F-2107

Using hiPSC-derived beta cells to advance diabetes drug discovery

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Abstract

By 2030, diabetes is predicted to be the seventh leading cause of death globally. Type I diabetes (T1D) is a chronic autoimmune variant of this disease characterized by pancreatic islet beta cell loss and dysfunction, which results in insufficient production of insulin and subsequent excess of blood glucose, leading to numerous complications. While advances in diabetes treatments, including the development of new classes of drugs, have made diabetes treatment more manageable, a gap in health outcomes between T1D patients and those without diabetes still remains. In addition to the development of new drugs, transplantation of pancreatic islets into patients, currently limited by donor availability, holds great promise for a diabetes cure. Stem cell-derived beta cells that faithfully recapitulate *in vivo* beta cell features have tremendous potential to advance diabetes treatments on both the drug discovery and regenerative medicine fronts.

Compounds known as incretins stimulate insulin production. Recent developments in therapeutics use incretin analogues, such as the GLP-1 analogue exenatide, to induce insulin secretion. Finding new target receptors to treat diabetes requires *in vitro* models that secrete insulin in response to stimulation, like their *in vivo* counterparts. In particular, free fatty acid receptors (FFARs/GPRs) are under high scrutiny as likely candidates for new therapies.

We have recently developed a hiPSC-derived beta cell line (ChiPSC12) displaying beta cell markers like insulin, C-peptide, MAFA, and NKX6.1. We present further characterization of these beta cells, showing mRNA expression data of beta-cell-specific marker genes together with analysis of insulin secretion upon stimulation with incretins and GPR agonists, to demonstrate their suitability for drug development. We also present data from another hiPS cell line, ChiPSC22, which carries the HLA type A*02:01 that is strongly associated with the susceptibility to develop T1D.

In addition, these newly developed beta cell lines can be used in a format suitable for high-throughput screening (HTS), enabling a fast, reliable, and robust beta cell *in vitro* system for finding new diabetes therapies.

1 Using hiPSC-derived beta cells as a tool for drug discovery

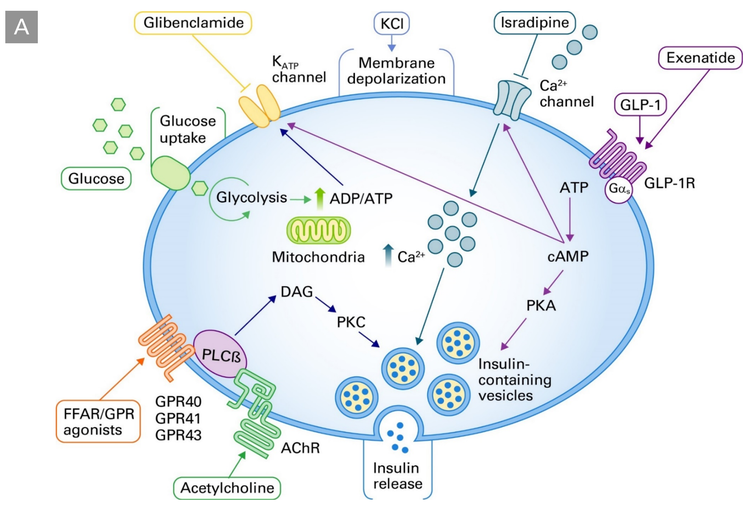

Figure 1. Beta cells derived from hiPSCs secrete insulin through multiple pathways. Panel A. Glucose enters the beta cell by a glucose transporter protein and is metabolized by glycolysis and oxidative phosphorylation, generating the ATP required for closing the potassium channel (K-ATP). When this ion channel closes, the membrane depolarizes and the calcium channel opens, allowing calcium influx into the cells and enabling insulin-containing vesicles to fuse to the membrane and secrete insulin. Insulin secretion can also be mediated by other pathways, such as the incretin system, where GLP-1 (or GLP-1 analogues, e.g. exenatide) binds to GLP-1R. Insulin secretion can also be stimulated by acetylcholine or by FFAR/GPR agonists. All of these mediators of insulin secretion are potential drug targets for the pharmaceutical industry. **Panel B.** Our protocol follows the typical beta cell developmental pathway, including hiPSC differentiation into definitive endoderm, pancreatic endoderm, and endocrine progenitor cells. hiPSCs are first differentiated into SOX17+ definitive endoderm. Following endodermal commitment, the cells are further differentiated into PDX1+/NKX6.1+ pancreatic endoderm. Next, differentiation continues into endocrine progenitor cells, which are NGN3+/NKX2.2+. Lastly, the progenitor cells mature into beta cells that are cryopreserved.

