Differentiation of COPAS-sorted non-endocrine pancreatic cells into insulin-positive cells in the mouse.

Kikugawa R, Katsuta H, Akashi T, Yatoh S, Weir GC, Sharma A, Bonner-Weir S.

Section of Islet Transplantation and Cell Biology, Joslin Diabetes Center, 1 Joslin Place, Boston, MA, 02215, USA.

AIMS/HYPOTHESIS: The regenerative process in the pancreas is of particular interest, since insulin-producing beta cells are lost in diabetes. Differentiation of new beta cells from pancreatic non-endocrine cells has been reported in vivo and in vitro, a finding that implies the existence of pancreatic stem/progenitor cells. However, while tissue-specific stem cells are well documented in skin, intestine and testis, pancreatic stem cells have been elusive. We hypothesised that pancreatic stem/progenitor cells within the non-endocrine fraction could be a source of new islets in vitro. METHODS: To test if there were such cells within the pancreas, we generated pancreatic cell aggregates from tissue remaining after islet isolation from mouse insulin promoter 1-green fluorescent protein (MIP-GFP) mice. To eliminate any contamination of insulin-positive cells, we deleted all GFP-positive aggregates using COPAS Select and cultured with Matrigel. Immunohistochemistry, quantitative real-time PCR and single-cell nested RT-PCR were performed to confirm formation of insulin-producing cells. RESULTS: The GFP-negative cells were expanded as monolayers and then differentiated into three-dimensional cystic structures. After 1 week of culture, GFP-positive cells were found as clusters or single cells. By quantitative real-time PCR, no insulin mRNA was detected immediately after COPAS sorting, but after differentiation insulin mRNA of the whole preparation was 1.91 +/- 0.31% that of purified MIP-GFP beta cells. All GFP-positive cells expressed insulin 1; most expressed insulin 2, pancreas duodenum homeobox-1 and cytokeratin 19 by single cell nested RT-PCR. CONCLUSIONS/INTERPRETATION: Our data support the concept that within the exocrine (acinar and ductal) pancreas of the adult mouse there are cells that can give rise to insulin-positive cells in vitro.