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PDGs act like stem cell niche in type 1 diabetes

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Context: Pancreatic duct glands (PDGs) have been proposed as a source of regeneration in response to exocrine pancreas injury, and thus may serve as an organ stem cell niche. There is evidence to suggest ongoing beta-cell formation in longstanding type 1 diabetes (T1D), but the source is unknown.

Objective: To investigate the pancreatic duct gland (PDG) compartment of the pancreas in humans with T1D for evidence of an active regenerative signature (presence of progenitor cells and increased proliferation) and, in particular, as a potential source of beta-cells.

Design, Setting and Participants: Pancreas from 46 brain dead organ donors (22 with T1D, 24 nondiabetic controls) were investigated for activation (increased proliferation) and markers of pancreatic exocrine and endocrine progenitors.

Results: PDG cell replication was increased in T1D (6.3 ± 1.6 vs. 0.6 ± 0.1%, $p < 0.001$, T1D vs. ND), most prominently in association with pancreatic inflammation. There were increased progenitor-like cells in PDGs of T1D, but predominantly with an exocrine fate.

Conclusion: The PDG compartment is activated in T1D consistent with a response to ongoing inflammation, and via resulting ductal hyperplasia may contribute to local obstructive pancreatitis and eventual pancreatic atrophy characteristic of T1D. However, there is no evidence of effective endocrine cell formation from PDGs.

PRECIS: PDGs serve as a stem cell niche in the pancreas. Replication in PDGs is increased in T1D, implying ongoing inflammation, but the majority of cells in PDGs have an exocrine rather than endocrine fate.

Abbreviations: ChrgA, Chromogranin A. T1D, type 1 diabetes. ND, nondiabetic. PDGs, pancreatic duct glands.

INTRODUCTION

In longstanding type 1 diabetes (T1D), there is a near complete loss of pancreatic beta-cells through autoimmune mediated cell death (1, 2). However, several lines of evidence suggest that there may be ongoing attempted beta-cell regeneration, although insufficient to be clinically meaningful. For example, even in individuals with longstanding T1D, there are still detectable insulin-expressing cells, often isolated and scattered in the exocrine pancreas or in small clusters in and around pancreatic ducts (3, 4). Consistent with this, by use of sensitive assays, residual insulin secretion is often present in individuals with longstanding T1D (5-8).

Theoretically, these observations may indicate that there are small populations of beta-cells that elude immune detection or that there is ongoing new beta-cell formation subject to continued autoimmune destruction. In favor of the latter explanation, auto-reactive T-cells targeted to pancreatic beta-cells are frequently detected many years after diabetes onset, implying sustained auto-reactivity (9). In pancreas of individuals with T1D who have sufficient residual beta-cells to evaluate, we previously reported immune cells in close proximity to insulin-expressing cells and an increased frequency of beta-cell apoptosis (3). Also, consistent with other diseases with ongoing autoimmune mediated inflammation, the pancreas in individuals with T1D is subject to progressive fibrotic changes and loss of volume, although this may also reveal that the target of autoimmunity in T1D includes the exocrine pancreas as well as beta-cells (4, 10, 11).

Given the possibility that there may be ongoing beta-cell formation in T1D, we considered potential sources of these cells. The pancreatic duct glands (PDGs) have been proposed as a potential pancreatic stem cell niche (12-14). The purpose of the present investigation was to investigate the PDG compartment of the pancreas in humans as a potential source of newly forming beta-cells in T1D.

Materials AND METHODS

Study subjects (Table 1).

Pancreata were procured from brain dead organ donors by the JDRF Network for Pancreatic Organ Donors with Diabetes (nPOD), administered by the University of Florida, Gainesville, Florida. All procedures were in accordance with federal guidelines for organ donation and the University of Florida Institutional Review Board. 22 pancreata from individuals with type 1 diabetes (T1D) and 24 from nondiabetic (ND) controls matched by age, sex and BMI were examined in this study. The T1D and control cases were age and BMI matched (Supplementary Figure 1). The BMI was not available in two T1D donors (6050 and 6068). Since the nature of the nPOD consortium is one of a shared resource, multiple publications have used sections from the same pancreata reported here. A full listing of the publications is available from the nPOD web site http://www.jdrfnpod.org/category/publications/.

