Isolation, culture, and imaging of human fetal pancreatic cell clusters.

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Authors: Ana D Lopez, Ayse G Kayali, Alberto Hayek, Charles C King

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Public Summary:
For almost 30 years, scientists have demonstrated that human fetal ICCs transplanted under the kidney capsule of nude mice matured into functioning endocrine cells, as evidenced by a significant increase in circulating human C-peptide following glucose stimulation(1-9). However in vitro, genesis of insulin producing cells from human fetal ICCs is low(10); results reminiscent of recent experiments performed with human embryonic stem cells (hESC), a renewable source of cells that hold great promise as a potential therapeutic treatment for type 1 diabetes. Like ICCs, transplantation of partially differentiated hESC generate glucose responsive, insulin producing cells, but in vitro genesis of insulin producing cells from hESC is much less robust(11-17). A complete understanding of the factors that influence the growth and differentiation of endocrine precursor cells will likely require data generated from both ICCs and hESC. While a number of protocols exist to generate insulin producing cells from hESC in vitro(11-22), far fewer exist for ICCs(10,23,24). Part of that discrepancy likely comes from the difficulty of working with human fetal pancreas. Towards that end, we have continued to build upon existing methods to isolate fetal islets from human pancreases with gestational ages ranging from 12 to 23 weeks, grow the cells as a monolayer or in suspension, and image for cell proliferation, pancreatic markers and human hormones including glucagon and C-peptide. ICCs generated by the protocol described below result in C-peptide release after transplantation under the kidney capsule of nude mice that are similar to C-peptide levels obtained by transplantation of fresh tissue(6). Although the examples presented here focus upon the pancreatic endoderm proliferation and beta cell genesis, the protocol can be employed to study other aspects of pancreatic development, including exocrine, ductal, and other hormone producing cells.

Scientific Abstract:
For almost 30 years, scientists have demonstrated that human fetal ICCs transplanted under the kidney capsule of nude mice matured into functioning endocrine cells, as evidenced by a significant increase in circulating human C-peptide following glucose stimulation(1-9). However in vitro, genesis of insulin producing cells from human fetal ICCs is low(10); results reminiscent of recent experiments performed with human embryonic stem cells (hESC), a renewable source of cells that hold great promise as a potential therapeutic treatment for type 1 diabetes. Like ICCs, transplantation of partially differentiated hESC generate glucose responsive, insulin producing cells, but in vitro genesis of insulin producing cells from hESC is much less robust(11-17). A complete understanding of the factors that influence the growth and differentiation of endocrine precursor cells will likely require data generated from both ICCs and hESC. While a number of protocols exist to generate insulin producing cells from hESC in vitro(11-22), far fewer exist for ICCs(10,23,24). Part of that discrepancy likely comes from the difficulty of working with human fetal pancreas. Towards that end, we have continued to build upon existing methods to isolate fetal islets from human pancreases with gestational ages ranging from 12 to 23 weeks, grow the cells as a monolayer or in suspension, and image for cell proliferation, pancreatic markers and human hormones including glucagon and C-peptide. ICCs generated by the protocol described below result in C-peptide release after transplantation under the kidney capsule of nude mice that are similar to C-peptide levels obtained by transplantation of fresh tissue(6). Although the examples presented here focus upon the pancreatic endoderm proliferation and beta cell genesis, the protocol can be employed to study other aspects of pancreatic development, including exocrine, ductal, and other hormone producing cells.

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