The Effect of Overexpression of Pdx1 on the Differentiation of Induced Pluripotent Stem Cells Derived From Fibroblast of Diabetic Patient into Pancreatic Cells

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Objectives: **Pancreatic and duodenal homeobox 1 (PDX1), a member of the homeodomain-containing transcription factor family, is a key transcription factor important for both pancreas development and mature β cell function. Induced overexpression of Pdx1 resulted in a significant upregulation of insulin and other pancreas-related genes. The generation of insulin-producing pancreatic β-cells from human iPS cells in vitro would provide an unprecedented cell source for cell transplantation therapy in diabetes without the ethical obstacle of embryonic stem cells and would bypass immune rejection.**

Materials and Methods: **Pdx1 overexpressed hiPS cells were produced by Lentiviral transduction system and the infected cells were selected by puromycin. A differentiation process was carried out according to kroon et al., 2008 protocol with some modifications that converts human induced pluripotent stem cells to endocrine cells capable of synthesizing the pancreatic hormones insulin, glucagon, somatostatin. This process mimics in vivo pancreatic organogenesis by directing cells through stages resembling definitive endoderm, primitive gut-tube endoderm, posterior foregut, pancreatic endoderm and endocrine precursor which leads to cells that express endocrine hormones. We characterized the differentiation process in each stages at the RNA and protein levels using real-time PCR, immunofluorescence and flow cytometry.**

Results: **The results indicated high expression level of each stage-specific markers including SOX17, FOXA2, and GSC in DE stage, HNF4A in PG stage, PDX1 in PF stage, NGN3 in PE stage and pancreatic hormones such as insulin were detected.**

