Sca-1 positive pancreatic progenitor cells as a cell transplant therapy for treatment of diabetes

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BACKGROUND

Diabetes is a disease that affects roughly 24 million people in the United States. Treatment options for these patients include administration of exogenous insulin , changes in diet and exercise, and drastic changes in lifestyle. One curative treatment for insulin dependent patients involves the transplant of insulin secreting Beta cells isolated from the islets of Langerhans in a donor pancreas. Due to the limited number of islets that can be isolated as well as the limited survival time of these cells in the recipients, much research is focused at finding a unique and proliferative β-like progenitor cell for clinical therapies. We have recently had success isolating and characterizing a pancreas derived progenitor cell population . This pancreatic progenitor cell (PPC) is purified by expression of stem cell antigen 1 (Sca-1); and demonstrates a phenotype similar to endocrine cells of the pancreas as well as tremendous proliferative ability.

HYPOTHESIS

•Sca-1 positive pancreatic progenitor cells are precursors to pancreatic beta cells and maintain insulin producing ability and pancreatic phenotype in extended culture, thus making them ideal for cell transplant therapies to treat diabetes

MATERIALS and METHODS

•Cells were isolated from pancreas of 2 week old mice using mechanical and enzymatic digestion. Pancreatic cells were purified for Sca-1 expression and cultured under select conditions

•A CyQUANT assay was performed to monitor cell proliferation

•RT-PCR was performed to determine if cultured cells maintained pancreatic phenotype

•Western blot was performed to detect pancreatic proteins

•ELISA was performed to access the cell populations ability to produce insulin in reaction to glucose stimulation

RESULTS

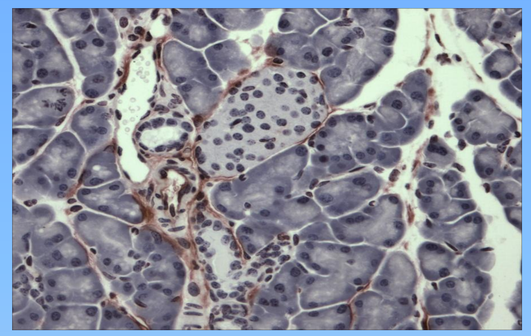
•Sca-PPC's were viable, highly proliferative, and capable of being maintained in culture through multiple passages

•RT-PCR and Western blot demonstrated that the cells maintained the expression of pancreatic markers

•ELISA for insulin production demonstrates basal production of insulin and in response to glucose stimulation

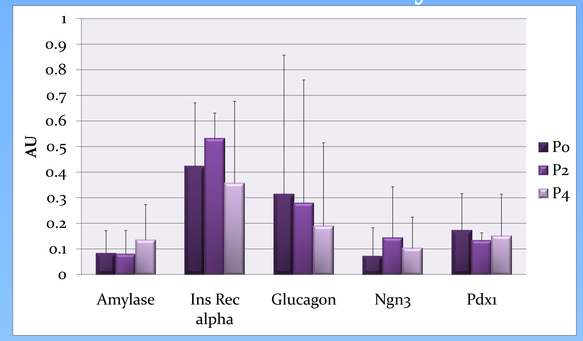
RESULTS

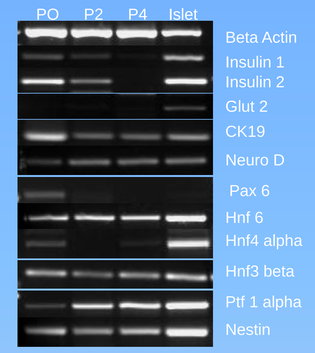
Immunohistochemistry for Sca-1 expression



Immunohistochemistry showing Sca-1+ cells residing around pancreatic ducts and beta cells

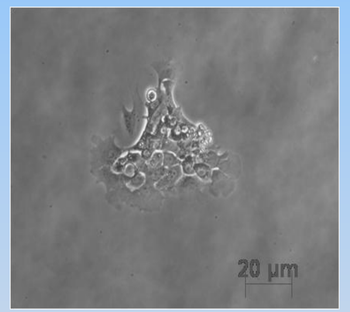
Western Blot and PCR analysis for select pancreatic markers



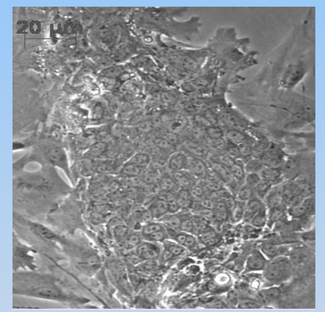


Assays were performed at passages 0, 2 and 4, as indicated. Three replicates were performed at each time point

Cell Culture showing early colony formation

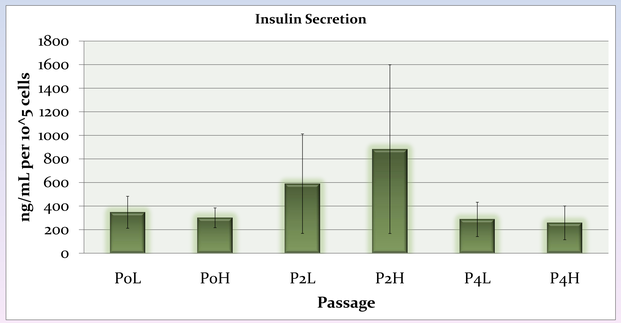


Sca-1 colony at day 3 of culture, 20X



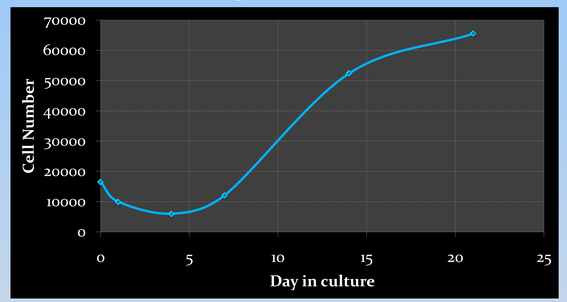
Sca-1 colony at day 4 of culture, 20X

ELISA for Insulin Production in ng/ml



Insulin ELISA results in response to high and low glucose concentrations at indicated passage number. Assay was performed with 3 replicates.

Proliferation Assay over 3 weeks of culture



CyQuant Assay demonstrating a rapid proliferation of cells beginning around day 4.

CONCLUSIONS

•Sca-positive pancreatic progenitor cells are a viable and proliferative pancreatic precursor cell

•PPC's show transcription and translation characteristic of early pancreatic endocrine cells, proving their Beta cell like properties. Fluctuation of factors could be indicative of gene regulation during development

•ELISA for insulin production demonstrates the ability of the cell population to release large amounts of insulin. This ability seems to decrease with passage

•Sca-PPCs offer a potential transplantable cell type that can be used for the treatment of diabetes