Pancreas sections and staining

A standardized preparation procedure for pancreata recovered from cadaveric organ donors is employed at the nPOD facility. Paraffin tissue sections from each region of pancreas (head, body and tail) were sequentially stained at the University of California, Los Angeles for Ki67, insulin and alcian blue by immunohistochemistry. Slides were incubated with the following primary antibodies; mouse anti-Ki67 (1:25, 4°C overnight, Dako, Carpinteria , CA , Ref # M7240), rabbit anti-insulin (1:200, 4°C overnight, Cell Signaling, Danvers, MA, Cat # 3014S). Slides were then incubated with alcian blue solution (4°C overnight, Electron Microscope Sciences, Hatfield, PA). Secondary antibodies used were biotin donkey anti-mouse (1:100, The Jackson Laboratory, Sacramento, CA) followed by peroxidase staining (Vector Peroxidase Standard PK-4000, Vector Laboratories Inc., Burlingame, CA); Biotin Donkey anti-rabbit (1:100, The Jackson Laboratory) using Alkaline Phosphatase for color development (Vector Laboratories Inc.).

Morphometric analysis.

Whole sections of pancreas stained for insulin, Ki67 and alcian blue with hematoxylin counterstain were digitally scanned using Aperio ScanScope (Aperio Technologies Inc., Vista, CA). Analysis was performed using Aperio ImageScope version 11.0.2.725. Three sections per case, 1 each from head, body and tail were analyzed, except in one T1D case [6031] and 7 control cases [6010, 6012,

6013, 6015, 6017, 6021, 6022] where only head and tail sections were available. All sections were examined and quantified in a blinded manner.

Interlobular ducts were defined as ductal structures embedded in mesenchyme and possessing a pancreatic duct gland compartment. PDGs were identified as coiled invaginations composed of columnar epithelium arising from interlobular ducts and lying within the mesenchyme surrounding those ducts. The number of interlobular duct epithelial cells and PDG cells were counted, as was the number of those cells with nuclei staining for Ki67 and cytoplasmic staining for insulin. Small ducts, defined as intralobular ducts not embedded in mesenchyme, were comparably analyzed. Numbers of cells counted for each compartment are shown in Table 2.

For assessment of PDGs as a potential pancreatic stem cell niche.
To assess the possible role of PDGs as an organ specific stem cell niche, we evaluated PDGs for expression of known lineage defining transcription factors for endocrine and exocrine fate. We evaluated sections from the 4 T1D donors that had a high frequency of replication in the PDG compartment (6064, 6089, 6135 and 6138) and 4 age and BMI-matched nondiabetic donors (6015, 6102, 6057 and 6098).

Slides were incubated at 4°C overnight with a cocktail of primary antibodies prepared in blocking solution (3% BSA in TBST) at the following combinations; rabbit anti-sox9 (1:500, EMD Millipore, Billerica, MA) plus mouse anti-Nkx6.1 (1:300, DHSB, F55A10 Iowa city, IA) and rabbit anti-sox9 (1:500) plus mouse anti-GATA4 (1:50, **Santa Cruz Biotechnology, Inc. sc-25310 X, Dallas, TX).** The primary antibodies were detected by a cocktail of appropriate secondary antibodies (Jackson ImmunoResearch Laboratories Inc., West Grove, PA); Alexa 647 conjugated donkey anti-rabbit (1:100), Cy3 conjugated donkey anti rabbit (1:200) and FITC conjugated donkey anti-mouse (1:100). Slides were counterstained to mark the nuclei using a mounting medium containing DAPI (Vectashield, Vector Labs, Burlingame, CA), and sections were viewed and analyzed as described previously (15).

To investigate the potential endocrine cell lineage of PDG cells, we evaluated Nkx2.2 as a pan-endocrine transcription factor and Nkx6.1 as a beta-cell transcription factor and combined this evaluation with a cocktail of all known pancreatic hormones as described previously (15). Sections were evaluated from 4 T1D donors (6050, 6138, 6051 and 6076) and 4 age and BMI-matched nondiabetic donors (6015, 6021, 6029 and 6134). Slides were blocked and incubated sequentially with the same antibodies as described in (15).

