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SHORT COMMUNICATION

Intrapancreatic delivery of human umbilical cord blood aldehyde dehydrogenase-producing cells promotes islet regeneration

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Abstract

Aims/hypothesis We sought to investigate the stimulation of islet regeneration by transplanted human umbilical cord blood (UCB) cells purified according to high aldehyde dehydrogenase (ALDH) activity (ALDH^{hi}), a conserved characteristic of multiple progenitor lineages. We hypothesised that direct intrapancreatic (iPan) delivery of ALDH^{hi} progenitors would augment islet regeneration via timely and localised exposure to islet-regenerative stimuli.

Methods Cells were purified from UCB based on flow cytometry for low ALDH activity (ALDH^{lo}) vs ALDH^{hi}. UCB ALDH^{lo} or ALDH^{hi} cells were compared for surface marker expression, as well as haematopoietic, endothelial and multipotent stromal progenitor content in vitro. UCB ALDH^{lo} or ALDH^{hi} cells were i.v. or iPan injected into streptozotocin-treated non-obese diabetic/severe combined immune-deficient mice temporally monitored for blood glucose, serum insulin and glucose tolerance. Human cell recruitment and survival in the pancreas, insulin content, islet-associated cell proliferation and islet vascularisation were documented in situ.

Results UCB-derived ALDH^{hi} cells were highly enriched for haematopoietic and endothelial progenitor frequency, and showed increased expression of progenitor and myeloid cell surface markers. Although i.v. transplantation of ALDH^{hi} cells demonstrated low pancreas engraftment and only transient blood glucose lowering capacity, iPan injected ALDH^{hi} cells reversed established hyperglycaemia, increased serum insulin and improved the response to a glucose challenge. iPan injected ALDH^{hi} cells surrounded damaged islets at early time points and increased islet-associated cell proliferation, resulting in the recovery of beta cell mass.

Conclusions/interpretation iPan delivery of UCB ALDH^{hi} cells potentiated islet-associated cell proliferation, insulin production and islet revascularisation, resulting in the recovery of host islet function. Elucidation of the progenitor-specific pathways stimulated during islet regeneration may provide new approaches to promote islet expansion during diabetes.

Keywords Aldehyde dehydrogenase · Beta cell proliferation · Cell therapy · Diabetes · Intrapancreatic transplantation · Islet regeneration · Islet revascularisation · Umbilical cord blood

Abbreviations

ALDH	Aldehyde dehydrogenase
BM	Bone marrow
CFC	Colony-forming cell
EdU	5-Ethynyl-2′-deoxyuridine
iPan	Intra-pancreatic
NOD/SCID	Non-obese diabetic/severe combined immune-deficient
STZ	Streptozotocin
UCB	Umbilical cord blood
vWF	von Willebrand factor

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Introduction

Residual islet function in diabetes patients with disease duration of >50 years suggests that the stimulation of islet regeneration may represent a viable strategy for diabetes treatment [1]. There exists increasing evidence that bone marrow (BM)-derived cells promote islet recovery after transplantation [2–4], and we have recently shown that the mechanisms of islet regeneration are modulated by the progenitor subtypes administered [5]. While transplanted human multipotent stromal cells stimulated the formation of small islets associated with ducts, i.v. transplanted BM with high aldehyde dehydrogenase (ALDH) activity ($ALDH^{hi}$) induced islet cell proliferation and led to the recovery of larger and highly perfused islets [5]. As a readily available alternative to BM, we postulated that transplantation of umbilical cord blood (UCB)-derived $ALDH^{hi}$ cells would stimulate islet expansion and vascularisation. Since human cell recruitment to the pancreas is inefficient following i.v. transplantation [5], we intended to determine if UCB $ALDH^{hi}$ cells delivered directly to the pancreas would permit timely exposure to regenerative stimuli and potentiate the recovery of islet function.

