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Stem Cell Based Models in Congenital Hyperinsulinism – Perspective on Practicalities and Possibilities

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Congenital hyperinsulinism (CHI) is a severe inherited neonatal disorder characterized by inappropriate insulin secretion caused by genetic defects of the pancreatic beta cells. Several open questions remain in CHI research, such as the optimal treatment for the most common type of CHI, caused by mutations in the genes encoding ATP-sensitive potassium channels, and the molecular mechanisms of newly identified CHI genes. Answering these questions requires robust preclinical models, particularly since primary patient material is extremely scarce and accurate animal models are not available. In this short review, we explain why pluripotent stem cell derived islets present an attractive solution to these issues and outline the current progress in stem-cell based modeling of CHI. Stem cell derived islets enable the study of molecular mechanisms of CHI and the discovery of novel antihypoglycemic drugs, while also providing a valuable model to study the biology of variable functional states of beta cells.

Keywords: congenital hyperinsulinism, stem cell derived islets, disease modeling, drug screening and discovery, insulin secretion, hypoglycemia, genetic defects

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INTRODUCTION

Congenital hyperinsulinism (CHI), characterized by inappropriate insulin secretion from the pancreatic beta cells, is the most common cause of persistent childhood hypoglycemia. At least 15 causative genes have been identified (1), with 30-55% of patients remaining without a genetic diagnosis (2–5). Over 50% of all CHI patients (2, 5) carry a recessive loss-of-function mutation in the K_{ATP}-channel genes *ABCC8* or *KCNJ11* (K_{ATP}HI), which leads to abnormal membrane depolarization and constitutive insulin secretion. Clinically, this leads to severe hypoglycemia for which there is no optimal treatment. This represents an ongoing clinical challenge as the hypoglycemia is life-threatening in the first days of life and despite best contemporary treatment, many continue to suffer from learning difficulties in the long term (6–10). A robust preclinical model would be required for studies aiming to discover improved treatment options for K_{ATP}HI and to pinpoint molecular mechanisms of rare newly identified forms of CHI.

Pluripotent stem cells (PSCs) represent the epiblast cells of the early embryo, capable of differentiation to any cell type in the human body. Tissue differentiated from PSCs holds enormous

promise in regenerative medicine to replace or repair a damaged or degenerated organ. PSCs can also serve as a powerful research tool by allowing limitless generation of difficult-to-procure tissue and would thus serve as an attractive solution for preclinical study of CHI. Pluripotent stem cell derived islets (SC-islets) could replace or complement rodent models and primary patient islet tissue, which both have important inherent weaknesses. Rodent islets are structurally and physiologically different from human islets (11–14) and these differences have manifested in K_{ATP}-channel knockout mouse models, which have presented with a much milder phenotype than the K_{ATP}HI patients (15–17). The availability of healthy primary islets is limited and faces issues of variable *in vitro* function (www.epicore.ualberta.ca/isletcore/). The availability of CHI patient islets presents an additional challenge due to the rarity of the disease. The limited tissue availability challenges any study that requires large amounts of tissue, such as screening for novel pharmacotherapeutics.

This review focuses on the use of PSC-derived pancreatic islets (SC-islets) for preclinical study of congenital hyperinsulinism. We outline the practical necessities in setting up SC-islet models and aim to identify relevant questions for CHI research where the SC-islets are particularly powerful.

SC-ISLET BASED DISEASE MODELING: KEY TECHNOLOGIES AND CURRENT PROGRESS

Genome Editing of Pluripotent Stem Cells

Pluripotent stem cells (PSCs) are derived from two main sources: preimplantation embryos (embryonic stem cells, ESCs) (18) and somatic cells that have been reprogrammed back to pluripotent state by overexpression of key genes (induced pluripotent stem cells, iPSCs) (19). iPSCs reprogrammed from a patient sample carry the disease-causing mutations of that individual and should thus phenocopy the disease, such as CHI, when differentiated. A similarly differentiated healthy iPSC line would serve as a non-isogenic control for this type of approach. Theoretically, this offers a disease model without the need for genome editing. In practice however, the differences in the donor genetic background exert a high degree of influence on the differentiation efficiency of stem cell lines, at least in islet differentiation, making it difficult to conclude whether the detected differences between patient and healthy cell lines are due to the disease gene or a differentiation-related artefact. Thus, it is often more practical to correct the disease-causing mutation with genome editing tools to yield an isogenic control cell line. Isogenic controls offer a clean look into the disease phenotype without differences in the genetic background. Generating isogenic controls with genome editing of the PSCs can be considered as the ideal approach for the effective modeling of a genetic disease such as CHI.