Statistical analysis.
Statistical analysis was performed using the student's *t* test or ANOVA analysis where appropriate (Graphpad Prism 6, La Jolla, CA). Data in graphs and tables are presented as means ± SEM. Findings were assumed statistically significant at the $p < 0.05$.

RESULTS

PDGs in human pancreas:
PDGs were readily identified in pancreatic sections from donors with T1D and controls (Figure 1). The extent of PDGs per unit area was increased in pancreas of humans with T1D (Figure 1B) with more PDG rings per section compared with the nondiabetic controls (71.0 ± 7.0 vs 55.6 ± 5.9 PDG rings per section, T1D vs ND, $p < 0.05$). The number of PDG cells/mm^2 of pancreas was also increased throughout the pancreas in T1D (head 14.5 ± 2.4 vs 9.7 ± 1.9, PDG cells/mm^2, body 10.4 ± 1.9 vs 6.5 ± 1.2, PDG cells/mm^2, tail 13.2 ± 2.0 vs 7.1 ± 0.9 PDG cells/mm^2, T1D vs ND).

However, since pancreas size is decreased in T1D, this increase in PDGs per unit cross-section of pancreas could be due to the relative loss of acinar tissue and preservation of PDGs. To address

this, we normalized the number of PDG cells/mm^2 to pancreatic weight in the cases where pancreatic regional weights were available (T1D: n = 11; ND: n = 10). Following this correction, there was no increase in PDG cells in T1D (484 ± 74 vs 548 ± 77 PDG cells/mm^2 x pancreas weight [g], T1D vs ND, p = ns) implying the apparent increase in PDG density in T1D may reflect a loss of pancreas volume rather than a selective expansion of the PDGs.

PDG cell replication in T1D.

Since a characteristic of a stem cell niche is increased proliferation under conditions of active tissue repair, we next quantified cell replication in PDGs in T1D versus controls by use of Ki67 nuclear immunoreactivity. By this approach, there was an eleven-fold increase in replication in the PDGs in T1D compared to nondiabetic controls (6.3 ± 1.6 vs. 0.6 ± 0.1%, p < 0.001, T1D vs. ND) (Figure 1C). However, on inspection of the data, it was apparent that PDG cell replication was markedly increased in pancreatic sections of 11 of the 22 individuals with T1D compared to nondiabetic controls, whereas in the pancreatic sections of the other 11 T1D donors the frequency of Ki67 positive PDG cells was comparable to that in nondiabetic controls. To probe the potential difference between these groups, T1D with high PDG replication and T1D with normal PDG replication, we subdivided the T1D donors into those with a mean PDG % positive for Ki67 of 1.2% or higher (range 1.6 to 49%) and those with a mean PDG % positive for Ki67 less than 1.2% (range 0.2 to 1.1%). This cutoff value was chosen as the mean Ki67 positive PDG % value in the "normal" PDG Ki67 positive T1D donor group then matched that of the nondiabetic donor group (0.7 ± 0.1 vs 0.6 ± 0.1%, T1D "normal" vs ND, p = ns).

To investigate the potential mechanisms accounting for the difference between the high versus normal PDG cell replication groups, we next considered both technical and patient characteristics, because of potential influences of the preterminal clinical condition (16). With respect to the former, order effect (nPOD pancreas donors are assigned case numbers sequentially); from the donor numbers used in this study (Table 1) it is apparent that nondiabetic and T1D donor pancreases were procured in a random fashion. Hospitalization stay was similar in both groups (6,026 ± 984 vs 4,915 ± 788 minutes, T1D vs ND, p = ns). The transport time (from cross clamping to processing) was, however, longer in the T1D donor group (1,107 ± 58 vs 711 ± 76 minutes, T1D vs ND, *p* < 0.001) but this, if anything, may have negatively affected Ki67 staining resulting in artificially low numbers (17). Length of hospitalization and transport time were not available in 3 of the T1D donors (6050, 6068 and 6085) and 3 of the nondiabetic donors (6029, 6091 and 6130). Slides were stained in batches of ten at UCLA, each batch including 5 T1D and 5 nondiabetic pancreas sections. The Ki67 antibody lot (Ki67 Cat# M7240, lot number 00070375) used was the same for all sections.