Methods

Cell isolation and characterisation Human UCB was obtained by venipuncture after informed consent at the London Health Sciences Centre. Within 24 h, mononuclear cells were isolated by Ficoll-Hypaque centrifugation, and cells with low ALDH activity ($ALDH^{lo}$), as well as $ALDH^{hi}$ cells, were purified by cell sorting based on ALDH activity using Aldefluor reagent (Stem Cell Technologies, Vancouver, BC, Canada) as previously described [6]. $ALDH^{lo}$ and $ALDH^{hi}$ cells were characterised for mature haematopoietic and primitive progenitor marker expression, and colony-forming cell (CFC) assays were performed for haematopoietic, endothelial and multipotent stromal progenitor functions as previously described [7].

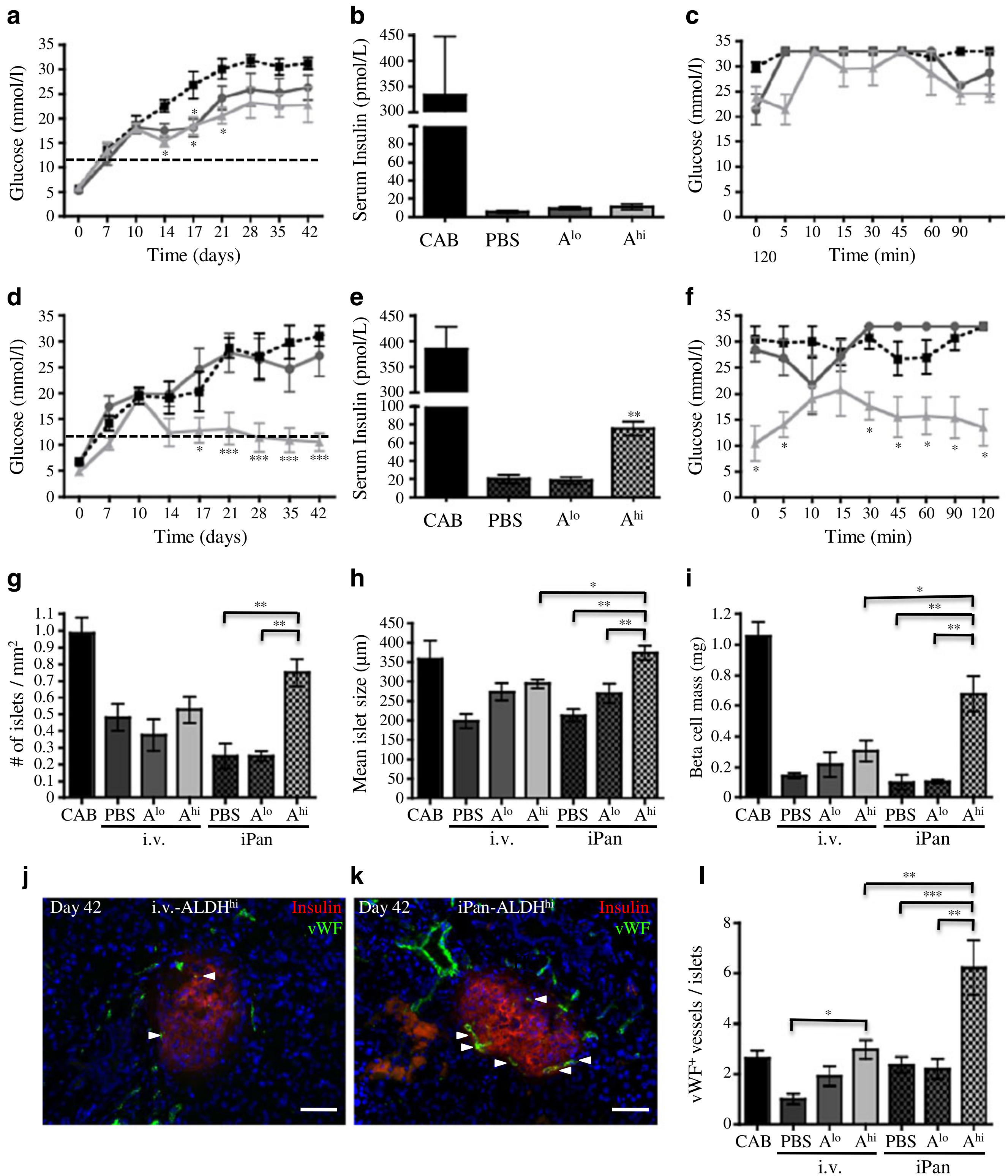
Transplantation of hyperglycaemic mice Non-obese diabetic/severe combined immune-deficient (NOD/SCID) mice (Jackson Laboratory, Bar Harbor, ME, USA) were injected with streptozotocin (STZ) 35 mg/kg per day i.p. on days 1–5. At day 10, mice were sublethally irradiated (300 cGy) to reduce residual innate immunity and transplanted by tail vein or intrapancreatic (iPan) injection with PBS or 2×10^5 $ALDH^{lo}$ or $ALDH^{hi}$ cells from a fresh UCB sample. For iPan injections, mice were anaesthetised, the pancreas and spleen exposed, and cells were microinjected (10 μl) into the splenic portion of the pancreas. Non-fasted blood glucose was monitored weekly. Twenty-four hours prior to being killed, each mouse received 200 μg 5-ethynyl-2′-deoxyuridine (EdU), and a fasted (4 h) glucose tolerance test (2.0 g/kg glucose) was performed for a duration of 2 h. Serum was collected for insulin ELISA (Alpco, Salem, NH, USA). BM and pancreas were analysed for human cells by flow cytometry as previously described [5, 7].