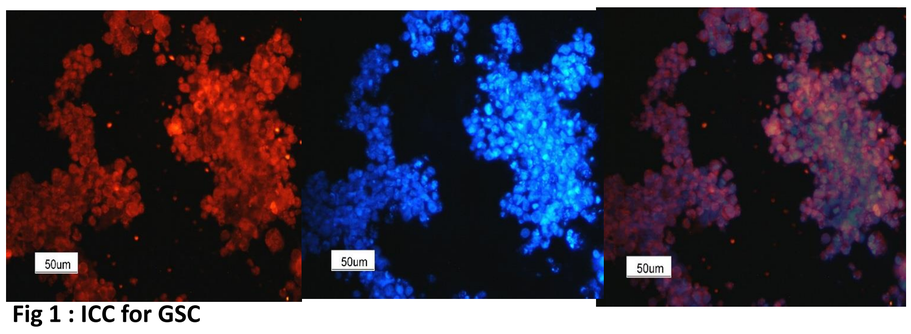

Fig 1 : ICC for GSC

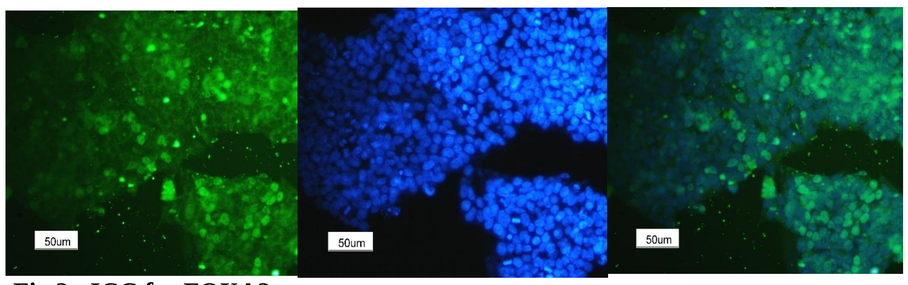

Fig 2 : ICC for FOXA2

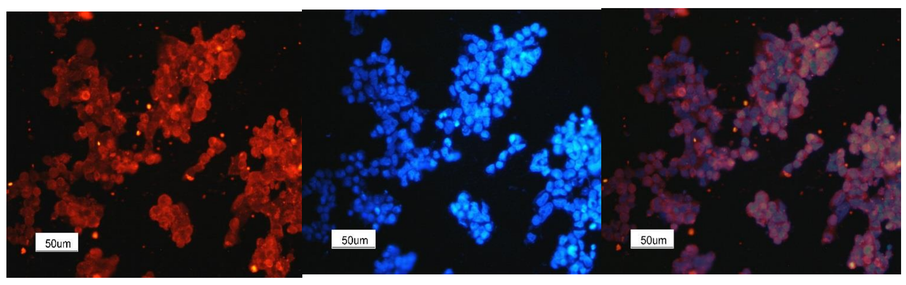

Fig 3 : ICC for SOX17

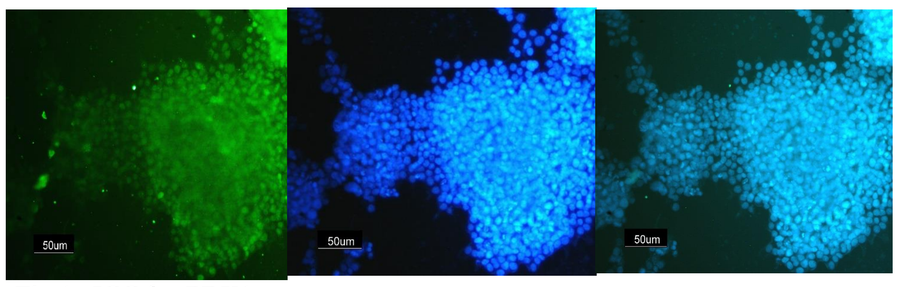

Fig 4 : ICC for PDX1

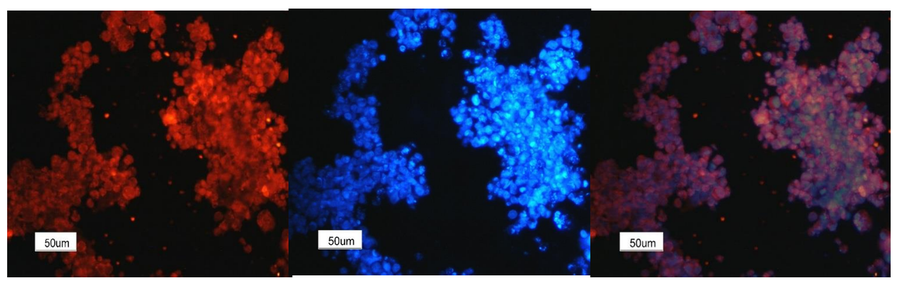

Fig 5 : ICC for INS

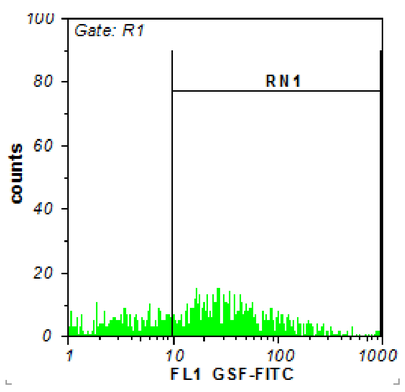

Fig 6: Flow cytometry analysis of GSC expressing cells (35.03%)

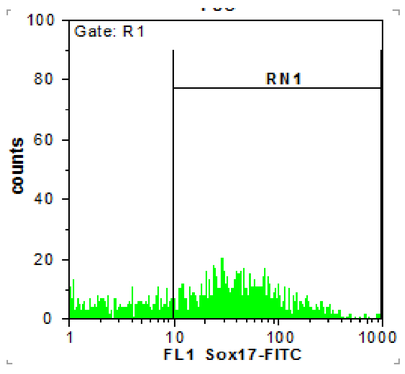

Fig 7: Flow cytometry analysis of SOX17 expressing cells (42.95%)

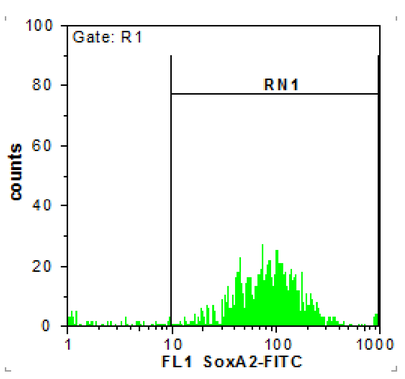

Fig 8: Flow cytometry analysis of FOXA2 expressing cells (47.67%)