Within the T1D donor group, with respect to the clinical characteristics, neither age (42.7 ± 7.6 vs 29.4 ± 3.9 years, high vs normal PDG replication, p = ns), duration of diabetes (27.8 ± 7.5 vs 23.3 ± 5.7 years, high vs normal PDG replication, p = ns), gender (High: 8 males, 3 females; normal: 6 males, 5 females), nor body mass index (24.3 ± 1.4 vs 25.0 ± 1.3 kg/m2, high vs normal PDG replication, p = ns) distinguished the groups. However, in those that did have increased PDG replication, this tended to be less markedly increased with age and duration of diabetes. Within the T1D donor group, hospitalization stay was similar in both high and normal PDG replication groups (7,380 ± 1490 vs 4,808 ± 1247 minutes, high vs normal PDG replication groups, p = ns). The transport time (from cross clamping to processing) was similar in the high and normal T1D PDG replication groups (1,032 ± 77 vs 1,175 ± 87 minutes, high vs normal PDG replication, p = ns). Within the T1D donor group, length of hospitalization and transport time were not available in two

of the donors who had high PDG replication (6050 and 6085) and in one of the donors with normal PDG replication (6068).

Having excluded technical or case ascertainment bias to account for large variance in PDG cell replication in T1D, we then turned to the possibility that pancreatic inflammation in T1D might account for the difference, since the PDG compartment has been shown to respond with increased proliferation in response to experimentally induced pancreatitis (13, 18). Interestingly, of the four donors with the highest PDG cell replication, (6045, 6064, 6089 and 6138), all had regions of discernable acute pancreatitis and one donor (6064) also had insulitis and peri-insulitis. Notably, none of these individuals had known clinical pancreatitis documented in life, consistent with accumulating data that subclinical subacute chronic pancreatitis is common in both T1D and T2D, the origin of which remains unknown (19). These findings are consistent therefore with the PDG compartment being activated by pancreatic inflammation, whether originating from the exocrine compartment or with sufficient intensity from the islet compartment and surrounds.

Interlobular and intralobular duct cell replication.
It has previously been reported that under conditions of increased PDG proliferation, the adjacent epithelium in interlobular pancreatic ducts is also increased (13, 18). Replication of interlobular duct epithelial cells as evaluated by Ki67 was increased 10-fold in T1D when compared with nondiabetic cases (5.9 ± 1.7 vs. 0.6 ± 0.1 % Ki67 positive, $p < 0.01$, T1D vs. ND) (Figure 2A). The increased frequency of Ki67 positive pancreatic epithelial cells correlated with that in PDG cells in both individuals with T1D and nondiabetic controls (Figure 2E, 2F).

The percentage of intralobular duct cells positive for Ki67 was also increased in T1D when compared with nondiabetic cases (1.9 ± 0.5 vs. 0.4 ± 0.0 % Ki67 positive cells, $p < 0.01$, T1D vs. ND) (Figure 2B). Taken together, based on Ki67 immunoreactivity, there is increased replication throughout the ductal tree in T1D, most prominent in the PDG compartment and then adjacent interlobular duct epithelium but still present even in the smaller intralobular ducts.

Insulin expressing cells in PDGs and pancreatic duct cells.
Having established that the putative pancreatic stem cell niche, the PDG compartment, has the increased cell proliferation anticipated in a stem cell niche engaged in repair, we then sought to investigate if the PDG compartment might be the source of newly forming beta-cells in T1D. Insulin immune-reactive cells, though infrequent, were detected in PDGs (Figure 1A). However, the frequency of these insulin expressing PDG cells as a percentage of PDG epithelial cells was decreased in T1D (0.003 ± 0.002 vs 0.40 ± 0.08 %, T1D vs ND, $p < 0.0001$) (Figure 1D). Likewise, the percentage of insulin immune-reactive interlobular duct cells (0.005 ± 0.002 vs 0.12 ± 0.03% T1D vs ND, $p < 0.0001$) and intralobular duct cells (0.02 ± 0.01 vs 0.34 ± 0.05 %, T1D vs ND, p < 0.0001) was decreased in T1D versus controls (Figure 2C, D).