Another approach to create isogenic cell lines is to engineer the disease-causing mutation in a healthy hPSC line. This approach is often more straightforward than the patient cell line approach because the differentiation protocol used to derive the SC-islets can be optimized for one cell line and all the interesting mutations can be engineered to it. Furthermore, from the genome editing point of view, it is easier to generate a knock-out than a knock-in. In correcting a patient line, a knock-in is always required, but for generating a disease cell line a simple knock-out is often enough since many diseases are caused by loss-of-function mutations.

Multiple technologies are available for the genome editing itself, but recently CRISPR-based technologies have started to dominate, due to their relative ease and high efficiency. The basic CRISPR system consists of a guide RNA, able to target the editing to a specific locus in the genome; a protein exhibiting nuclease activity such as Cas9 or Cas12a creating a double stand break; and an RNA template containing the mutation-corrected sequence or a sequence knocking out the healthy gene which can be read during homology directed repair of the double strand break. This basic system has been expanded and optimized further in many ways, as reviewed here (20). Regardless of the specifics, genome editing technology has matured to a state where generating disease relevant stem cell lines for further differentiation is practical.

Differentiation of PSCs to Islets

Due to the curative potential of PSCs in cell replacement therapy for insulin deficient diabetes, enormous effort has been expended in developing protocols that can drive PSCs to differentiate *in vitro* to pancreatic islets (SC-islets). Starting with the breakthrough protocol published in 2006, showing for the first time that insulin positive cells can be differentiated from hPSCs through steps mimicking normal development (21), the progress in the field has been rapid. First evidence of glucose-regulated insulin release was provided in 2014 (22, 23), Since then, many further improvements have been made (24–26) and in the past two years the first protocols giving robust, dynamic glucose stimulated insulin secretion have been reported, achieving beta cell maturity at least in terms of insulin secretory function (27–35). This recent progress in the field has identified multiple conditions related to optimal late-stage maturation. These include keeping SC-islets appropriately sized by resizing or by culture format, keeping the proliferation rate low, normoglycemic culture conditions, lack of ALK5-inhibition, addition of WNT4, circadian entrainment, and reducing the number of unwanted cells that might compromise function on the islet level by sorting or by addition of aurora kinase inhibitor.

Given the recapitulation of the adult function in the state-of-the-art protocols and the fact that the protocols used to derive the mature SC-islet use the same signaling cues as the fetal islets during their *in vivo* development, SC-islets represent an excellent avenue for modeling CHI pathophysiology, including both developmental and insulin secretory defects. We have shown that all the main components of the stimulus-secretion coupling machinery of beta cells: metabolic processing of glucose, currents of the critical ion-channels, the insulin-secretion modulating amplifying mechanisms and the exocytosis machinery, are present in SC-islets (30). As most forms of CHI manifest in

the neonatal period, achieving adult-like function might not even be necessary for some study questions. This is exemplified by existing SC-islet models for CHI, which have been successful even using less efficient differentiation protocols, as described in the following section.

Stem Cell-Based Models for CHI

Thus far, two studies have taken advantage of the SC-islet differentiation and genome editing technologies in modeling K_{ATP}-channel related CHI (K_{ATP}HI) (36, 37), as summarized in **Table 1**. Guo and colleagues (36), used healthy hESCs and introduced a knockout of the *ABCC8* gene, encoding the K_{ATP}-channel subunit SUR1. The *ABCC8* KO beta cells secreted around 2-fold more insulin *in vitro* and failed to respond to K_{ATP}-channel acting pharmaceuticals. Their beta-like cells could be inhibited with octreotide, and to a lesser degree with nicorandil and nifedipine. Thus, they replicated the K_{ATP}HI insulin secretion phenotype *in vitro*.