2 hiPSC-derived beta cells express relevant mRNAs

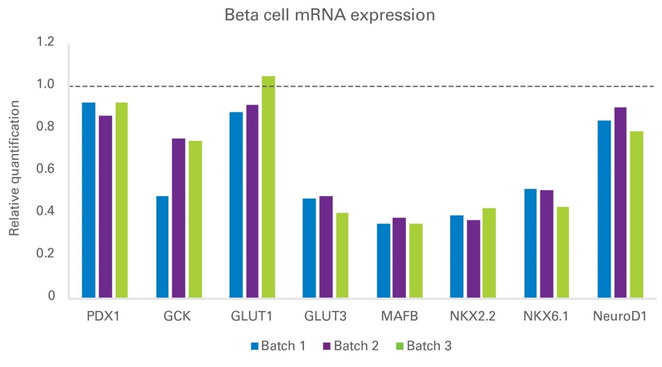

Figure 2. hiPSC-derived beta cells express key beta cell mRNAs. Three lots of beta cells were separately differentiated and gene expression was analyzed from ChiPSC12 using MagMax total isolation kit and TaqMan probes. Beta cells consistently expressed mRNA in similar quantities for eight markers across all batches tested. The dashed line represents relative expression levels compared to a batch of primary islets.

3 hiPSC-derived beta cells secrete insulin in response to KCl

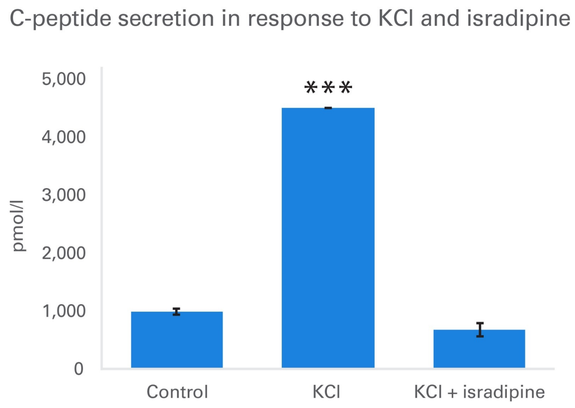

Figure 3. hiPSC-derived beta cells secrete insulin in response to KCl. Cells from ChiPSC12 were grown in medium containing 2.8 mM glucose, then treated sequentially with the indicated treatments. Cells increase secretion of C-peptide following exposure to 30 mM KCl, and this effect is reversed upon addition of the calcium channel blocker isradipine (10 µM). C-peptide secretion was measured by Mercodia C-peptide ELISA. The experiment was performed on two batches of hiPSC-derived beta cells and three biological samples from each batch (n = 6). Mean ± SEM (n = 6); asterisks indicate p<0.001.

4 hiPSC-derived beta cells secrete insulin in response to incretins

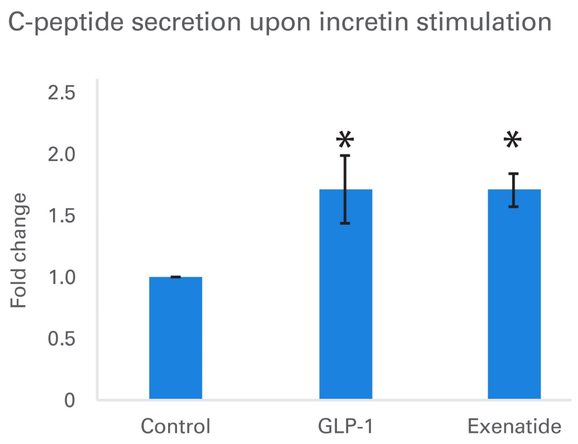

Figure 4. hiPSC-derived beta cells secrete insulin in response to incretins. Cells from ChiPSC12 were exposed to glucose (5.5 mM) and incretins (100 nM GLP-1 or 100 nM exenatide) for 45 min exhibited increased C-peptide secretion. C-peptide secretion was measured by Mercodia C-peptide ELISA. Mean ± SEM (n=5, GLP-1; n=4, exenatide); asterisk indicates p<0.05.

