Highly Efficient and Reproducible Differentiation of Human Pluripotent Stem Cells to PDX1+/NKX6.1+ Pancreatic Progenitors

Michael J. Riedel[1], Stephanie Lam[1], Yvonne Luu[1], Allen C. Eaves[1,2], Terry E. Thomas[1], and Sharon A. Louis[1]
[1] STEMCELL Technologies Inc., Vancouver, Canada [2] Terry Fox Laboratory, BC Cancer Agency, Vancouver, B.C., Canada

Introduction

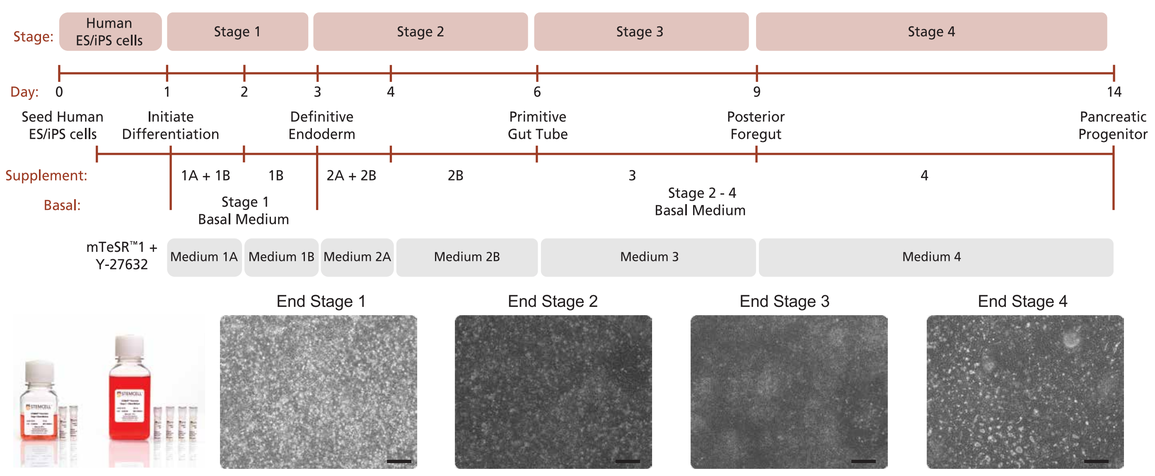
Type 1 diabetes is characterized by a loss of the insulin-producing beta cells of the pancreatic islets. Transplanting cadaveric donor islets into the portal vein of type 1 diabetic individuals can induce insulin independence. However, a shortage of donor islets precludes this cell therapy from widespread clinical use and typically only those individuals who cannot control their glucose homeostasis by exogenous insulin delivery qualify for the procedure.

An alternative source of tissue for transplantation may be insulin-producing cells derived from human embryonic stem (hES) and human induced pluripotent stem (hiPS) cells. Recent advances in protocols for the derivation of pancreatic cell types from hES and hiPS cells have resulted in the initiation of clinical trials in North America, whereby immature pancreatic progenitor cells are loaded into a device and implanted under the skin[1,2]. Maturation of these progenitor cells to functional endocrine cells occurs over months after which this surrogate pancreas can regulate glucose homeostasis. Additional research programs are now aimed at developing protocols to mature these pancreatic progenitor cells in vitro as well as using standardized protocols for generating pancreatic cells for disease modeling and developmental studies.

Several protocols have been developed to generate pancreatic progenitors from hES and hiPS cells but with varying efficiency and reproducibility across cell lines. To standardize generation of hES and hiPS cell-derived pancreatic progenitors, we developed the serum-free, defined STEMdiff™ Pancreatic Progenitor Kit that supports efficient and reproducible generation of pancreatic progenitors from multiple hES and hiPS cell lines. Cells generated using this optimized medium and protocol express key markers including PDX-1, NKX6.1 and SOX9, thus providing researchers with a standardized tool for pancreatic cell research.

