Discovery of CEP1410, a small molecule inducer that promote proliferation of pancreatic beta cells using novel micro-3D histo-culture assay procedure.

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Introduction

Identification of novel small molecule inducers that promote proliferation of pancreatic beta-cell is an important approach to treat diabetes. This unique approach represents a potential regeneration strategy for the treatment of type 1 diabetes. Unfortunately, the lack of availability of suitable human beta cell lines makes such a discovery a challenge. Here, we adapted and successfully developed the human islets micro-3D histo-cultures for high-throughput cell based assays. This micro-3D Histo-culture system of culturing human islet for high-throughput screening utilizing a thymidine analog, EdU, to detect beta-cell replication during screening. This culture system allows simultaneous monitoring of Ki67, EdU incorporation and beta cell numbers provides robust assay for beta-cell replication. Importantly, this micro-3D histo-culture system preserved the beta-cell physiological function, as measured by glucose-stimulated insulin secretion. We then performed a pilot screen of 384 compounds, observing some phenotypic effects on cells. This high-throughput human islet cell culture method can be used to assess various aspects of beta-cell biology on a relatively large number of compounds. From this screen we were able to characterize 10 lead compounds CEP1410, 1412, 1413, 1414, 1415, 1416, 1417, 1418, 1419 and 1420 that were able to increase the cell mass of beta cells and responded to glucose –stimulated insulin secretion. CEP1410, our lead compound was tested in-vivo. In diabetic rat model, once a day oral administration of CEP1410 increased the mass of Beta cells. The gene expression profile of pancreatic Beta-cells revealed high gene expression of Insulin, glucagon, somatostatin, Glut-2 and Isl-1 on Day 20 when study was terminated.

Methods:

The human pancreatic samples were obtained under IRB with consent after surgery or biopsy,(transplant rejects) and were placed immediately in Celprogen Pancreatic Transportation Media [M35002-04S] and shipped at 4-8°C for further processing. Tissues were washed with 1XPBS solution and aseptically cut and diced into sections and then 3D cultured utilizing Celprogen Complete Growth Media (M35002-04S). The cells were cultured in 384 well black with clear bottom tissue culture plates with filtered insert pre-coated with ECM E35002-384W. The cells were selected with Flow Cytometer for Pancreatic Beta cells based on the biomarkers and real time PCR :GAD, Islet Cell antibodies (ICA), CD152, Insulin, glucagon, somatostatin, pax6, Glut-2,and Isl-1. Upon selection the cells were cultured in their respective media and ECM

In-vitro study: Human Pancreatic cell culture, were isolated from 100 Normal donor patients that had under gone biopsy procedure and the tissues were not cancerous. The ages ranged from 45 years old to 65 years old, female genders. The tissues were consented and obtain under IRB and HIPPA regulations and guidelines. The tissues were transported from the surgical suites to Celprogen in Human Pancreatic complete growth media (Cat# M35002-04S) within 24 hours after it had been surgically removed from the patients together with 10cc of peripheral blood in purple top tubes. Upon receipt of the tissue the tissue was sectioned into two halves and processed into monolayer cell cultures whereas the other tissue was cultured as 3 D culture system.. One section was maintained as the heterogeneous normal population of cultured cells. The other section was processed further and isolated with Normal Pancreatic Islets cell's biomarkers, in Celprogen Media (M35002-04S) and ECM (E35002-04-384 wells) combination. Once the monolayer cell cultures were established within 7-14 days the cells were characterized by Flow, IHC, Western Blot and Real Time PCR. Once the cells were characterized they were seeded at 5,000 cells per well in a 384 well format, pre-coated with Celprogen ECM and cultured in complete growth Phenol free media with low serum concentration. The drugs were tested by incubating at various concentrations for 72 hours at 5% carbon dioxide, humidified 37°C incubator only for the 3D cell culture system. At the end of 72 hours, cell viability was obtained utilizing Alamar blue Cell based assay and also Cellometer Auto 200 [Nexcelom Bio Science Cell counter. The 3D culture system allows for simultaneous monitoring of Ki67, EdU incorporation and beta cell numbers provides robust assay for beta-cell replication. We then performed a pilot screen of 384 compounds, observing some phenotypic effects on cells. This high-throughput human islet cell culture method can be used to assess various aspects of beta-cell biology on a relatively large number of compounds. From the initial screen of 384 compounds we were able to narrow it down to 10 lead compounds.

In-vivo study: We tested the 10 lead compounds at the following concentrations 10, 15 and 20 mg/kg once daily oral administration in rat diabetic model. There were 11 groups of 6 rats per group, two rats per dose, one female and one male rat. The control group only received 1X PBS carrier vehicle, whereas, the experimental groups received once daily oral gavage [10,15& 20 mg/kg]. After 20 days post gavage administration, the study was terminated and the rats were sacrificed and their pancreas was surgically removed and beta cell mass was calculated and gene expression profile of beta cells for genes insulin, glucagon, somatostatin, Glut-2 and Isl-1. At the end of the study the rats were sacrificed and the pancreas tissues were sectioned into three compartment; 1. One section was fixed and H&E stained, 2. One section was cultured into monolayer and IHC studies and flow studies with various pancreatic markers was performed, 3. One section was stored liquid nitrogen for genomic DNA and total RNA analysis for Real-time PCR.