5 hiPSC-derived beta cells express and show functional response to FFARs/GPRs

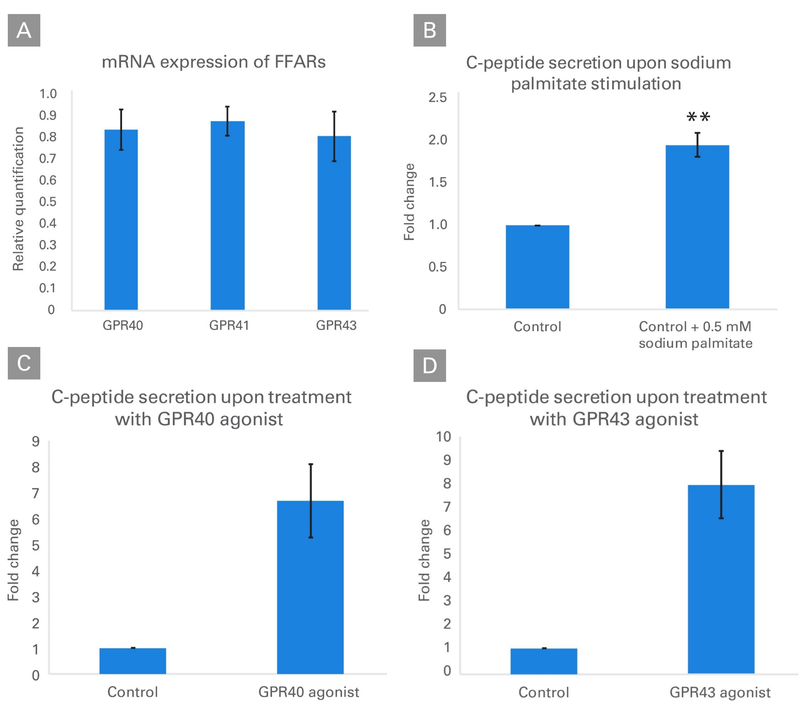

Figure 5. Expression and insulin secretion of FFARs/GPRs. Panel A. hiPSC-derived beta cells from ChiPSC12 consistently express free fatty acid receptors, also known as the G-protein-coupled receptor 40 (GPR40) family. Four lots of beta cells were separately differentiated, *GPR40, GPR41*, and *GPR43* gene expression were analyzed, and the relative expression was normalized to one of the batches. Cells from all four batches express these three free fatty acid receptors. Mean ± SEM (n = 4). **Panel B.** Beta cells derived from ChiPSC12 were first incubated in glucose (2.8 mM; control) followed by a sodium palmitate (0.5 mM) exposure for 45 min. Upon palmitate treatment, the cells increased their C-peptide secretion. ** indicates p<0.01. Beta cells from ChiPSC12 were first incubated in glucose (5.5 mM; control) followed by stimulation with **(Panel C)** 200 µM of the GPR40 agonist AS2034178 or **(Panel D)** 150 µM of the GPR43 agonist 4-CMTB for 45 min. The agonist-treated cells displayed an upregulation in C-peptide secretion, as measured by ELISA. Mean ± SEM (GPR40 agonist n = 3, GPR43 agonist n = 4).

6 hiPSC-derived beta cells respond to acetylcholine

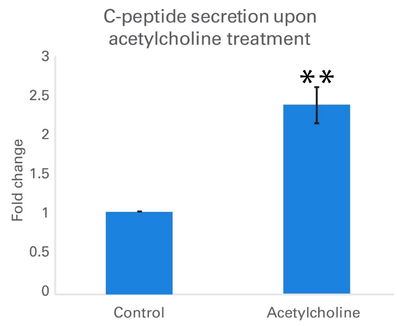

Figure 6. Acetylcholine response. Beta cells derived from ChiPSC12 were stimulated with acetylcholine (25 µM) for 45 min in 2.8 mM glucose. The levels of secreted C-peptide was measured by Mercodia C-peptide ELISA and found to be significantly upregulated after stimulation. Mean ± SEM (n = 4); asterisks indicate p<0.01.