We used iPSCs derived from a patient carrying the homozygous V187D-mutation (38) in the *ABCC8* gene and compared them to mutation-corrected controls (37). The *ABCC8* mutant beta-like cells secreted around 3-fold more insulin in low glucose compared to the corrected counterparts. They also failed to respond to K_{ATP}-channel acting pharmaceuticals but could be inhibited with clonidine and EGTA. Upon transplantation and *in vivo* maturation under the kidney capsule of immunocompromised mice, the mutant grafts secreted 7-fold higher levels of human C-peptide and caused 38% lower blood glucose upon fasting than the control grafts. We could thus replicate the cardinal phenotypic features of K_{ATP}HI both *in vitro* and *in vivo*. In addition to these features of the secretory function, we found that the K_{ATP}-inactivation directed the development of endocrine cells towards beta cells at the expense of alpha cells in the *in vitro* differentiation. This may have been due to the increased proliferation we detected in the *ABCC8* mutant beta cells. The role of K_{ATP}-channel inactivation in proliferation was also found in a previous study using pancreas-derived mesenchymal stem cells derived from a K_{ATP}HI patient (39).

TABLE 1 | Summary of published studies on stem cell based modeling of congenital hyperinsulinism.

***In vitro* phenotype**	K_{ATP}-mutant vs. control
Studies 1&2	
Insulin secretion in low glucose	2-3 fold higher secretion
Response to diazoxide	No response vs. 50% reduction
***In vivo* phenotype**	
Study 2	
Fasting C-peptide	7 fold higher level in circulation
Fasting glucose	40% lower
Developmental phenotype	
Study 2	
Beta cell proportion	32% more beta cells
Beta cell proliferation	61% more proliferating beta cells

Study 1: Guo et al. Scientific Reports (36).
Study 2: Lithovius et al. Diabetologia (37).

POTENTIAL RESEARCH AVENUES FOR SC-ISLET CHI MODELS

Development of Pharmacotherapy for Diazoxide-Resistant CHI

The first-line antihypoglycemic drug used in CHI, diazoxide, is an opener of the K_{ATP}-channel and as such, most of the recessive K_{ATP}HI patients are unresponsive to it (40, 41). Second-line treatments, such as somatostatin receptor agonists are widely used, but still the most severe patients must undergo pancreatectomy to control hypoglycemia. This is a suboptimal treatment which rarely results in euglycemia (42–44). There is an obvious need for more effective antihypoglycemic medication for diazoxide-resistant CHI.

SC-islets can be generated in limitless quantities and when derived with state-of-the-art differentiation protocols (27, 29, 30), they harbor the stimulus-secretion coupling machinery of adult primary islets: the K_{ATP}-channel related triggering pathway, as well as the neurohormonal and metabolic amplifying pathways (30). This covers the key pathways that modulate insulin secretion and could thus serve as pharmacological targets.

Potential antihypoglycemic targets on the beta cell include ion-channels, G-protein coupled receptors and transcription factors controlling beta cell function, as summarized in **Figure 1**. Depending on the genetic cause of CHI, some of these might be more advantageous than others. In the most common type of diazoxide resistant CHI, K_{ATP}HI, insulin hypersecretion occurs because the K_{ATP}-channel is inactive leading to constitutive depolarization and constantly elevated intracellular calcium (45). In this intracellular environment, a molecule acting upstream of the K_{ATP}-channel in the stimulus-secretion coupling machinery (ie. glucose uptake, glycolysis and the TCA cycle) is likely to be ineffective. Potential antihypoglycemic ion-channel acting drugs would act by decreasing the membrane potential independently of the K_{ATP}-channel (ie. by acting on K^+, Na^+ and Cl^- channels) or by reducing intracellular Ca^{2+}, the final trigger of exocytosis. Nifedipine and other Ca^{2+} channel blockers have been used in the treatment of K_{ATP}HI but have proven ineffective (46). Sikimic and colleagues identified DCEBIO, acting as an agonist of the repolarizing $K_{Ca3.1}$ channel, as a molecule abolishing the glucose induced Ca^{2+} oscillations in two K_{ATP}HI islet preparations (47). A potential downside with ion-channel acting molecules is that many of the target channels are also expressed in non-islet cells, such as cardiomyocytes and neurons, increasing the likelihood of serious side effects.

