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β-Cell dedifferentiation, reduced duct cell plasticity, and impaired β-cell mass regeneration in middle-aged rats

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The incidence of type 2 diabetes (T2D) increases with aging (8), but the factors contributing to this higher risk are not well established. Although age-related impairments of β-cell function and insulin action have been associated largely with the development of T2D (11), limitations in β-cell regenerative capacity could also contribute to increase the risk of diabetes in aged individuals. β-Cell mass expands throughout life in rodents and humans (14, 31, 38), and in young adults it increases in response to two different experimental conditions, the 90%-Px model and partial pancreatectomy (Px + V) and gastrin administration (Px + G). Pancreatic remnants were analyzed 3 and 14 days after surgery. β-Cell mass increased in young animals after Px and was further increased after gastrin treatment. In contrast, β-cell mass did not change after Px or after gastrin treatment in middle-aged rats. β-Cell replication and individual β-cell size were similarly increased after Px in young and middle-aged animals, and β-cell apoptosis was not modified. Nuclear immunolocalization of neurog3 or nkx6.1 in regenerative duct cells, markers of duct cell plasticity, was increased in young but not in middle-aged Px rats. The pancreatic progenitor-associated transcription factors neurog3 and sox9 were upregulated in islet β-cells of middle-aged rats and further increased after Px. The percentage of chromogranin A+/hormone islet cells was significantly increased in the pancreases of middle-aged Px rats. In summary, the potential for compensatory β-cell hyperplasia and hypertrophy was retained in middle-aged rats, but β-cell dedifferentiation and impaired duct cell plasticity limited β-cell regeneration.

Aging; regeneration; β-cell replication; duct cell plasticity; β-cell dedifferentiation; gastrin

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surgery. All animals were injected subcutaneously every 12 h from the day of surgery until the end of the study.

Fed morning plasma glucose levels were monitored daily from the snipped tail with a portable meter (Accu-Chek; Roche Diagnostics, Mannheim, Germany). Blood samples were taken ~12 h after the previous gastrin injection and just before the next injection.

Pancrectomy and Pancreatic Remnant Harvesting

Ninety percent Px was performed as described previously (50). Briefly, animals were anesthetized with 5% isoflurane (Forane; Abbott) and maintained anesthetized with an isoflurane (1.5%) air mixture. Ninety percent of the pancreas was removed by gentle abrasion with cotton applicators, being careful to leave major blood vessels intact; the pancreatic remnant was the tissue between the common bile duct and the first loop of the duodenum and corresponded to 9.25 ± 0.48 and to 9.9 ± 0.61% of the total pancreas of young and middle-aged animals, respectively. Sham pancreatectomy was accomplished by breaking splenic and duodenal mesenteric connections and gently handling the pancreas with the fingertips. In sham-operated animals, the equivalent of the remaining tissue left after Px (remnant equivalent) was collected.

For immunohistochemical analysis, pancreatic remnants and remnant equivalents were harvested on days 3 (n = 13 and 5, respectively) and 4 (n = 13 and 5, respectively) after surgery, weighed, and fixed in 4% phosphate-buffered saline-buffered paraformaldehyde and processed for paraffin embedding. For gene expression analysis, pancreatic remnants and remnant equivalents were harvested on day 3 after surgery (n = 4 for each group). Tissues were rapidly immersed in RNAlater solution (Ambion; Applied Biosystems, Warrington, UK), chopped into small pieces (<5 mm), and kept overnight at 4°C until total RNA extraction.

Immunostaining and Image Analysis

Primary antibodies are listed in Table 1. For Nkx6 homeobox 1 (nkx6.1) immunofluorescence the biotin-streptavidin amplification system was used (Invitrogen, Carlsbad, CA). Neurogenin 3 visualization required TSA amplification (TSA Plus Cyanine3/Fluorescein System; Perkin-Elmer, Boston, MA).

