Cell Therapy for Diabetes: Expansion and re-differentiation of adult human pancreatic islet cells for transplantation (Ramot)

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The insulin-producing pancreatic islet β cells are destroyed in type 1 diabetes (T1D) by autoimmunity, and malfunction in type 2 diabetes (T2D), which together afflict >5% of world population. Restoration of a functional β-cell mass by regeneration or transplantation represents the ultimate therapeutic goal in both types of diabetes. Currently cell transplantation is severely limited by the availability of deceased human pancreas donors.

UNMET NEED

Efforts for developing abundant β-cell sources for transplantation have focused on 3 main approaches: Deceased donor β-cell expansion in vitro, differentiation of pluripotent stem cells, and reprogramming of other cell types into β-like cells. Our group has developed ways for expansion of adult human islet cells in vitro. However, these conditions result in loss of β-cell phenotype [1]. Using cell-lineage tracing, we tracked the fate of β cells in these cultures [2], and demonstrated that β-cell-derived (BCD) cells significantly proliferate in vitro for up to 16 population doublings, in a process involving dedifferentiation and epithelial-mesenchymal transition EMT [3]. These results were reproducible with islet cells obtained from multiple adult human donors, aged 19-62.

OUR SOLUTION

Our studies revealed the key role of activation of the NOTCH, WNT, and TGFβ pathways signaling in BCD cell dedifferentiation [4-6]. Blocking signaling of the activated pathways using shRNAs directed against transcripts encoding their mediators significantly reduced cell dedifferentiation, but also prevented induction of cell proliferation, suggesting that the bulk of the replication effect of growth factors in cultured β cells is exerted through signaling pathways which also induce EMT and dedifferentiation. Thus, significant replication of BCD cells in this system can only be achieved at the cost of cell dedifferentiation. Despite loss of β-cell gene expression, BCD cells maintain open chromatin structure in key β-cell genes [7], suggesting they may be amenable to redifferentiation. Blocking the NOTCH, WNT, and TGFβ pathways in BCD cells following in-vitro expansion using shRNAs induced cell growth arrest, morphological and gene expression changes characteristic of mesenchymal-epithelial transition (MET), and activation of expression of β-cell genes [5,6,8]. Each of these treatments synergized with a combination of soluble factors, termed Redifferentiation Cocktail (RC) [9,10], to further improve redifferentiation efficiency, up to 60% of BCD cells. The redifferentiated cells were monohormonal, contained about 10% of the insulin content of normal β cells, manifested glucose-stimulated insulin secretion, and were capable of correction of hyperglycemia in immunodefficient mice. Combined inhibition of several pathways also enhanced BCD redifferentiation.

OUR PRODUCT

Based on our results using shRNA-mediated pathway inhibition, we have developed a combination of small-molecule inhibitors of signaling pathways involved in EMT, which can induce redifferentiation of >90% of BCD cells. The cell phenotype is stable following compound removal. Thus, we now have in place a complete process of in-vitro expansion and redifferentiation of human β-cells, which does not require gene transfer. The resulting cells can be used for cell therapy, drug evaluation, and toxicity studies.

DIFFERENTIATION

Our approach provides a robust and highly reproducible process for in-vitro expansion and redifferentiation of human β-cells, in contrast to alternative approaches for development of abundant β-cell sources, which have been difficult to reproduce with cells from multiple human donors.

PATENTS

WO2006054305 titled “Populations of expanded and re-differentiated adult islet beta cells capable of producing insulin and methods of generating same”

WO2009078012 titled “Methods of generating expanded and re-differentiated adult islet beta cells for use in the treatment of diabetes”

WO2012035539 titled “Methods of expanding and redifferentiating islet beta cells”

US provisional patent application

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