Several islet specific G-protein coupled receptors (GPCRs) have been identified (48). The role of these receptors in beta cells is to fine-tune the glucose-stimulated insulin secretion by affecting the amplifying pathways. They exert their action through cAMP and other second messengers, which sensitize or desensitize insulin granule exocytosis. Human islets express around 300 GPCRs (49). Highly expressed ones include receptors for the other islet hormones, glucagon and somatostatin, and the gut-derived incretins, GLP1 and GIP, but many beta cell GPCRs also have no known ligand (orphan receptors). This offers the potential for novel antihypoglycemic compounds to be found by screening. Many of the most potent GPCR-coupled amplifying pathways,

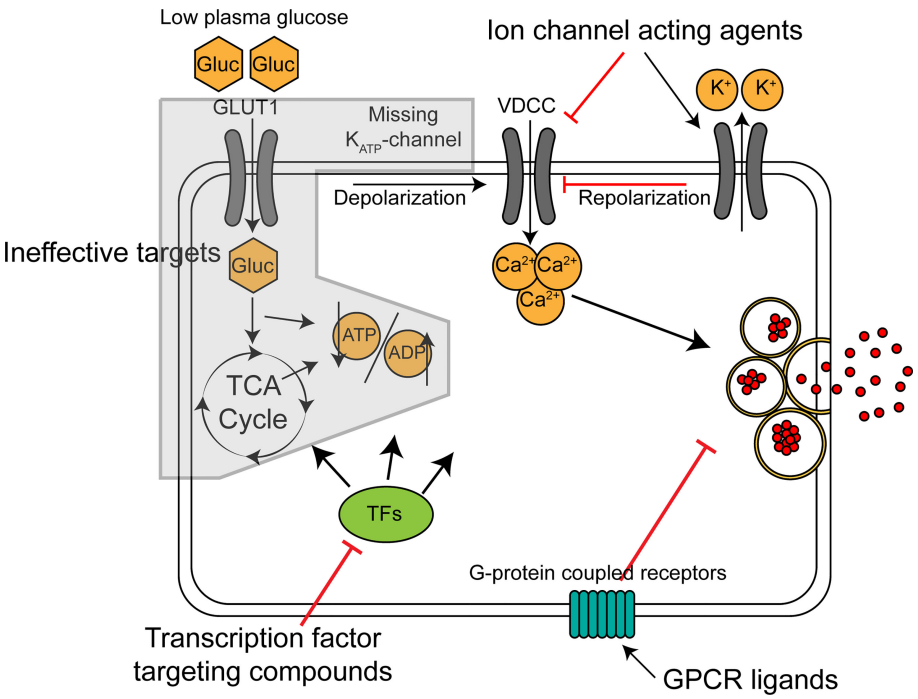

FIGURE 1 | Mechanism of action for classes of potential antihypoglycemic agents for treatment of K_{ATP}-channel related CHI. Broad, schematic depiction of insulin secretion machinery in K_{ATP}-channel deficient beta cells in low glucose. GLUT1, glucose transporter; Gluc, glucose; VDCC, voltage-dependent calcium channel; TCA cycle, tricarboxylic acid cycle; TFs, transcription factors.

such as the GLP1-receptor coupled pathway, are primarily stimulated only when the beta cells are already triggered by the K_{ATP}-channel closure and subsequent influx of calcium, restricting their activity mostly to high glucose conditions (50). The K_{ATP}HI cells are constantly in the state of high $[Ca^{2+}]_i$, which should allow the inappropriate activation of these pathways even in low glucose. Targeting GPCRs in K_{ATP}HI has been shown to be effective by the well-established use of somatostatin receptor agonists such as octreotide. More recently, studies by De León and associates have identified the GLP1-receptor inverse agonist exendin-(9-39) as an effective antihypoglycemic agent in K_{ATP}-KO mice (51) and in adult K_{ATP}HI patients (52).

Transcription factors controlling beta cell insulin secretion could serve as a third group of targets for antihypoglycemic medication. Senniappan and colleagues demonstrated the validity of this approach using mTOR inhibitor rapamycin on diazoxide-resistant CHI patients (53). Since the initial report however, the use of rapamycin has been questioned due to low efficacy and high incidence of serious side effects (54, 55). These issues are likely due to mTOR being an important regulator of wide variety of cellular processes in most tissues, and as such, an agent acting on a transcription factor more specifically controlling beta cell insulin secretion related gene expression would be more ideal. Despite the existence of several beta cell-specific transcription factors that control the expression of insulin secretory machinery genes (e.g., MAFA or RFX6), small molecules specifically targeting them are missing, and thus the ion-channel and GPCR-related strategies are likely to provide more accessible targets.