While these data argue against the PDG compartment being an effective source of new beta-cells in T1D, it is plausible that there are activated endocrine progenitors within the PDG compartment, but that any insulin expressing cells thus formed are rapidly removed through the beta-cell specific autoimmune surveillance. Therefore, we next examined PDGs for evidence of potential progenitor cells.

Pancreatic progenitors in PDGs.
Resident stem cell pools in the adult tissues emulate the progenitor cells that gave rise to the organ during embryogenesis (20). To investigate if PDGs recapitulate embryonic pancreatic progenitors we selected transcription factors that mark early pancreatic progenitors, GATA4 predominantly marks an acinar fate (21), and Sox9 predominantly marks a pancreatic duct cell fate (21)(Figure 3).

Cells expressing these transcription factors were commonly present in PDGs, and comparable in T1D and ND controls: Sox9 (68.02 ± 7.05 vs 66.78 ± 3.25 % of cells, T1D vs ND, p = ns) and GATA4 (49.6 ± 8.4 vs 37.5 ± 11.3 % of cells, T1D vs ND, p = ns,). Sox9/GATA4 double positive cells (34.7 ± 11.6 vs 35.8 ± 10.6 % of cells, T1D vs ND, p = ns) were also common. These findings are consistent with the PDG compartment serving as a source of pancreatic duct cells, and plausibly acinar cell regeneration.

To probe the PDG compartment as a potential source of endocrine cells, we also examined PDGs for cells expressing Nkx6.1, a transcription factor that first appears in the pancreatic bud (21, 22) but is later restricted to the beta-cell lineage, and Nkx2.2, a transcription factor that marks pancreatic cells with an endocrine lineage. We further refined this evaluation by simultaneously immunostaining PDGs with these transcription factors and for known islet hormones by use of an "endocrine cocktail" of antibodies. We identified very occasional NKx6.1positive/hormone cocktail positive or NKx2.2 positive/hormone cocktail positive cells in PDGs in both nondiabetic and T1D donors (Figure 4), but these were too infrequent to quantify and much less frequent than the GATA4 or Sox9 expressing PDG cells. Taken together these findings lend support to the proposal that the PDG compartment may serve as a source of exocrine pancreas regeneration in response to pancreatic injury, but there is no evidence that it serves as a meaningful source of endocrine cells.

DISCUSSION

The PDG compartment has been proposed as a potential pancreatic stem cell niche, based on its anatomic properties and its proliferative response to experimentally induced pancreatic injury (12). We investigated the PDG compartment as a potential source of newly forming beta-cells. These studies, inevitably limited by the constraints of evaluating human tissue, support the concept that the PDG compartment may serve as a source of regeneration for the exocrine pancreatic ductal tree; however, the PDG compartment does not appear to be a significant source of endocrine cells.

PDGs have been recognized for many years (23, 24) and their extent in human pancreas has recently been delineated (14). Like Carpino et al, we have noted that the pancreatic duct gland compartment is present in head, body and tail regions, thus extending throughout the length of the organ. Attention was drawn to PDGs because they proliferate in response to acute injury and express genes characteristic of early pancreatic endoderm (12, 13). Similar glandular structures (peribiliary glands) are present as crypt like outpouchings off the biliary tree and have been reported to bear multiprogenitor cells with potential ductal epithelium or endocrine fate (25, 26)

While new beta-cells can form in adult humans, for example during pregnancy (27), the source of these new beta-cells is unknown. It was proposed that new endocrine cells form from pancreatic ducts in rats based on the intimate relationship between islets and adjacent ducts (ductulo-insular complexes) (28). However, most (29-34) but not all (35) lineage tracing studies have ruled out pancreatic duct or acinar cells as precursors of beta-cells in postnatal mice. While it is plausible that lineage studies focused on exocrine ducts were negative because ducts are a committed derivative of PDG pluripotential cells, to date there is no known specific marker defined that could be used to lineage trace the fate of cells arising from PDGs. As such the role of PDGs as a pancreatic stem cell niche remains speculative, although the present study supports the possibility that the PDG compartment serves as a source of ductal epithelium and possibly acinar cells following pancreatic injury.