Immunohistochemistry Frozen pancreas sections were stained for immunofluorescent analyses to detect murine insulin with human cell engraftment (HLA-A,B,C), blood vessel density (von Willebrand factor; vWF) and EdU incorporation as previously described [5].

Results

UCB $ALDH^{hi}$ cells possessed haematopoietic and endothelial progenitor phenotypes and functions We first characterised UCB $ALDH^{lo}$ vs $ALDH^{hi}$ cells for cell surface marker expression and for haematopoietic, endothelial and multipotent stromal colony formation in vitro. Compared with UCB $ALDH^{lo}$ cells that primarily expressed lymphocyte markers, $ALDH^{hi}$ cells highly expressed myeloid (CD33) and haematopoietic/endothelial progenitor markers (CD34, CD117, CD133; see electronic supplementary material

Fig. 1 iPan delivery of UCB $ALDH^{hi}$ cells augments the recovery of endocrine functions via increased islet size and vascularisation. Blood glucose, serum insulin and glucose tolerance measurements were performed on i.v. transplanted vs iPan transplanted mice. **a** Compared with mice i.v. injected with PBS (black squares, dashed line, n=6) or mice i.v. injected with 2×10^5 $ALDH^{lo}$ cells (dark grey circles, n=8), mice i.v. injected with 2×10^5 $ALDH^{hi}$ cells (light grey triangles, n=9) showed a transient reduction (days 14–21) in blood glucose that returned to hyperglycaemic levels by days 28–42. The dotted line marks hyperglycaemia (>12 mmol/l), defined as a twofold increase from basal glucose concentrations. (*p<0.05 vs PBS). **b** At day 42, all i.v. transplanted mice showed reduced serum insulin concentrations, and (**c**) were unable to respond to a glucose challenge. **d** Mice iPan injected with 2×10^5 $ALDH^{hi}$ cells (light grey triangles, n=6) showed significantly improved blood glucose from days 17 to 42, compared with mice iPan injected with PBS (black squares, dashed line, n=6) or $ALDH^{lo}$ cells (dark grey circles, n=7). (*p<0.05, ***p<0.001 vs PBS). **e** Mice iPan injected with $ALDH^{hi}$ cells showed elevated serum insulin, and (**f**) improved response to glucose challenge at day 42 (*p<0.05 vs PBS). Compared with mice iPan injected with PBS (n=3) or $ALDH^{lo}$ cells (n=4), mice iPan injected with $ALDH^{hi}$ cells (n=4) also showed increased (**g**) islet number, (**h**) islet size and (**i**) total beta cell mass at day 42. iPan delivery of $ALDH^{hi}$ cells also increased islet size and total beta cell mass compared with i.v. injected $ALDH^{hi}$ cells. Representative photomicrographs of mouse pancreas stained for murine insulin and vWF at day 42 after (**j**) i.v. injection or (**k**) iPan injection with 2×10^5 $ALDH^{hi}$ cells. Arrowheads mark vWF^+ vascular structures in islets. Scale bars, 50 μm. **l** Compared with mice iPan injected with PBS or $ALDH^{lo}$ cells, mice iPan injected with $ALDH^{hi}$ cells increased islet vascularisation at day 42. iPan delivery of $ALDH^{hi}$ cells also increased islet vascularisation compared with i.v. injected $ALDH^{hi}$ cells. Cells from a total of five UCB samples were used for day 42 analyses. Data are presented as mean ± SEM ($^*p$<0.05, $^{**}p$<0.01, $^{***}p$<0.001). A, ALDH; CAB, citric acid buffer vehicle control