Morphometry

β-Cell, duct cell, and acinar cell mass was obtained by multiplying its relative area by the pancreas weight. β-Cell and acinar cell relative area was determined using a software based semiautomatic method [Leica Application Suite (LAS); Leica Microsystems, Heerbrugg, Switzerland]. Pancreatic sections stained for insulin or amylase (see Table 1 for primary antibody description) were visualized with Alexa fluor 488 under a fluorescent microscope with a ×20 objective. Consecutive fields were captured along the x-axis acquiring the entire row, and one out of every two fields was captured along the y-axis. Microscopy-derived images were automatically calibrated by the LAS software. Insulin- and amylase-positive areas were semiautomatically selected, based on wide-range tones of green positive pixels, to define the regions of interest (ROIs). ROIs were then automatically identified and quantified in each image by LAS software. The ROI identification process was verified manually and corrected if required. Total pancreatic tissue area was measured in DAPI-labeled low-contrast images that allowed the identification of the tissue by the blue background. To assess the accuracy of the semiautomatic method, the relative β-cell area was determined in five pancreases using both the semiautomatic and the traditional point-counting method (32) with similar results (0.58 ± 0.12% vs. 0.57 ± 0.10%, respectively). The β-cell and acinar cell relative area was calculated by dividing the insulin- or amylase-stained area by the total pancreatic area.

The higher nuclear/cytoplasmic ratio of duct cells compared with β-cells or acinar cells leads to inappropriately low duct cell relative area measurements with the semiautomatic method (duct cell relative area measured by point counting: 4.7 ± 0.72% vs. semiautomatic: 0.71 ± 0.17%; P = 0.008). Thus, duct cell mass was determined using the traditional point-counting method on keratin 20-labeled sections (Table 1) (50). A nomogram relating number of points counted to volume density and expected relative standard error in percentage of mean (<10%) was used to determine the number of intercepts needed for a representative sampling (32). A minimum of two to three sections 150 μm apart were included for each animal.

Areas of regeneration were identified based on mesenchymal cell infiltration and observation of tubular complexes. The relative area was manually determined on slides stained for keratin 20/vimentin using the Leica Application Suite (LAS) software (Leica Microsystems). All measurements were performed by a blinded observer.

Cell Replication

Rats were injected with the thymidine analog 5-bromo-2′-deoxyuridine (Sigma, St. Louis, MO), 100 mg/kg ip, 6 h before the pancreas remnant was harvested. To assess β-cell replication, sections were double stained with immunoperoxidase for BrdU using a Cell Proliferation Kit (GE Health Care, Amersham, UK) and for insulin using a

Table 1. Primary antibodies used for immunostaining

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<th>Name</th>
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<th>Dilution</th>
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BrdU, 5-bromo-2′-deoxyuridine; CCKBR, cholecystokinin B receptor; MW, microwave; DSHB, Developmental Studies Hybridoma Bank nkx6.1, Nk6 homeobox 1; PanCK, pan-cytokeratin; PHH3, phosphohistone H3. *Biotin-streptavidin-conjugated Alexa fluor 555 amplification was used; #tyramide signal amplification fluorescein system was used.
rabbit anti-human insulin antibody. Acinar and duct cell replication was determined by double immunofluorescence (Amylase/BrdU or KRT20/BrdU). A minimum of 1,200 cells/pancreas were counted.

### Individual β-Cell Area

The mean cross-sectional area of individual β-cells, a measurement of β-cell size, was determined on the immunoperoxidase-stained sections for insulin with the image-analytical software (AnalySIS 3.0, Soft Imaging System) (32). The perimeter of each islet of the section was carefully traced on the computer’s monitor to exclude any other tissue, and β-cell nuclei of each islet were counted. To calculate the area of the individual β-cells, the total β-cell area in the section was divided by the number of β-cell nuclei.