It is possible that insulin-expressing cells produced in the PDGs in the setting of T1D might be selectively removed by the autoimmune response, and through epitope spreading any potential precursors of beta cells might also be eliminated (36). With this in mind, we did examine the PDG

compartment for any evidence of increased immune cell infiltrate in T1D, but did not find a consistent difference. Arguably, however, given the generally low immune infiltrate in humans with T1D, there could be such a difference that we were unable to find in the available samples studied.

An area of unresolved interest is why pancreata in long standing T1D are decreased in volume and mass in comparison to nondiabetic controls (37). Consistent with that data, exocrine function is also impaired in T1D (38). Hypotheses that have been put forward to account for these findings include loss of the growth promoting properties of insulin and subclinical pancreatitis (19). Chronic inflammation is a characteristic of autoimmune mediated disease states where the target of autoreactive T-cells is replenished, such as gut epithelium in ulcerative colitis and synovial epithelium in rheumatoid arthritis (39, 40). Under those conditions the chronic inflammation results in accumulating fibrosis and distortion of the architecture of the underlying targeted tissue. The duration of detectable autoantibodies characteristic of T1D and the decline in beta-cell function long precedes diabetes onset (41). A prolonged period of insulitis preceding diabetes onset may lead to cytokine mediated proliferation of the PDGs and pancreatic duct tree in the affected lobes. The high frequency of replication noted in PDGs in the present study was not accompanied by an increase in apoptosis, PDG cell apoptosis by TUNEL being rarely detected in even PDGs with the highest detected Ki67 (data not shown). As in the case of epithelia derived from the gut stem cell niche, it appears that the excess epithelial cells derived from the PDG are either sloughed off into the ductal lumen or contribute to ductal hyperplasia distorting and obstructing ducts. The latter is known to lead to local areas of pancreatitis and likely subsequent atrophy of the affected region of acinar tissue (18, 42). This would be consistent with the findings of a distorted exocrine ductal tree with stenosis, tortuosity and obstruction documented in patients with long term autoimmune mediated diabetes, but not those who developed diabetes after short term islet inflammation (43). An alternative hypothesis previously advanced is that autoimmunity directed towards beta-cells in T1D is only one facet of the autoimmune process, with autoimmunity also directed towards the exocrine pancreas. Autoimmune pancreatitis is well described but does not typically involve the islets (44).

The present studies have limitations, in large part because they are exclusively undertaken in human pancreas and are therefore inevitably cross sectional. While brain dead organ donors have the advantage of being transplant grade, this does not mean they are unaffected by the final illness of the donor who was likely subject to numerous potent therapies. Because of the nature of procurement of organ donors, there is also necessarily a limited prior clinical history in comparison to a formal clinical trial. While the study of human pancreas has obvious advantages in terms of potential clinical relevance, given our primary goal here, there is no means to undertake the gold standard lineage tracing approach required to properly define a stem cell niche and its derivatives. Also, by definition, studies of human pancreas are cross-sectional and necessarily descriptive.

In conclusion the PDG compartment of the pancreas and the ductal tree in T1D are characterized by increased proliferation consistent with a role in pancreatic duct and possibly acinar cell regeneration, but without evidence of effective beta-cell regeneration.

Acknowledgments

AUTHOR CONTRIBUTIONS

ASM and AEB performed the studies, undertook the microscopy, and performed the morphological analysis. ASM, AEB and PCB researched data, wrote the manuscript, reviewed the manuscript, edited the manuscript, and contributed to the discussion. The authors have no known conflict of interest.

Disclosure Statement: The authors have nothing to disclose.

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Figure 1. Examples of PDG compartments in nondiabetic (A) and subjects with type 1 diabetes (B). Pancreas sections were stained by immunohistochemistry for Insulin [pink], Ki67 [brown] and alcian blue with hemotoxylin counterstain. Insets, magnified areas of the low power images marked by black rectangle. Black arrows indicate Ki67 positive nuclei in PDG cells. Pink arrows indicate insulin positive PDG cells. Black stars indicate the interlobular duct lumen. (C), the frequency of replicating cells in the epithelium of PDGs is higher in T1D compared to ND (6.3 ± 1.6 vs $0.6 \pm 0.1\%$, T1D vs ND, $p < 0.001$). (D), the frequency of insulin positive cells in the epithelium of PDGs is higher in ND compared to T1D (0.003 ± 0.002 vs $0.40 \pm 0.08\%$, T1D vs ND, $p < 0.0001$). White bars = nondiabetic [ND], black bars = Type 1 Diabetic [T1D]. Scale bar = 100µm.