[ESM] Table 1). In contrast to BM ALDH^{hi} cells that possessed CFC capacity for all three-progenitor lineages [7], UCB ALDH^{hi} cells were enriched for multipotent haematopoietic and endothelial CFC (ESM Fig. 1a–e), but did not establish multipotent stromal colonies (ESM Fig. 1c, f). Thus, UCB ALDH^{hi} cells represent a mix of early myeloid cells and haematopoietic progenitors with endothelial progenitor content.

iPan delivery of UCB ALDH^{hi} cells improved endocrine function To investigate whether direct iPan delivery of UCB progenitors could induce islet regeneration, STZ-treated NOD/SCID mice were i.v. or iPan injected with PBS or dose-matched UCB ALDH^{lo} or ALDH^{hi} cells. After i.v. transplantation of ALDH^{hi} cells on day 10, blood glucose was transiently reduced in comparison with that of PBS controls. However, from days 17 to 42, blood glucose gradually increased towards severe hyperglycaemia (>20 mmol/l; Fig. 1a). Serum insulin (Fig. 1b) and response to glucose challenge (Fig. 1c) at day 42 were low for all i.v. transplanted groups. By contrast, iPan delivery of UCB ALDH^{hi} cells reduced hyperglycaemia within 7 days ($^{*}p<0.05$), and blood glucose remained lower than pretransplant levels for >1 month (Fig. 1d), indicating immediate and stable recovery from established hyperglycaemia. Compared with mice iPan-injected with PBS or ALDH^{lo} cells, iPan ALDH^{hi} transplanted mice showed increased serum insulin (Fig. 1e) and improved response to glucose challenge at day 42 (Fig. 1f).

iPan delivery of UCB ALDH^{hi} cells increased islet number, size and vascularisation We postulated that iPan delivery of UCB ALDH^{hi} cells would augment pancreas islet content. Compared with delivery-matched PBS or ALDH^{lo} cells, iPan injection of ALDH^{hi} cells increased both the number and size of islets, and augmented total beta cell mass (Fig. 1g–i). Furthermore, islet size and total beta cell mass were increased compared with i.v. injection of ALDH^{hi} cells ($^{*}p<0.05$). Although i.v. delivery of ALDH^{hi} cells modestly increased islet-associated vascularisation (Fig. 1j, l), iPan delivery of ALDH^{hi} cells further improved islet vascularisation (Fig. 1k, l). These effects were observed despite low-frequency human cell engraftment in the pancreas of mice i.v. injected (four of nine mice) or iPan injected (four of five mice) with ALDH^{hi} cells at day 42 (ESM Fig. 2a–b). By contrast, ALDH^{lo} cells were rarely detected in the pancreas after i.v. or iPan injection. Only ALDH^{hi} cell i.v. transplanted mice showed haematopoietic reconstitution in the murine BM (ESM Fig. 2c–d). Collectively, these data suggested that further experiments were warranted to characterise islet regenerative processes in relation to human cell pancreatic engraftment at early time points (days 14–17).