### β-Cell Apoptosis

Sections were double stained by immunofluorescence for apoptotic nuclei (Alexa fluoro 488) with the TUNEL technique (In Situ Cell Death Detection Kit, ApopTag; Intergene, Oxford, UK) and for insulin (Alexa fluoro 555) using a rabbit anti-human insulin antibody. A minimum of 1,200 cells/pancreas were counted (50).

### RNA Isolation, Quantification, and Retrotranscription

Pancreatic tissue was resuspended with 1 ml of TRIZol Reagent (Sigma) for homogenization (Ultraturrax; Janke & Kunkel, Stauffen, Germany). Total RNA was purified according to the manufacturer’s instructions (PureLink Micro-to Midi System; Invitrogen) (50). RNA quality was assessed with the Bioanalyzer 2100 (Agilent Technologies) and the RIN score ranged from 7.4 to 9.2. cDNA synthesis was performed from 5 μg of total RNA using the Superscript III First-strand cDNA synthesis system (Invitrogen).

### Quantitative PCR

PCR was run in a 7900HT Fast Real-Time PCR system (Applied Biosystems) with 384-well optical plates, allowing all samples to be amplified in the same run for each gene. Reactions were performed using TaqMan Gene Expression Assays and TaqMan Gene Expression Master Mix (Applied Biosystems), following the manufacturer instructions in a final volume of 20 μl with 250 ng of cDNA in each reaction. A full listing of assays (Applied Biosystems), gene names, and assay identification numbers is given in Table 2. Relative quantities were calculated using ABI SDS 2.2.2 RQ (relative quantitative value) for 7900HT software (Applied Biosystems) and the 2−ΔΔCT analysis method with TATA box-binding protein (tbp) as the endogenous control. Gene expression was expressed as a RQ resulting from the calculations performed in the SDS2.2.2. RQs were normalized to give a mean of 1 for control rats to facilitate the comparison across genes with varying basal abundance.

### Statistical Analysis

Results are expressed as means ± SE. Statistics were performed using GraphPad Prism 6 software, and differences among means were evaluated using the Student’s t-test or the one-way analysis of variance (ANOVA) combined with Tukey’s test for post hoc analysis as appropriate. β-Cell apoptosis data were not distributed normally (KS normality test, P < 0.05). Thus, results are expressed as median with interquartile range, and the Kruskal-Wallis one-way analysis of variance combined with the post hoc Dunn’s test was used for multiple comparisons analysis. A P value of <0.05 was considered significant.

### RESULTS

#### Acinar and Duct Cell Replication in Young And Middle-Aged Rats

Gastrin treatment had minor effects on the expansion of the exocrine compartment of the pancreas. In young and middle-aged sham-operated animals, pancreatic weight, acinar and duct cell replication, and acinar cell mass were not changed after gastrin treatment (Fig. 1, A–D). Duct cell mass increased in young sham-operated rats treated with gastrin (S + V: 3.1 ± 0.4 mg; S + G: 4.4 ± 0.3 mg; P = 0.024), whereas it remained unchanged in middle-aged rats (S + V: 15.9 ± 2.4; S + G: 13.6 ± 1.6 mg; P = 0.44) (Fig. 1E).

After Px, pancreatic remnant weight was similarly increased in young and middle-aged rats compared with the pancreatic remnant equivalent of sham-operated counterparts (Fig. 1F). Acinar and duct cell replication and mass were similarly augmented in young and middle-aged Px rats (Fig. 1, G–J). These results indicate that young and middle-aged rats have similar acinar and duct cell regenerative potential.

#### β-Cell Replication after Px in Young and Middle-Aged Rats

**Blood glucose.** In young animals, blood glucose levels were similar in S + V, Px + V, and Px + G rats throughout the study, as expected (50). In middle-aged animals, 50% of Px + V and 43% of Px + G rats were hyperglycemic on day 14 after Px (S + V: 92 ± 4.3 mg/dl; Px + V: 275 ± 88 mg/dl; Px + G: 282 ± 83 mg/dl; Fig. 2A). Short-term gastrin treatment had no effect on blood glucose levels in sham-operated young or middle-aged rats (young: S + V 109 ± 3.2 mg/dl, S + G 105 ± 2.7 mg/dl; middle-aged: S + V 92 ± 28 mg/dl, S + G 85 ± 3.5 mg/dl).