Figure 2. The frequency of replicating cells in the epithelium of interlobular duct cells (5.9 ± 1.7 vs $0.6 \pm 0.1\%$ Ki67 positive cells, T1D vs ND, $p < 0.01$) (A) and intralobular duct cells (1.9 ± 0.5 vs. $0.4 \pm 0.0\%$ Ki67 positive cells, $p < 0.01$, T1D vs. ND) (B) in type 1 diabetic and nondiabetic subjects. The frequency of insulin positive cells in the epithelium of interlobular ducts (0.005 ± 0.002 vs $0.12 \pm 0.03\%$ insulin positive cells T1D vs ND, $p < 0.0001$) (C) and intralobular ducts (0.02 ± 0.01 vs $0.34 \pm 0.05\%$ insulin positive cells, T1D vs ND, $p < 0.0001$) (D) in type 1 diabetic and nondiabetic subjects. The relationship between frequency of Ki67 positivity in interlobular duct cells and in PDG cells from subjects with type 1 diabetes (E) and nondiabetic subjects (F). The percentage of Ki67 positive cells in the Pancreatic Duct Glands [PDGs] (the number of Ki67+ PDG cells/ total number of PDG cells) and the percentage of Ki67 positive cells in the interlobular pancreatic ducts (the number of Ki67+ interlobular duct cells/ total number of interlobular duct cells) show a positive correlation in both the Type 1 Diabetic donors ($r = 0.84$, $p < 0.0001$) and in the nondiabetic donors ($r = 0.45$, $p = 0.0002$). Data were present as mean $\pm$ SEM, n = 22 donors for T1D, the data points representing 22 sections of head of pancreas, 21 sections of body of pancreas and 22 sections of tail of pancreas; n = 24 donors for ND, the data points representing 24 sections of head of pancreas, 16 sections of body of pancreas and 24 sections of tail of pancreas. White bars = nondiabetic [ND], black bars = Type 1 Diabetic [T1D].

Figure 3. Examples of PDG cells expressing Sox9-GATA4 in pancreas from an adult ND (A) and in an adult T1D donor (B). Individual layers stained for Sox9 (green) and GATA4 (red) are shown along with merged (with DAPI) images. Insets, higher magnification of individual layers as marked by the yellow square in the low power images. Yellow arrows indicate a PDG cell co-staining for Sox9 and GATA4. Scale bars, 50 µm (for low power images) and 25 µm (for insets).

Figure 4. Examples of PDG cells expressing chromogranin A (ChrgA), NKx6.1 and endocrine cocktail (insulin, glucagon, somtostatin, pancreatic polypeptide and ghrelin) in pancreas from an adult ND donor (A) and chromogranin A (ChrgA), NKx2.2 and endocrine cocktail in an adult T1D donor (B). Individual layers stained for NKx6.1/NKx2.2 [red], endocrine cocktail (Insulin, Glucagon, Somatostatin, Pancreatic Polypeptide and Ghrelin) [white], Chromogranin A [green] are shown along with the merged (with DAPI) image. Insets, higher magnification of individual layers as marked by the yellow square in the low power images. Yellow arrows indicate a PDG cell co-staining for Nkx6.1, Chromogranin A and a cocktail of all the endocrine hormones (A) or Nkx2.2, Chromogranin A and a cocktail of all the endocrine hormones (B). Scale bars, 50 µm (for low power images) and 25 µm (for insets).

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TABLE 1. Characteristics of human subjects with and without type 1 diabetes.