Protocol

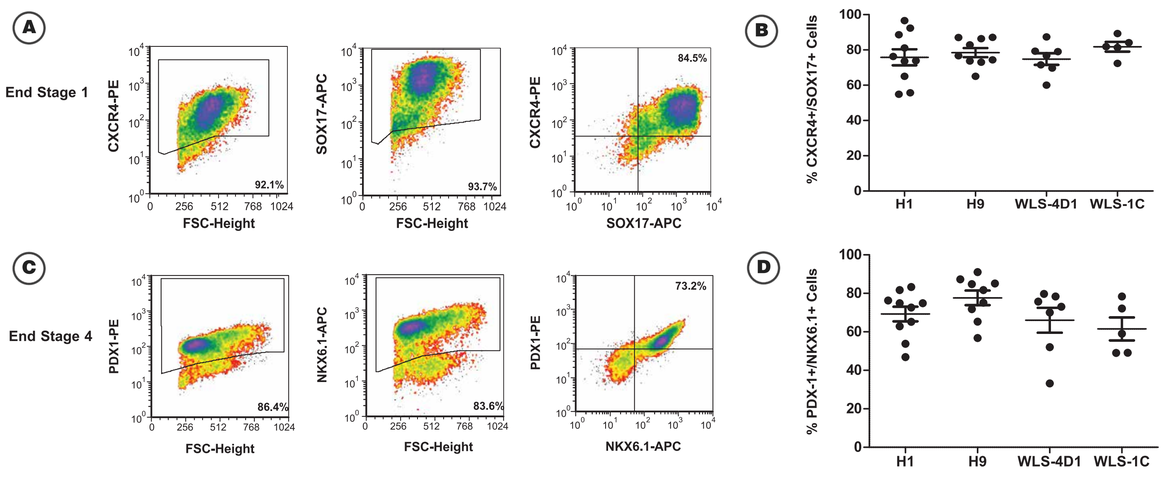
FIGURE 1: Product Format and Protocol



The STEMdiff™ Pancreatic Progenitor Kit promotes the differentiation of hES and hiPS cells through 4 stages: definitive endoderm (End Stage 1), primitive gut tube (End Stage 2), posterior foregut endoderm (End Stage 3) and pancreatic progenitors (End Stage 4). The kit comprises 2 basal media and 6 supplements that are used over the course of 14 days to promote pancreatic progenitor cell formation. Representative images of cell morphology are shown at the end of each stage of differentiation (Scale bar, 50 µm).

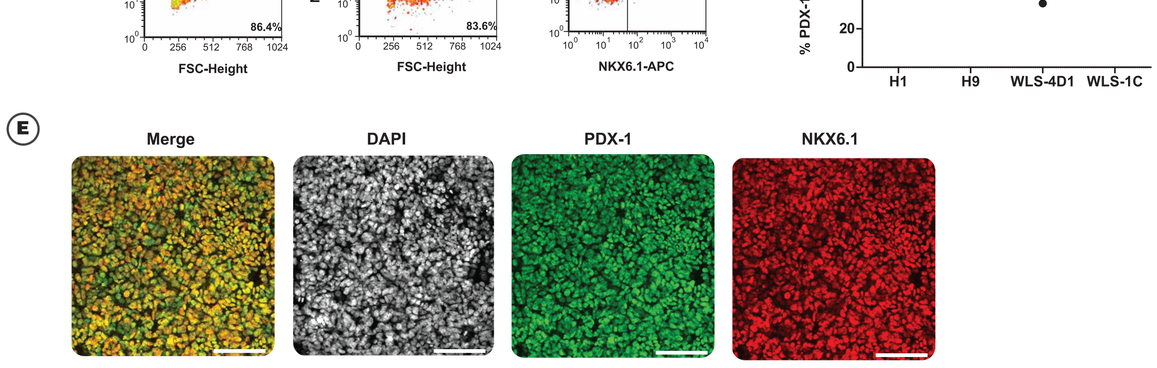
Results

FIGURE 2: Efficient Generation of Pancreatic Progenitor Cells Across Multiple Cell Lines