iPan-injected UCB ALDH^{hi} cells surrounded islets and stimulated islet cell proliferation To investigate human cell engraftment during hyperglycaemic reduction, mouse pancreases were isolated at days 14 and 17 and stained for cells producing insulin and HLA-A,B,C. At day 14, HLA^{+} cells were very infrequent or absent in mice iPan injected with ALDH^{lo} cells (ESM Fig. 2e). By contrast, iPan injected ALDH^{hi} cells showed improved survival and HLA^{+} cells were numerous throughout the pancreas (Fig. 2a). By day 17, when insulin production was sufficient to clearly identify regenerating islets, iPan delivered ALDH^{hi} cells were localised surrounding islets (Fig. 2b). Although few human cells remained at day 42 (arrows, Fig. 2c), HLA^{+} cells never produced insulin (Fig. 2a–c). At days 14 and 17, human cell engraftment was not detected in the pancreas of any i.v. transplanted mice (data not shown). These data suggested that improved ALDH^{hi} cell survival and recruitment to islets correlated with the temporal recovery of insulin production.

Next we assessed islet-associated cell proliferation using EdU labelling 24 h prior to sacrifice. While islet-associated proliferation was minimal in mice iPan injected with ALDH^{lo} cells (ESM Fig. 2f), mice iPan injected with ALDH^{hi} cells demonstrated EdU labeling in both insulin^{-} (arrowheads) and insulin^{+} cells (arrows, Fig. 2d, e). By day 17, the percentage of EdU^{+} cells within islets was increased (Fig. 2f), as was the frequency of insulin^{+} EdU^{+} cells (ALDH^{hi} 29.5±3.4% vs ALDH^{lo} 6.3±1.2%; $^{*}p<0.05$). Corresponding to islet proliferation at early time points, iPan ALDH^{hi} transplanted mice showed an increase in non-fasted serum insulin (Fig. 2g, i). However, serum insulin levels remained below threefold lower than citric acid buffer controls. Although iPan transplanted mice did not demonstrate a measurable response to glucose bolus at day 14 (Fig. 2h), iPan ALDH^{hi} transplanted mice demonstrated improved glucose tolerance by day 17 (Fig. 2j). Collectively, these data suggested that within 4–7 days of iPan administration, increased ALDH^{hi} cells surrounding islets correlated with increased proliferation of insulin^{+} and insulin^{-} cells, resulting in augmented insulin production and improved endocrine functions.

Discussion

This study demonstrates the capacity of UCB-derived ALDH^{hi} cells to promote islet regeneration when transplanted directly into the murine pancreas. UCB ALDH^{hi} cells represented a heterogeneous mixture of myeloid cells and haematopoietic/endothelial progenitors readily available for the development of novel cellular therapies for type 1 or type 2 diabetes through the recent establishment of UCB registries for allogeneic transplantation. While i.v. transplantation of UCB ALDH^{hi} cells leads to minimal recovery of islet function, iPan delivery of UCB ALDH^{hi} cells leads to reversal of established hyperglycaemia, increased serum insulin and improved glucose tolerance within 7 days of transplantation. To our knowledge this work represents the first report documenting potent islet recovery after iPan delivery of clinically applicable progenitors isolated from human UCB.