The most clinically relevant parameter for identifying the potential drug would be its effectiveness in reducing the CHI SC-islets' insulin secretion in low glucose. Measuring just intracellular calcium fluxes with a dye or a genetically encoded sensor could lead to failure in identifying a potential drug if the drug acts on the amplifying mechanisms of insulin secretion, whose activity does not result in further calcium fluxes. Any candidate identified in the *in vitro* screens should be validated *in vivo*. The first line strategy for this is to use CHI SC-islet grafts, which lead to hypoglycemia in the recipient mice (37). Conceivably measurements of mouse blood glucose and C-peptide secreted by the graft in drug-treated and non-treated mice engrafted with CHI SC-islets should reveal the effectiveness of a candidate molecule *in vivo*.

Discovery of Novel CHI Pathomechanisms

Around 50% of new CHI patients do not present a mutation in the genes previously identified as causative for CHI (2). Unraveling of the exact pathogenic mechanism should direct selection of the most appropriate treatment for each patient. Pinpointing the molecular mechanism could also aid discovery of antihypoglycemic pharmaceuticals and shed light to the role of the identified genes in the regulation of beta cell insulin secretion. Answering these questions requires a model system with sufficient fidelity to capture disease pathophysiology on many levels. SC-

islets have been used to discover the molecular mechanism of multiple genes causing different types of monogenic diabetes, ranging from mechanisms related to beta cell development (56–58) to function (59, 60) and degeneration (61–63), as reviewed recently (64). In the case of novel CHI genes, several parameters can be used to unravel disease mechanisms. These include, at least, development of the endocrine populations during differentiation; insulin secretion under different stimuli *in vitro*; transcriptomic, epigenomic proteomic, and dynamic metabolomic studies. The stem cell based approach can provide even the large amounts of tissue required for these analyses.

The Use of CHI SC-Islets as a Model for Chronic Beta-Cell Hyperfunctionality and Glucotoxicity

The beta cell is highly specialized, focusing on the production and secretion of insulin. It is understandable that CHI mutations, which by definition accelerate this process, have profound consequences for the cellular biology of the beta cells. Huopio and colleagues established that, in addition to causing hypoglycemia in infancy (65), dominant K_{ATP}HI mutations predispose to T2DM later in life (66), providing clinical evidence for the detrimental consequences of long-term beta cell hyperactivity. Similar T2DM predisposing effect has been discovered in carriers of activating glucokinase mutations (GCK-HI) (67, 68).

Li and colleagues found that diverse cellular functions are disturbed or altered in CHI patient beta cells lacking K_{ATP}-channels, including glucose dependent metabolic pathways, expression of key transcription factors and receptors and the regulation of cell cycle (69). Additionally, the chronically elevated $[Ca^{2+}]_i$ characteristic of K_{ATP}HI and GCK-HI compromises beta cell identity in (70) and causes double strand breaks and p53 activation (71). Related to these findings, the increased workload of CHI beta cells initially increases their proliferation and mass while later leading to beta cell dysfunction and apoptosis *via* glucotoxicity (71–74), thus paralleling the natural course of beta cells in type 2 diabetes. These examples highlight the possibility of using CHI SC-islets as a model to discover further consequences of the influence of chronically altered beta cell functional states on their biology. Indeed, we could capture the initially increased beta cell mass and proliferation *in vitro*, while the proliferation normalized *in vivo* (37). Again, the possibility for isogenic comparison between healthy and CHI SC-islets provides a specific means to link any alterations to specific mutations.

CONCLUSIONS

Stem cell derived islets represent a powerful tool for modeling diseases of the pancreatic beta cell, due to the potential to produce them in limitless quantities with high consistency and with high disease phenotype fidelity. In the case of CHI, the SC-islets can be harnessed to discover novel antihypoglycemic medications, to study molecular mechanisms of newly discovered CHI genes and to study the basic biology of a hyperactive beta cell. Thus, we believe that modeling of CHI with SC-islets will serve as a critical next step required for the development of specific and efficient antihypoglycemic drugs.

AUTHOR CONTRIBUTIONS

VL wrote the initial draft and edited the manuscript. TO edited the manuscript and provided resources. All authors contributed to the article and approved the submitted version.

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