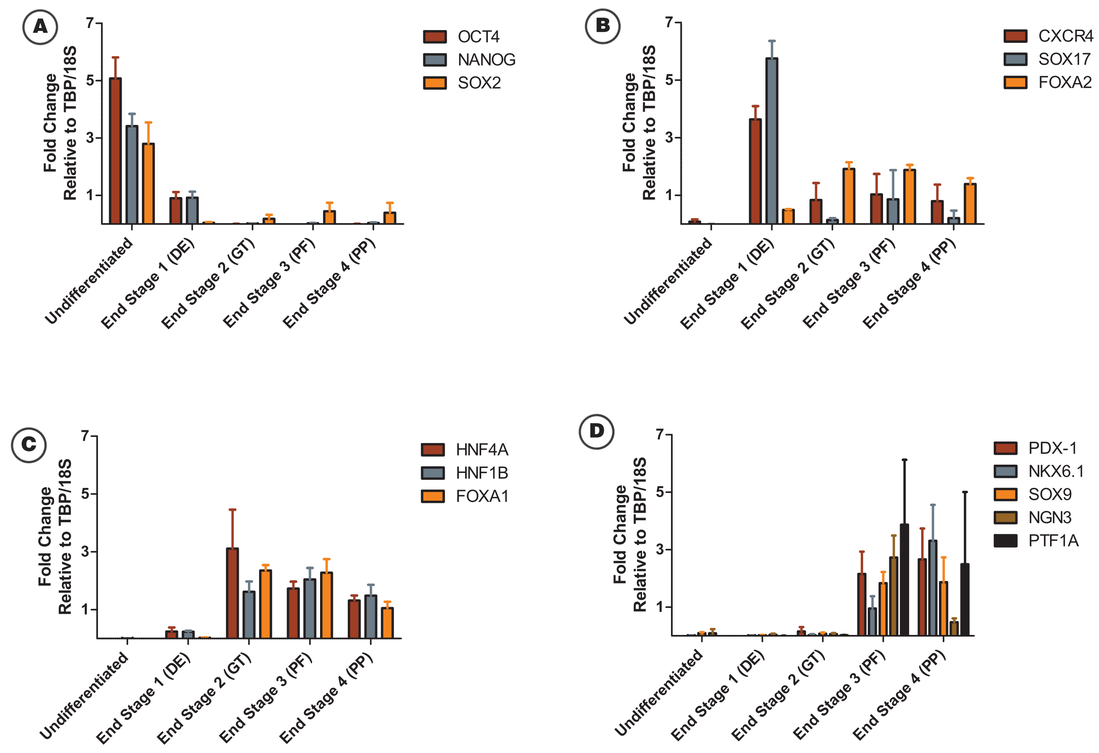
The STEMdiff™ Pancreatic Progenitor Kit promotes highly efficient generation of definitive endoderm (End Stage 1) and pancreatic progenitor cells (End Stage 4). **(A)** Representative flow cytometry plots for CXCR4 and SOX17 co-expression in differentiated H9 hES cells. Gates are set based on isotype controls. **(B)** Quantitative data for CXCR4/SOX17 co-expression in two hES and two hiPS cell lines. Data are plotted as individual points representing the mean of duplicates within a single experiment. The horizontal line represents the mean of all experiments, with error bars indicating the SEM. n = 5-10 per cell line. **(C-D)** Cells were immediately carried forward from the end of Stage 1 into Stages 2 - 4 without passaging, resulting in highly efficient conversion of definitive endoderm cells into PDX-1+/NKX6.1+ pancreatic progenitors at the end of Stage 4. **(C)** Representative flow cytometry plots for PDX-1 and NKX6.1 co-expression in differentiated H9 hES cells. **(D)** Quantitative data for PDX-1/NKX6.1 co-expression in two hES and two hiPS cell lines. Data are plotted as in panel B (n = 5 - 10 per cell line). The average efficiency of pancreatic progenitor differentiation ranged from 61.5% to 77.7% depending on the cell line. **(E)** Representative images of PDX-1 (green) and NKX6.1 (red) immunoreactivity in pancreatic progenitor cells at the end of Stage 4 (Scale bar, 100 µm). Nearly all NKX6.1+ cells also expressed PDX-1 as is observed in the developing human pancreas[3].