**β-cell mass.** In young rats, β-cell mass was increased 14 days after Px (S + V: 0.4 ± 0.07 mg; Px + V: 0.79 ± 0.12 mg; P = 0.008; Fig. 2B) and further increased by gastrin (Px + G: 1.4 ± 0.18 mg; P < 0.0001). In contrast, in middle-aged rats β-cell mass was similar in all three groups (day 14: S + V: 1.9 ± 0.20 mg; Px + V: 1.47 ± 0.36 mg; Px + G: 1.45 ± 0.20 mg; Fig. 2B).

**β-cell hyperplasia.** β-Cell replication was significantly increased on day 3 after Px in young and middle-aged rats. On day 14, it remained increased in middle-aged hyperglycemic Px rats and was reduced to sham levels in normoglycemic young Px rats (Fig. 2D). Gastrin treatment further increased β-cell replication in middle-aged Px rats but not in young rats (Fig. 2, C and D). Gastrin did not stimulate β-cell replication in sham-operated rats at any age (young rats: S + V 1.2 ± 0.37%, S + G 0.87 ± 0.27%; middle-aged rats: S + V 0.1 ± 0.03%, S + G 0.1 ± 0.04%). Phosphohistone H3 labeling showed similar results (Fig. 2, E and F), indicating that replicating β-cells in young and middle-aged Px rats underwent mitosis after DNA replication.

**β-Cell hypertrophy and apoptosis.** Individual β-cell size was similarly increased 14 days after Px in young and middle-aged rats (Fig. 2G), and β-cell apoptosis did not change significantly (Fig. 2H).

### Table 2. Gene expression assays used for real-time qPCR

<table>
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<th>Gene Name</th>
<th>Gene Symbol</th>
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<td>Neurogenin 3</td>
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<td>Nkx6.1</td>
<td>Rn00581973_ml</td>
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<td>Pancreatic and duodenal homeobox 1</td>
<td>Pdx-1</td>
<td>Rn00755591_ml</td>
</tr>
<tr>
<td>TATA box-binding protein</td>
<td>Tbp</td>
<td>Rn01455646_ml</td>
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qPCR, quantitative PCR.
In the absence of consistent cell tracing tools for direct analysis and quantification of β-cell neogenesis in rat and human pancreases, the number of extraislet β-cells scattered throughout the pancreatic tissue has been classically used as an indication of β-cell generation outside the islet (7, 10, 28, 56). The number of extraislet β-cells, single or forming small clusters, was similar in sham-operated young and middle-aged rats and was not modified by short-term gastrin treatment (S, 0.16 ± 0.08 vs. age-matched S group for Tukey’s test). Values are means ± SE; n = 5. ANOVA, P < 0.05; *P < 0.05 vs. other age-matched groups; #P < 0.05 vs. age-matched S + V group for Tukey’s test. Values are means ± SE; n = 5.

Duct Cell Plasticity After Px in Young and Middle-Aged Rats

After 90% Px, new β-cells appear within focal areas of regeneration in the pancreatic remnant. These areas contain regenerative ducts surrounded by mesenchymal cells and undergo proliferation and differentiation into all different pancreatic cell types, forming new lobes of pancreas (3, 24, 50). The size and cell composition of focal areas of regeneration were similar in young and middle-aged Px rats, and accordingly the duct cell relative area was similarly increased in young and middle-aged Px groups compared with sham-operated rats (Fig. 3, A–C).

