis. A deeper understanding of islet differentiation and maturation is critical for generating fully functional human islets in vitro. Integrating the microenvironmental cues that mimic in vivo situations may help in further improving the protocol.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

EY conceptualized and wrote the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcell.2022.854604/full#supplementary-material
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Conflict of Interest: EY is an inventor on licensed patents and patent applications related to the HILO technology described in this manuscript.

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