FIGURE 3: Rapid Expansion Yields High Numbers of Pancreatic Progenitor Cells



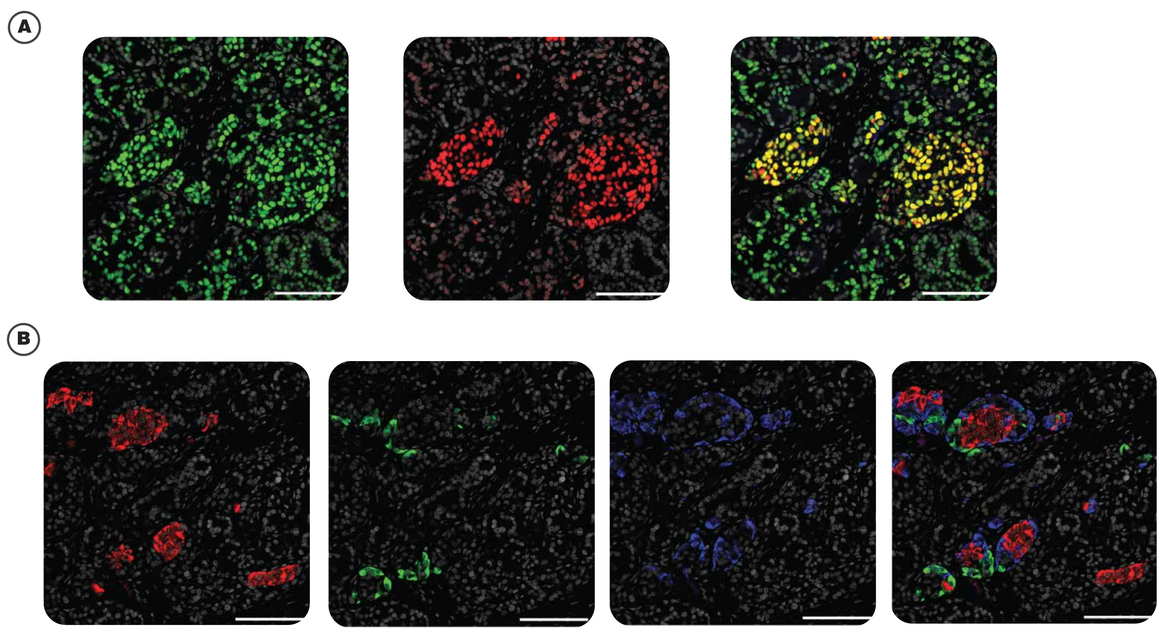
(A, B) Undifferentiated hPSCs were seeded at 8 x 10^5 cells (2.1 x 10^5 cells/cm^2) into wells of a 12-well culture plate containing mTeSR™1 + 10 µM Y-27632. Plating efficiency was approximately 50-60%, yielding a nearly confluent monolayer of approximately 4 - 5 x 10^5 cells per well on Day 1. By the end of Stage 1, there was an approximate 4-fold increase in total cell number, of which >75% are CXCR4+/SOX17+ definitive endoderm. By the end of Stage 4, the average number of pancreatic progenitor cells (PDX-1+/NKX6.1+ cells) ranges from 3.2 x 10^6 to 3.9 x 10^6 cells, yielding 4 to 5 pancreatic progenitor cells per input hPSC, depending on the cell line used. Each well of a 12-well plate therefore contains sufficient pancreatic progenitor cells to transplant into a single mouse.

FIGURE 4: Gene Expression Profile is Indicative of Consistent Transition of Definitive Endoderm to Pancreatic Progenitor Cells



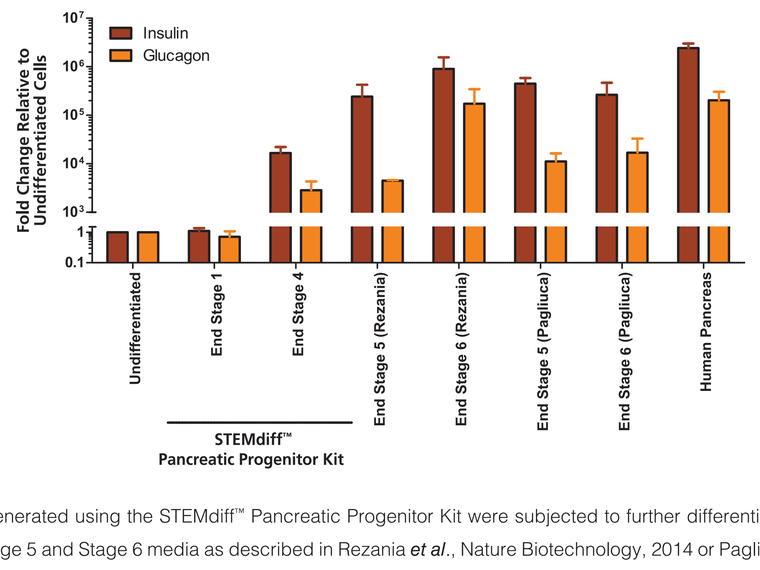
Gene expression profile at the end of each stage of differentiation for key markers of **(A)** the pluripotent state, **(B)** definitive endoderm, **(C)** primitive gut tube and posterior foregut and **(D)** pancreatic progenitor cells. Expression was normalized to 18S ribosomal RNA and TATA Binding Protein (TBP). Data are the mean ± SEM for 3 - 5 experiments. Expression pattern is consistent with published data[4].

