Title

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Journal

Transplantation, 107(10S2)

ISSN

0041-1337

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Publication Date

2023-10-01

DOI

10.1097/01.tp.0000994680.51057.c0

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ILV-001 a highly promising therapeutic agent for islet transplantation

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Introduction: Our team has shown a bimodal effect on β-cells of muscle-secreted myokines from normal or insulin-resistant human skeletal muscle. We identified ILV-001, a component of the muscle secretome regulated by muscle fiber type and physical exercise, moreover naturally present in the extracellular matrix of the islet niche. Thus, we aimed at investigating the therapeutic potential of ILV-001 in protecting β-cell function and survival during islet transplantation.
Methods: In vitro: Rat islets were incubated 24h ± ILV-001 (1µg/mL). Islet survival was assessed by TUNEL assay ± cytomix. Islet function was evaluated using GSIS test.
In vivo: 10000 IEQ/kg ± ILV-001 were injected intraportaly in diabetic syngeneic rats. For 3 months body weight gain, glycaemia, c-peptidemia and graft function (IPGTT) were followed. Inflammation was evaluated titrating plasmatic α2-macroglobulin. Histology was performed on grafted islets.
Results: In vitro: ILV-001 improves islet function and survival and potentiates insulin secretion after GSIS test.
In vivo: ILV-001 improves graft function in terms of glycaemia ($p<0.001$) and c-peptidaemia ($p<0.01$) and decreases exogenous insulin intake requirements. ILV-001 reduced inflammation 24h post graft with a lower plasmatic α2-macroglobulin versus control with respectively 459±70 versus 903±191 µg/mL ($p<0.05$). Finally, histology showed a preservation of insulin positive cells with ILV-001 versus control at the end of study (69±3% versus 39±3% of β-cells/islet, $p< 0.001$).
Conclusion: ILV-001 is a highly promising candidate for the achievement of a functional cure in T1D thanks to potent β-cell preservation and obtaining long term graft maintenance following islet transplant.

410.8

Improving the safety of stem-cell-derived beta cell transplantation with an inducible safety switch

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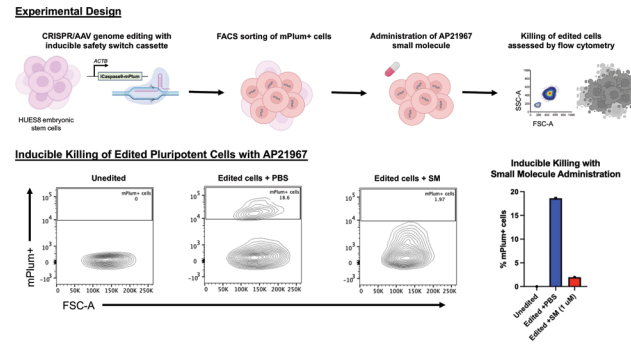
Introduction: In patients with type 1 diabetes mellitus, glucose homeostasis can be re-established with allogeneic islet transplantation. This approach has the advantage of overcoming the surgical morbidity of whole pancreas transplant. However, widespread applicability of both therapies is limited by a paucity of deceased donors. Pluripotent stem cell-derived islets offer a potentially renewable source of functional beta cells, but a major limitation of stem cell-derived therapies is the risk of teratoma formation following transplantation. Therefore, for stem cell-derived islet transplantation to reach its full therapeutic potential, safety concerns over iatrogenic tumorigenesis and teratoma formation must be overcome. Here, we describe the results of a CRISPR/AAV-mediated genome engineering strategy to integrate an inducible safety switch into the HUES8 embryonic stem cell line. This approach may impart a drug-inducible safeguard into stem cell-derived beta cells, allowing for eradication of the complete allograft in the case of an adverse event.
Methods: We identified a Cas9 guide RNA specific to the 3' UTR of the ACTB housekeeping gene, which is ubiquitously expressed in all cells. We then designed an iCaspase9-mPlum expression cassette, whose expression is induced by the small molecule A/C heterodimerizer, AP21967. This cassette was then integrated by an AAV6 vector into luciferase-expressing HUES8 cells, linking expression of our integration cassette to ACTB expression. Edited HUES8 cells were sorted by fluorescence activated cell sorting and expanded in vitro. Small molecule was then added and killing of edited cells was assessed by flow cytometry.
Results: HUES8 cells were successfully edited as seen by mPlum positivity on flow cytometry, with both monoallelic and biallelic edited populations obtained. Addition of AP21967 led to killing of both populations, demonstrating the ability to induce apoptosis in edited cells expressing our transgene cassette via the control of a small molecule.



Conclusions: We demonstrate successful editing of HUES8 embryonic stem cells and integration of an inducible safety switch into the ACTB locus, allowing inducible killing of pluripotent cells. Further testing to validate that genome engineering has not compromised efficiency of downstream stem cell-derived beta cell differentiation or function is underway. These encouraging findings support further testing to validate the ability of this ACTB-driven switch to reduce teratoma risk of edited stem cell-derived beta cells using in vivo mouse transplantation experiments.

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Expansion and differentiation of human pancreas-derived islet cell precursors into functional islet cell organoids

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Background: Emerging technologies in human embryonic stem cell (hES) and inducible pluripotent stem cell (iPSC) differentiation protocols designed to mimic stages of islet cell development have led to promising sources of stem-cell derived β-like cells for potential islet cell transplantation. We recently isolated adult human islet cell precursors (hICPs) from COBE bag remnants obtained during islet cell isolation procedures. We hypothesized that hICPs could be expanded and differentiated into functional islet-like organoids for use in islet cell transplantation.
Methods: Adult hICP gene marker expression was compared to stages of hES-derived β- and α-like cells single-cell transcriptome profiles. Proliferation of hICPs was stimulated by cytokines, stressed islet exosomes, and GSK3β/DYRK inhibitors. Small molecule ISX9 was used to stimulate calcineurin (CN)/NFAT signaling and islet cell differentiation. High throughput QPCR arrays measured cell replication and survival genes. QPCR was used to validate and measure expression of cell cycle genes and islet cell differentiation transcription factors. Flow cytometry was used to identify RGS16+ and Nestin+ hICPs and determine β:α-cell ratios in differentiated islet cell organoids. ELISA was used to measure release of insulin and glucagon from islet cell organoids upon high and low glucose stimulation, respectively.
Results: Adult hICPs were determined to be equivalent to stages 5-7 of hES-derived pancreatic endocrine precursors by reference to islet progenitor markers RGS16 and Nestin. Treatment of hICPs with exosomes purified from stressed human islet cells induced cell cycle gene expression and expansion of islet cell organoids. Gene profiling analyses of hICPs treated with ISX9, harmine, and GNF4877 promoted NFAT signaling and induction of several cell cycle and anti-apoptotic genes. Stimulated CN/NFAT signaling directly upregulated transcription factors identified to drive efficient islet cell differentiation. Hh/SMO inhibition promoted β-cell differentiation and Hh agonists induced genes to favor α-like cells. Differentiated islet cell organoid products released 16.8 ± 1.7 pg/IEQ/30 min insulin in response to high glucose and 40.5 ±8.5 pg/IEQ/30 min glucagon in response to low glucose which were comparable to those of human islets.
Conclusion: These results demonstrate feasibility to selectively target CN/NFAT, AKT/GSK3β, and Hh/SMO signaling components in adult hICPs to induce expansion and differentiation into islet cell organoids with functional β- and α-like phenotypes. Further standardization of protocols to generate functional islet organoids from adult hICPs could provide widespread utility for islet cell replacement strategies for islet transplantation.