Islet cell proliferation correlated with the presence of UCB ALDH^{hi} cells surrounding damaged islets, and islet

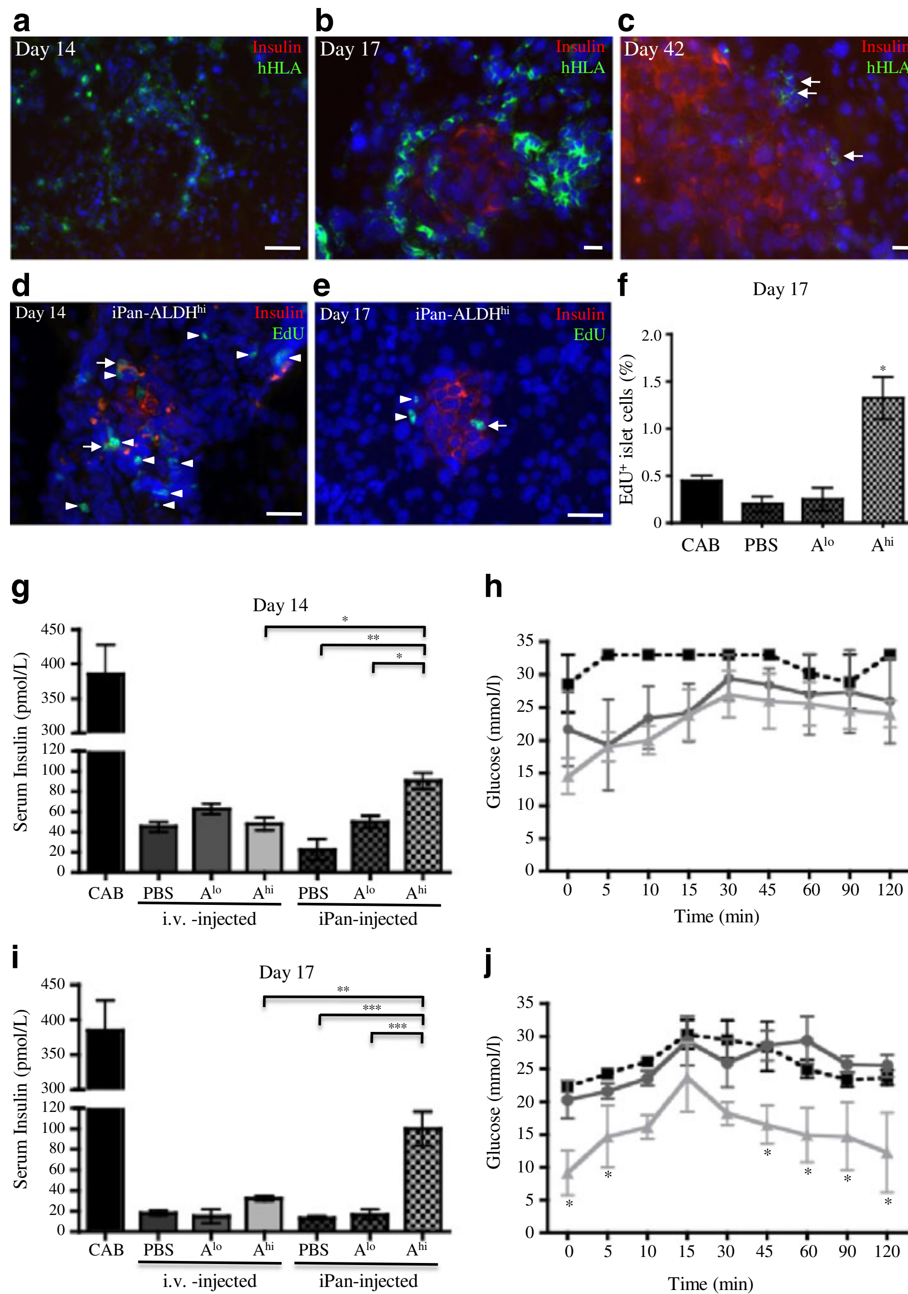


Fig. 2 iPan delivery of UCB ALDH^{hi} cells formulates a niche for islet regeneration. **a,b** Representative photomicrographs of human cell engraftment within the pancreas 4 (day 14) and 7 (day 17) days after iPan injection of UCB ALDH^{hi} cells. **c** At day 42, few HLA^{+} cells were detected surrounding insulin^{+} islets. Arrows mark HLA-A^{+}, -B^{+} and -C^{+} cells. **d,e** Representative photomicrographs demonstrating EdU^{+} cells within regenerating islets at 4 (day 14) and 7 (day 17) days following iPan injection of ALDH^{hi} cells. Arrowheads mark insulin^{-}/EdU^{+} cells associated with islets. Arrows mark insulin^{+}/EdU^{+} cells within islets. Scale bars, 50 µm. **f** At day 17, mice iPan injected with ALDH^{hi} cells (n=4) demonstrated an increased frequency of EdU^{+} cells within islets compared with mice iPan injected with PBS (n=3) or ALDH^{lo} cells (n=4) (*p<0.05 vs PBS). **g** At day 14, mice iPan injected with ALDH^{hi} cells (n=3) showed increased non-fasted serum insulin compared with mice iPan injected with PBS (n=3) or ALDH^{lo} cells (n=3). **h** However, at day 14, mice iPan injected with ALDH^{hi} cells (light grey triangles) did not respond to a glucose challenge. **i** At day 17, mice iPan injected with ALDH^{hi} cells (n=4) showed increased non-fasted serum insulin compared with mice iPan-injected with PBS (n=3) or ALDH^{lo} cells (n=4). iPan delivery of ALDH^{hi} cells also increased non-fasted serum insulin compared with i.v. injected ALDH^{hi} cells (n=3). **j** By day 17, mice iPan injected with ALDH^{hi} cells (light grey triangles) demonstrated improved glucose tolerance compared with mice iPan-injected with PBS (black, n=3) or ALDH^{lo} cells (dark grey circles, n=3) (*p<0.05 vs PBS). Cells from a total of three UCB samples were used for day 14 and 17 analyses. Data are represented as mean ± SEM ($^{*}p$<0.05, $^{**}p$<0.01, $^{***}p$<0.001). CAB, citric acid buffer vehicle control; A, ALDH