Results:

The results are indicated in the figures and graphs below:

Figure 1: 3D Model for Human Pancreatic Islet Cell Culture. Confocal microscopy of triple-labeled immunofluorescence islets of Langerhan Beta cells cultured for 7days depicted for (A) insulin (green), (B) nestin (red) and (C) DAPI labeling for nuclei (blue). The superposition of A, B and C is shown in D. Insulin in the center of the islet (A), nestin-positive cells among the insulin-positive cells and of regions of co-localization of both insulin and nestin (in yellow). Scale bar in µm

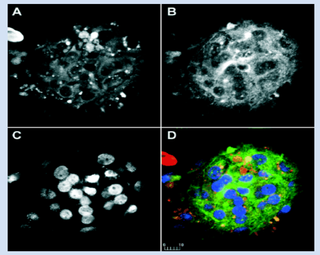


Figure 2 A. Islet of Langerhan cells after 20 days post treatment group Rat diabetic model stained with Hematoxylin and Eosin. The lightly stained area indicates the islet of langerhan cells. **B**. Beta cell culture stained for insulin – FITC.

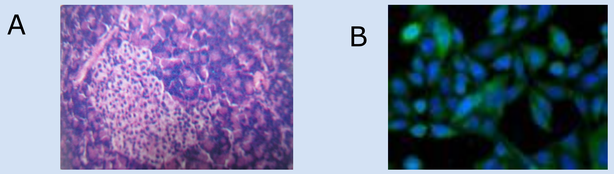


Table 1. Positive Cells Markers for Human Pancreatic cells

H. Pancreatic Cells	IHC	Real Time PCR
Insulin	‡	‡
Glucagon		‡
somatostatin	‡	‡
Pax6, Isl-1,		‡
Glut-2		‡
GAD, Ki67, Nestin	‡	
ICA (Iselt Cell Antibodies)	‡	

Figure 3: Comparison of treated and untreated human pancreas 3D culture for evaluation of lead compound screen for Beta Cell Proliferators. A. Control, B. 24hours Incubation with CEP1410, C. 48 hours incubation with CEP1410, at concentration 1 uM. The cell density of Beta cells increases incubation time, as indicated in the images when compared to the controls. D. Proliferation index.

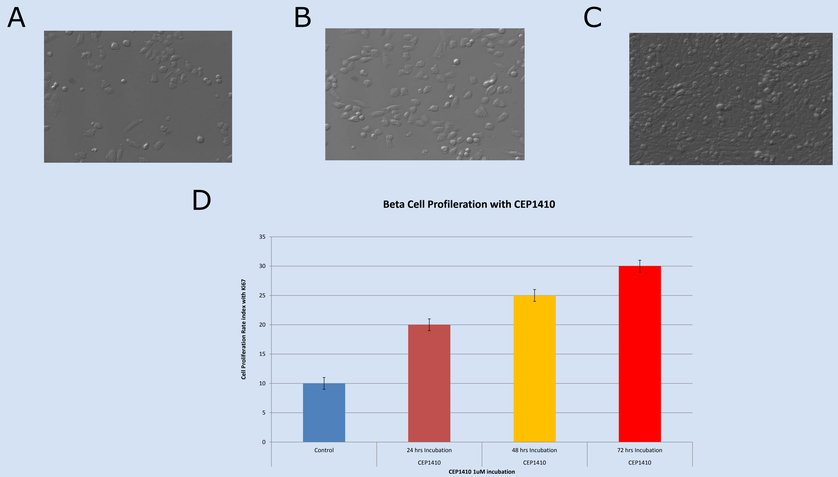
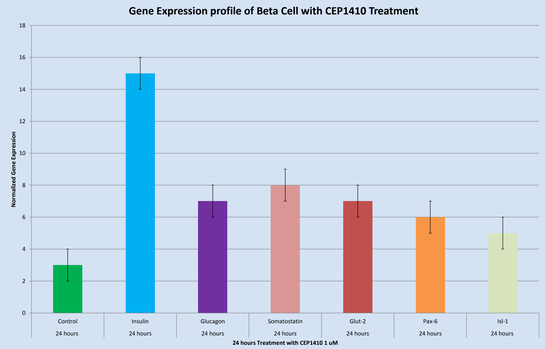


Figure 4: Gene expression profile of Beta Cells with CEP1410 treatment, The gene expression profile of pancreatic Beta-cells revealed high gene expression of Insulin, glucagon, somatostatin, Glut-2 and Isl-1 on Day 20 when study was terminated.



Conclusions:

In this study we screened 384 compounds, observing some phenotypic effects on cells. This high-throughput human islet cell culture method can be utilized to assess various aspects of beta-cell biology on a relatively large number of compounds. From this screen we were able to characterize 10 lead compounds CEP1410, 1412, 1413, 1414, 1415, 1416, 1417, 1418, 1419 and 1420 that were able to increase the beta cell mass and responded to glucose –stimulated insulin secretion. CEP1410, our lead compound was tested in-vivo. In diabetic rat model, once a day oral administration of CEP1410 increased the mass of Beta cells. The gene expression profile of pancreatic Beta-cells revealed high gene expression of Insulin, glucagon, somatostatin, Glut-2 and Isl-1 on Day 20 when study was terminated.

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