Differentiation of the focal areas of regeneration into an endocrine phenotype involves the expression of pancreatic and endocrine progenitor markers (such as nkh6.1) in regenerative ducts, recapitulating embryonic pancreas development (24), and is fostered by gastrin treatment (50). Gene expression of pdx-1 and nkh6.1 was significantly increased in pancreatic remnants of young Px rats treated with gastrin but not in middle-aged Px rats (Fig. 3, D and E) and was significantly lower in the middle-aged group: pdx-1 (middle-aged Px + G vs. young Px + G): 1.03 ± 0.06 vs. 2.16 ± 0.29, P = 0.008; nkh6.1: 1.88 ± 0.38 vs. 3.42 ± 0.63, P = 0.01. The expression of nkh6.1 and neurog3 in regenerative duct cells was 55 and 70% lower, respectively, in middle-aged Px animals (Fig. 3, F–I). In gastrin-treated animals, the expression of both progenitor markers in duct cells was more than threefold higher in young rats (Fig. 3, F–I). Overall, the lower expression of pdx-1, nkh6.1, and neurog3 in the regenerative ducts of focal areas of regeneration in pancreatectomized and gastrin-treated pancreatectomized rats suggests that duct cell plasticity is reduced in middle-aged rats.

Fourteen days after surgery, focal areas of regeneration were identified infrequently in pancreatic remnants from both young and middle-aged rats (not shown), suggesting that cell differentiation was not delayed in middle-aged rats.

Expression of Immaturity Markers in β-Cells After Px in Young and Middle-Aged Rats

β-Cell dedifferentiation, based on β-cell insulin loss and the expression of neurog3 or sox9 in adult insulin-expressing islet β-cells, has been identified in animal models of metabolic stress (2, 48) and deregulated hedgehog function (20). The gastrin high-affinity receptor cholecystokinin B receptor (CCKBR), which is expressed in the embryonic pancreas and is excluded from mature islets, was confined to duct cells in
focal areas of regeneration and excluded from islets in young Px rats. In contrast, it was expressed in \( \beta \)-cells from middle-aged rats and significantly increased after Px (Fig. 4, A and B), suggesting a degree of \( \beta \)-cell immaturity.

To further investigate \( \beta \)-cell dedifferentiation in middle-aged rats, we determined the expression of neureglin 3 and sox9 in islet \( \beta \)-cells from middle-aged Px rats. These transcription factors are expressed in embryonic pancreas progenitor cells, they are excluded from the embryonic insulin-expressing cells (25, 40), and their expression in adult \( \beta \)-cells has been associated with \( \beta \)-cell dedifferentiation (20, 48). In middle-aged rats, neureglin 3 mRNA was significantly increased in gastrin-treated Px rats 3 days after surgery (Fig. 4C). Double immunofluorescence of neuregln3 and insulin revealed an in-
increased percentage of double-positive cells in islets of middle-aged rats (S + V: 40.4 ± 8.9%; S + G: 42.6 ± 6.9%) compared with young animals (S + V: 6.7 ± 2.7%; S + G: 4.6 ± 1.2%; P = 0.02) (Fig. 4, D and E). In young rats, the percentage of islets with neurog3/insulin double-positive cells was significantly increased in vehicle- (21 ± 2.5%) and gastrin-treated (24 ± 3.2%) rats 3 days afterPx, in agreement with previous reports (19, 55). In middle-aged rats, the percentage of islets with neurog3/insulin double-positive cells was significantly increased in the Px + V (66.4 ± 6.1%; P = 0.02) and Px + G (76.5 ± 3.8%; P = 0.001) groups (Fig. 4E).

