Use of Embryonic Stem Cells to Produce Islets in Cell Replacement Therapy as a Treatment for Type 1 Diabetes

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Embryonic stem(ES) cells are undifferentiated, pluripotent cells, which are isolated from the inner cell mass of the blastocyst, with a capacity for proliferation and self-renewal as well as differentiation. When ES cells grow to high density or on non-adherent plates they form aggregates referred to as embryoid bodies(EBs). Within EBs, cells undergo spontaneous differentiation into cells from all three embryonic lineages, ectoderm, endoderm, and mesoderm. Our long-term goals are to use ES cells to generate islet-like structures capable of reversing hyperglycemia through the regulated secretion of insulin and to define the mechanism for ES cell differentiation into islets. This study explored whether the expression of PDX-1, a transcription factor important for both pancreatic development and β-cell function, in ES cells will facilitate the generation of islets with a capacity for insulin secretion that approximates that of native islets.

Expression of PDX-1 was achieved using an adenoviral vector. Ad-β-Gal, which is a replication defective adenovirus 5 (Ad5) vector containing an expression cassette consisting of the cytomegalovirus(CMV) promoter and β-galactosidase gene, was used as a control for adenoviral infection in these studies. The adenoviral vector that was used to express PDX-1 is Ad-PDX-1, which expresses rat PDX-1 under control of the CMV promoter. For these studies, the mouse ES cell line R1 was maintained in an undifferentiated state by growth on gelatin-coated plates in ES cell medium supplemented with leukemia inhibitory factor(LIF). The cells were then treated for 19 hrs with 500 or 1,000 multiplicities of infection of either Ad-PDX-1 or Ad-β-Gal.

Virus was removed, and the cells were incubated for 12 hrs in ES cell medium supplemented with LIF. After that the cells were replaced onto nonadherent bacterial plates in ES cell medium without LIF to allow for formation of EBs and cell differentiation. The resulting EBs were collected after incubation for 5 days. Immunocytochemistry was used to demonstrate PDX-1 and insulin expression. The cells infected with Ad-β-Gal were stained with X-gal, which turns blue in the presence of β-galactosidase. To complement the immunocytochemistry studies, reverse transcriptase(RT)-polymerase chain reaction(PCR) was used to demonstrate PDX-1, insulin, and as a control, glyceraldehyde 3-phosphate dehydrogenase(GAPDH) mRNA in Ad-PDX-1-infected cells and other cell types.

PDX-1 as well as insulin proteins were clearly present in Ad-PDX-1-infected cells, but not in Ad-β-Gal-infected cells. Consistent with the immunocytochemistry results, PDX-1 and insulin mRNAs were present in Ad-PDX-1-infected cells, and the expression was similar to that in native mouse islets and MIN-6 cells, a mouse β-cell line that produces insulin. In contrast to Ad-PDX-1-infected cells, PDX-1 and insulin mRNA were not present in Ad-β-Gal-infected cells. These data demonstrate that PDX-1 expression induces insulin production in EBs.

References

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