number, size and vascularisation were increased 1 month post-transplantation. Although iPan injected ALDH^{lo} cells were occasionally detected in the pancreas at early time points, these cells failed to induce islet-associated proliferation and subsequent recovery of function. Thus, islet regeneration was promoted after timely local exposure to ALDH^{hi} cells, suggesting that islet proliferative or proangiogenic stimuli were provided specifically by iPan injected ALDH^{hi} cells.

Clinical evidence suggests that strategies employing BM or UCB stem cells can potentially benefit diabetic patients via beta cell regenerative or immunomodulatory mechanisms [8, 9]. However, Haller and colleagues recently reported that i.v. infusion of unfractionated UCB cells induced changes in regulatory T lymphocyte frequency but failed to preserve C-peptide [9]. Although our study did not directly address potential immunomodulatory mechanisms, or the activation of putative islet-derived beta cell precursors that may survive STZ toxicity [10], these experiments establish proof of concept that iPan delivery of purified ALDH^{hi} progenitor cells can formulate a regenerative niche that impacts the behaviour and function of regenerating host islets. Further investigation is warranted to elucidate the molecular pathways activated in signal-receiving beta cells or beta cell precursors during ALDH^{hi} cell-stimulated islet regeneration, as iPan delivery of UCB ALDH^{hi} cells may represent a viable strategy to 'tip the balance' in favour of islet expansion vs destruction during diabetes.

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

Contribution statement GIB and DAH contributed to the conception and design, analysis and interpretation of data, and writing and revision of the manuscript. DMP and JMH-L contributed to the analysis and interpretation of data, and revising the manuscript critically for important intellectual content. All authors approved the final manuscript. All authors are affiliated with the Krembil Centre for Stem Cell Biology, Robarts Research Institute, Vascular Biology Group, Department of Physiology and Pharmacology, University of Western Ontario.

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