Sox9, whose function is required in pancreatic progenitor cells, is normally excluded from mature β-cells (15, 19, 25, 41). Sox9/nkx6.1/insulin triple-positive cells were very rare in young pancreases (S + V: 0.06 ± 0.04%; S + G: 0.05 ± 0.02%), and they increased in pancreatic islets of middle-aged rats (S + V: 0.22 ± 0.08%; S + G: 0.23 ± 0.07%; P = 0.04). On day 3 afterPx, sox9/nkx6.1/insulin triple-positive cells were similarly increased in vehicle- (0.38 ± 0.15%) and gastrin-treated (0.43 ± 0.12%) young Px rats. In middle-aged rats, sox9/nkx6.1/insulin triple-positive cells increased five- and seven-old in the Px + V (1.07 ± 0.21%; P = 0.006) and Px + G (1.69 ± 0.5%; P = 0.01) groups, respectively (Fig. 4, F and G).

Double immunostaining of chromogranin A (chga), a pan-endocrine marker, and islet hormones [insulin (ins), glucagon (gcg), somatostatin (sst), and pancreatic polypeptide (ppy)] revealed a 10-fold increase in chga+/ins−/gcg−/sst−/ppy− cells in middle-aged Px rats (Fig. 4, H and I), indicating the loss of hormone expression in some islet endocrine cells. In contrast, in islets from young animals the percentage of chga+/ins−/gcg−/sst−/ppy− cells did not significantly differ among the groups (Fig. 4I).

**DISCUSSION**

In this study, we have found that β-cell mass expansion in response to Px and gastrin treatment was impaired in middle-aged rats. The capacity to undergo compensatory β-cell hyperplasia and β-cell hypertrophy was retained in middle-aged rats, and the limitation in β-cell regeneration resulted from impaired duct cell plasticity and β-cell dedifferentiation.

β-Cell-replicative response was retained in middle-aged Px rats. β-Cell replication has been considered the major mechanism for β-cell mass expansion in the postnatal pancreas (12).
As aging progresses, β-cell replication declines, reaching minimal levels when 40% of the life expectancy is achieved (~9 mo in rodents and ~30 yr in humans) (28, 31, 34). It has been suggested recently that the adaptive β-cell replicative response may be completely blunted in middle-aged mice (35, 51). We show that even though basal β-cell replication was indeed very low in middle-aged rats, it was strongly stimulated after Px and was further increased by gastrin treatment. Thus, the similar β-cell replicative levels in young and middle-aged Px rats indicates that middle-aged β-cells are capable to rejuvenate in terms of cell proliferation dynamics in response to external stimuli.

β-Cell neogenesis from the ductal epithelium is the major mechanism for β-cell mass expansion during embryonic life (18, 19, 42). Indirect evidence of β-cell neogenesis has been found in rodents and humans in postnatal and adult life (7, 24, 26, 45, 46). However, its contribution on β-cell mass expansion remains controversial because of the lack of specific markers for the identification of pancreatic progenitors and the discordant observations in genetic lineage-tracing experiments (4, 16).
Stimulation of gastrin signaling in duct cell-enriched human cell populations or in diabetic young mice has been shown to promote β-cell neogenesis and increase β-cell mass (39, 43, 45, 53, 57). In the absence of consistent cell-tracing tools for direct analysis and quantification of β-cell neogenesis in rats, we have examined the number of extrasil β-cells scattered throughout the pancreatic tissue as an indication of β-cell generation outside the islet. Our results suggest a reduced generation of new β-cells in middle-aged Px animals. According to the previous studies analyzing β-cell neogenesis induced by gastrin treatment, we have found that in young rats, surrogate markers of β-cell neogenesis were induced after Px and were further increased by gastrin treatment; however, in middle-aged Px rats they were blunted.

An age-related decline in stem cell differentiation potential, rather than in stem cell expansion capacity, has been shown to hamper muscle and neural regeneration after extensive damage (9, 52). In line with these reports, we found that duct cell expansion and formation of focal areas of regeneration was preserved in middle-aged Px rats. However, duct cell plasticity and progenitor competence appears to be reduced in middle-aged Px rats, based on the lower nkx6.1 and neurog3 expression in regenerative duct cells, compared with young Px rats. The disappearance of focal areas of regeneration in both age groups 2 wk after pancreatectomy suggests that the reduced duct cell progenitor competence found on pancreatic remnants of middle-aged rats initially after surgery could not be attributed to delayed cell differentiation potential in this group.