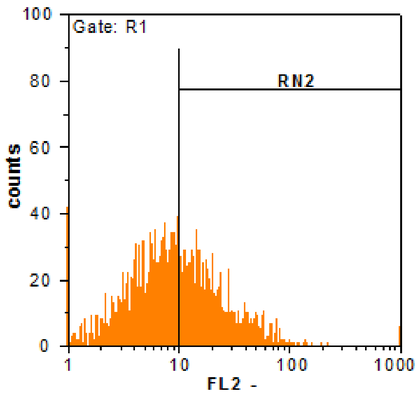

Fig 9: Flow cytometry analysis of PDX1 expressing cells (45.54%)

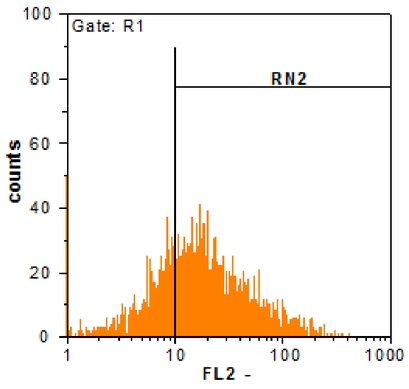

Fig 10: Flow cytometry analysis of c-PEP expressing cells (70.23%)

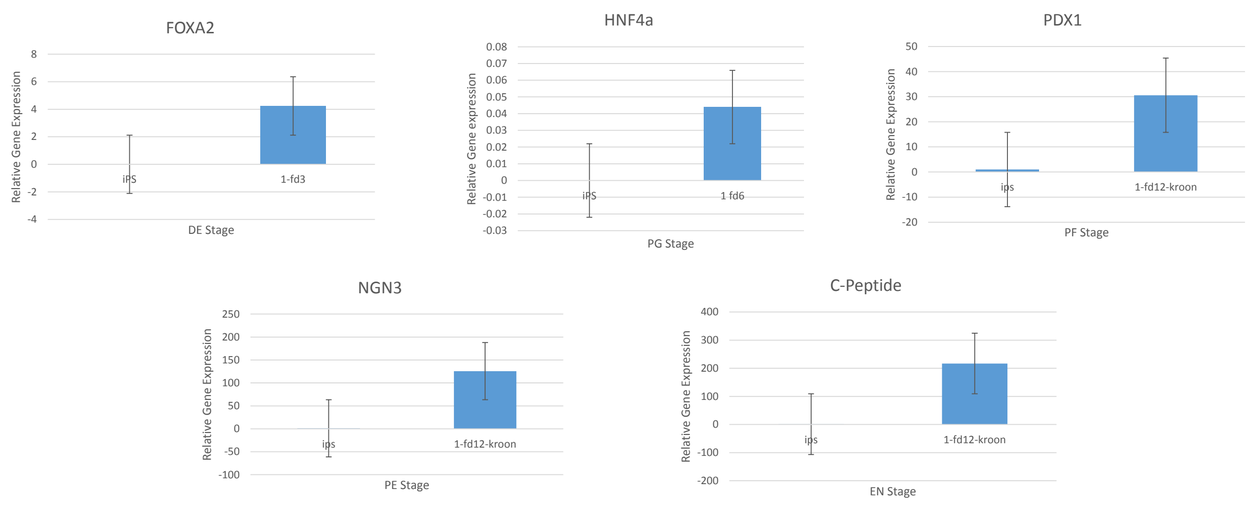

Fig 11: Expression Levels of Stage Specific Markers

Conclusion: **These results demonstrated that overexpression of Pdx1 is an important new strategy for the efficient generation of functionally immature insulin-producing β-islet cells from hiPS cells.**

Keywords: **Human-induced pluripotent stem cells (hiPSCs), Definitive endoderm (DE), Primitive Gut-tube (PG), Posterior Foregut (PF), Pancreatic Endoderm (PE)**

References:

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