T1D							CONTROLS				
nPOD Case No.	Age [years]	Sex	BMI	Duration [years]	Diabetes Medications	% Ki67 in PDG cells	nPOD Case No.	Age [years]	Sex	BMI	% Ki67 in PDG cells
6031	39	M	24.5	35	Insulin	0.51	6010	47	F	19.7	0.55
6035	32	M	27.1	28	Insulin	0.54	6012	68	F	23.7	0.57
6036	49	F	25.5	34	Insulin	2.07	6013	65	M	24.2	0.82
6040	50	F	31.6	20	Insulin	1.07	6015	39	F	32.2	0.13
6045	26	M	23.1	8	Insulin	7.04	6017	59	F	24.8	0.46
6050*	82	M	N/A	58	Insulin	5.12	6021	72	F	24.5	0.86
6051	20	M	22.7	13	Insulin	0.33	6022	75	M	30.6	0.40
6054	35	F	30.4	30	Insulin	1.14	6029	24	F	22.6	0.11
6061	28	M	22.1	23	Insulin	2.20	6030	30	M	27.1	0.26
6062	11	M	21.9	6	Insulin	1.62	6034	32	F	25.2	0.59
6063	4	M	23.8	3	Insulin	0.82	6047	8	M	23.9	1.80
6064	20	F	22.6	9	Insulin	48.70	6048	30	M	20.6	0.49
6068*	72	F	N/A	69	Insulin	1.08	6057	22	M	26.0	0.30
6070	23	F	21.6	7	Insulin	0.36	6060	24	M	32.7	1.14
6076	26	M	18.8	15	Insulin	0.51	6091	27	M	35.6	0.75
6085*	89	M	20.3	84	Insulin	2.32	6096	16	M	18.8	0.27
6088	31	M	27.0	5	Insulin	1.12	6098	18	M	22.8	0.11
6089	14	M	26.0	8	Insulin	22.18	6102	45	F	35.1	0.38
6119	28	M	19.4	14	Insulin	1.98	6104	41	M	20.5	0.38
6128	34	F	22.2	31.5	Insulin	0.21	6126	25	M	25.1	0.29
6135	44	M	28.7	21	Insulin	6.40	6130	5	M	18.5	0.05
6138	49	F	33.7	41	Insulin	29.03	6131	24	M	24.8	0.92
							6134	27	M	20.1	0.73
							6140	38	M	21.7	0.78
Mean	**36.6**		**24.7**	**25.6**		**6.20**	**Mean**	**35.9**		**25.0**	**0.55**
SEM	**4.7**		**0.9**	**4.6**		**2.55**	**SEM**	**4.0**		**1.0**	**0.08**

* Denotes Type 1 Diabetes Medalist

TABLE 2. The numbers of cells counted in the PDGs, interlobular, and intralobular ducts in the donors with T1D and nondiabetic controls.

T1D	Head	Body	Tail	Total
PDG cells, total	43,718	21,443	29,057	94,218
PDG cells, mean per donor (range)	1987 (430-5780)	1021 (152-2531)	1321 (545-3461)	
Interlobular Duct cells, total	56,183	33,706	37,298	127,187
Interlobular Duct cells, mean per donor (range)	2554 (202-7335)	1605 (280-3628)	1695 (310-4657)	
Intralobular Duct cells, total	59,923	48,628	49,681	158, 232
Intralobular Duct cells, mean per donor (range)	2724 (752-4942)	2316 (990-3988)	2258 (810-3359)	
ND	Head	Body	Tail	Total
PDG cells, total	32,396	19,185	22,823	74,404
PDG cells, mean per donor (range)	1350 (197-4835)	1199 (117-5580)	951 (70-2071)	
Interlobular Duct cells, total	53,857	32,859	37,711	124,427
Interlobular Duct cells, mean per donor (range)	2244 (199-7997)	2054 (436-6169)	1571 (289-2950)	
Intralobular Duct cells, total	68,952	61,284	66,407	196,643
Intralobular Duct cells, mean per donor (range)	2873 (840-4354)	3605 (1626-4937)	2767 (988-4303)	

A ND, 6140

B T1D, 6138

100 μm

C

Ki67 positive PDG cells [%]

ND   T1D

**

D

Insulin Positive Cells in PDGs [%]

ND   T1D

***

A

B

Ki67 positive intralobular duct cells [%]

C

% of insulin positive interlobular duct cells

D

% of insulin positive intralobular duct cells

E

T1D

r = 0.84, p < 0.0001

% of Ki67 positive PDG cells

% of Ki67 positive interlobular duct cells

F

ND

r = 0.45, p = 0.0002

% of Ki67 positive PDG cells

% of Ki67 positive interlobular duct cells