FIGURE 5: In Vivo Maturation of Pancreatic Progenitor Cells to Mono-Hormonal Endocrine Cells



The STEMdiff™ Pancreatic Progenitor Kit generates cells that are capable of maturation towards mono-hormonal pancreatic endocrine cells following transplantation into a SCID-Beige mouse. 1 - 2 x 10^6 end Stage 4 cells were transplanted under the kidney capsule and allowed to engraft and mature over 24 weeks. **(A)** Representative image of PDX-1 (green) and NKX6.1 (red) immunoreactivity in the matured graft. Nuclei were stained with DAPI (gray) (Scale bar, 100 µm). **(B)** Representative image of insulin (red), glucagon (green) and somatostatin (blue) immunoreactivity in the matured graft. Nuclei were stained with DAPI (gray) (Scale bar, 100 µm). We gratefully acknowledge assistance from members of the laboratory of Dr. Timothy J. Kieffer (University of British Columbia, Vancouver, Canada) for transplant studies and immunohistochemical analysis of grafts, particularly Shannon O'Dwyer and Ali Asadi.

FIGURE 6: In Vitro Maturation of Pancreatic Progenitor Cells Increases Insulin Gene Expression



PDX-1+/NKX6.1+ cells generated using the STEMdiff™ Pancreatic Progenitor Kit were subjected to further differentiation by treating end Stage 4 cultures with Stage 5 and Stage 6 media as described in Rezania ***et al.***, Nature Biotechnology, 2014 or Pagliuca ***et al.***, Cell, 2014. Cells were harvested at the end of Stage 1 and at the end of Stage 4 (STEMdiff™ Pancreatic Progenitor Kit) as well as at the end of Stage 5 and Stage 6 following in vitro maturation[4,5] and analyzed for expression of insulin and glucagon by RT-qPCR. Expression was normalized to 18S ribosomal RNA and then to undifferentiated cells. Data are the mean ± SEM for 3 experiments.

Summary

- The STEMdiff™ Pancreatic Progenitor Kit promotes efficient and reproducible generation of NKX6.1+/PDX-1+ pancreatic progenitor cells from human pluripotent stem cells maintained in mTeSR™1.
- Differentiation is robust across multiple human pluripotent stem cell lines, producing high yields of pancreatic progenitor cells.
- Pancreatic progenitor cells generated with the STEMdiff™ Pancreatic Progenitor Kit exhibit a gene expression profile similar to state-of-the-art published protocols, including up-regulation of key transcription factors PDX-1, NKX6.1 and SOX9.
- Cells generated using the STEMdiff™ Pancreatic Progenitor Kit are capable of maturing towards mono-hormonal insulin-producing cells following in vivo or in vitro maturation.

1. Kroon E., *et al.* Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells *in vivo.* Nature Biotechnology 26 (4): 443 - 52, 2008.
2. Schulz T.C., *et al.* A scalable system for production of functional pancreatic progenitors from human embryonic stem cells. PLoS One 7 (5): e37004, 2012.
3. Riedel M.J., *et al.* Immunohistochemical characterization of cells co-producing insulin and glucagon in the developing human pancreas. Diabetologia 55 (2): 372 - 81, 2010.
4. Rezania A., *et al.* Reversal of diabetes with insulin-producing cells derived *in vitro* from human pluripotent stem cells. Nature Biotechnology 32 (11): 1121 - 33, 2014.
5. Pagliuca., ***et al.*** Generation of functional human pancreatic ß cells in vitro. Cell 159 (2): 428-39, 2014.