In middle-aged rats, β-cells expressed markers of immaturity (cckbr, neurogenin3, and Sox9), suggesting that β-cell identity was compromised. Cckbr and gastrin are expressed in pancreatic endocrine cells during late embryonic pancreas organogenesis and decline early after birth (33, 37, 47). Accordingly, cckbr expression was undetectable in β-cells from young control rats and was unaffected after Px, as shown previously (49). However, in middle-aged rats, cckbr was expressed in a small proportion of islet β-cells in control rats and significantly increased after Px, which could indicate a degree of β-cell dedifferentiation of mature β-cells or the presence of newly formed immature β-cells. The increased expression of neurogenin3 and Sox9 in insulin-expressing β-cells and the increased proportion of “empty” endocrine cells (chga− hormone−) in islets of middle-aged Px rats supports the loss of β-cell maturity in older animals. Furthermore, since neither Sox9 nor neurogenin 3 expression overlaps with insulin during embryonic β-cell neogenesis (25, 40), the expression of these transcription factors in insulin-expressing adult β-cells is an additional indication of the presence of β-cell dedifferentiation rather than β-cell neogenesis.

Dedifferentiated β-cells were identified in 40–70% of the islets, but only 2–3% of the β-cells showed dedifferentiation features, indicating that β-cell dedifferentiation was not common in contrast to what has been described in genetic models of β-cell dedifferentiation (20, 48, 54). Nevertheless, the extent of β-cell dedifferentiation was sufficient to preclude the β-cell mass expansion anticipated by the increased β-cell replication observed in middle-aged Px rats. The upregulation of cckbr in β-cells of middle-aged Px rats provides a mechanistic basis for gastrin-induced β-cell replication in these animals. A possible explanation for β-cell dedifferentiation in middle-aged rats could be β-cell glucotoxicity, since sustained hyperglycemia induces β-cell dedifferentiation in young rodents (17, 22, 54). However, in our middle-aged Px animals the signs of β-cell immaturity were already identified after short-term (48–72 h) exposure to hyperglycemia. β-Cell dedifferentiation after short exposure to high blood glucose levels suggests an increased vulnerability of middle-aged β-cells to hyperglycemia.

The differences between rodent and human pancreas regenerative capacity have usually been attributed to the genetic background. Partial pancreatectomy in young rodents significantly stimulates β-cell mass growth, as reported repeatedly (3, 6, 13, 23), whereas no β-cell regeneration was identified in partly pancreatectomized humans (30). However, we now show that in 12-mo-old rats (at ~50% of their life expectancy), partial pancreatectomy does not induce β-cell mass expansion, similar to what was described in adult humans at ~65% of their life expectancy (30). Therefore, some of the reported differences in β-cell regeneration between humans and rodents could be attributed to differences in age rather than to the genetic background.

In summary, we have found that impaired duct cell plasticity and β-cell immaturity limited the expansion of the functional β-cell mass in middle-aged Px rats. This impaired expansion took place despite increased β-cell hypertrophy and hyperplasia after pancreatectomy. Strategies aiming to increase the functional β-cell mass in adult individuals with diabetes may require the prevention or reversal of β-cell dedifferentiation.

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DISCLOSURES

The authors have nothing to disclose.

AUTHOR CONTRIBUTIONS

N.T. conception and design of research; N.T., M.V., Y.M., and A.P. performed experiments; N.T., M.V., Y.M., and A.P. analyzed data; N.T., M.V., Y.M., and A.P. interpreted results of experiments; N.T. prepared figures; N.T. drafted manuscript; N.T. and E.M. edited and revised manuscript; N.T., M.V., Y.M., and E.M. approved final version of manuscript.

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