7 Robust protocol produces insulin-positive cells from multiple hiPSC lines

A

hiPSC line	Karyotype (from banked cells)	HLA type	Insulin positive cells (%)
ChiPSC12	46, XY	A*01:01	50%
ChiPSC18	46, XY	A*23:01	45%
ChiPSC22	46, XY	A*02:01**	67%

**HLA A*02:01 is correlated with a higher suseptibility to developing type 1 diabetes.

B

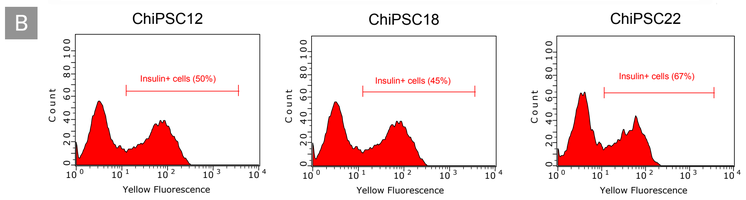

Figure 7. Insulin expression in hiPSC-derived beta cells from multiple lines. Panel A. Cells derived from three different hiPSC lines have normal karyotypes, different HLA types, and varying populations of insulin-positive cells**. Panel B.** Beta cells derived from three different hiPSC lines were fixed and stained for insulin.

8 hiPSC-derived beta cells are suitable for high-throughput drug screening

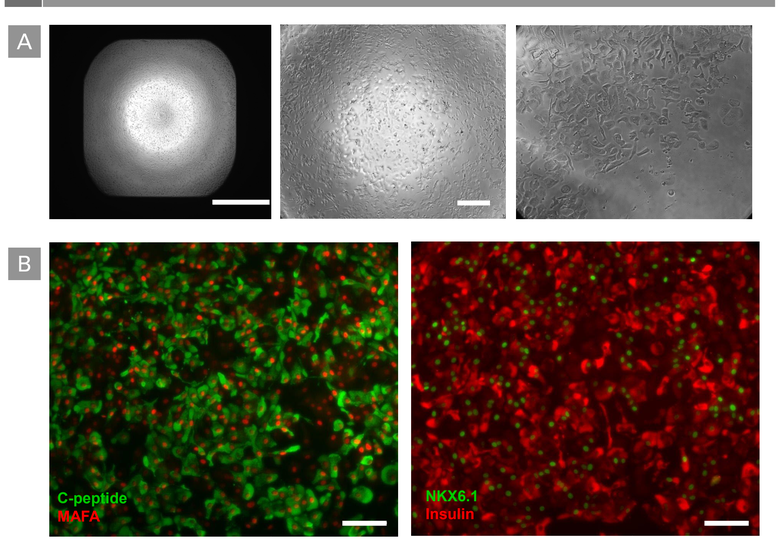

Figure 8. hiPSC-derived beta cell line in high-throughput format. Cells from ChiPSC22, a line carrying the HLA type A*02:01, conferring T1D susceptibility, were thawed and plated in a precoated 384-well plate at a seeding density of 10,000 cells/well. The beta cells were cultured according to the user manual for two weeks before fixation and further immunocytochemistry. **Panel A.** Morphology images of cultured cells at 4X, 10X, and 20X magnification in a 384-well plate. Far left, scale bar = 1 mm; middle scale bar = 200 µM. **Panel B.** After plating in a 384-well plate, fixed cells were stained for; C-peptide and MAFA (left), or insulin and NKX6.1 (right). Scale bar = 100 µM.

Conclusions

- hiPSC-derived beta cells provide a reliable and reproducible model to be used in drug discovery.
- hiPSC-derived beta cells consistently demonstrate high expression of known beta cell mRNA transcripts and proteins.
- hiPSC-derived beta cells display appropriate responsiveness to incretin and KCl stimulation.
- hiPSC-derived beta cells secrete insulin in response to GPR agonists.
- hiPSC-derived beta cells expressing insulin can be generated from multiple iPS cell lines.
- hiPSC-derived beta cells can be scaled up for high-throughput drug screening.

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