Expression of c-Kit receptor tyrosine kinase and effect on β-cell development in the human fetal pancreas

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Li J, Quirt J, Do HQ, Lyte K, Fellows F, Goodyer CG, Wang R. Expression of c-Kit receptor tyrosine kinase and effect on β-cell survival in the human fetal pancreas. Am J Physiol Endocrinol Metab 293: E475–E483, 2007. First published May 22, 2007; doi:10.1152/ajpendo.00172.2007.—The receptor, c-Kit, and its ligand, stem cell factor (SCF), are critical for hematopoietic stem cell differentiation and have been implicated in the development, function, and survival of rodent islets. Previously, we reported that exogenous SCF treatments of cultured human fetal (14–16 wk fetal age) islet-epithelial clusters enhanced islet cell differentiation and proliferation (Li J, Goodyer CG, Fellows F, Wang R. Int J Biochem Cell Biol 38: 961–972, 2006). In the present study, we examined the expression pattern of c-Kit in early to midgestation human fetal pancreata and the relevance of c-Kit receptor tyrosine kinase for insulin gene expression and β-cell survival. c-Kit is expressed in the intact pancreas in a cell-specific manner, with a significant decrease in immunoreactivity in the duct regions from 8 to 21 wk fetal age, paralleled by a significant increase in expression within endocrine regions. These c-Kit-positive cells are highly proliferative and show frequent coexpression with insulin and glucagon. Treatment of islet-epithelial clusters with anti-ACK45 antibody stimulates c-Kit phosphorylation paralleled by a significant increase in PDX-1 and insulin expression, increased cell proliferation, and reduced β-cell death. In contrast, transient transfection with c-Kit siRNA results in a three- to fourfold decrease in c-Kit, PDX-1, and insulin expression and decreased cell proliferation. This study describes important changes in the distribution and dynamics of c-Kit-expressing cells during human fetal pancreatic neogenesis, suggesting that c-Kit may be a marker for human pancreatic islet progenitor cells. Functional analysis of the c-Kit receptor tyrosine kinase provides evidence that phosphorylation of c-Kit receptor may be involved in mediating early β-cell differentiation and survival.

islet-epithelial cluster; anti-c-Kit antibody; c-Kit small interfering ribonucleic acid

ISLET CELL TRANSPLANTATION offers a promising approach to the treatment of type 1 diabetes, and recent successful clinical trials (14, 23) indicate that this cell-based therapy is indeed feasible. However, the scarcity of islets prevents it from being a routine treatment. One solution is to use progenitor cells to create a continually expandable pool of new islet cells. Before this can occur, however, we need a better understanding of human islet progenitor identities and the factors controlling their growth and differentiation.

One factor believed to be involved in the development of β-cells is c-Kit, a type 3 receptor tyrosine kinase of the platelet-derived growth factor subfamily (1, 12). The c-Kit receptor consists of an extracellular portion with five immunoglobulin-like regions and an intracellular tyrosine kinase domain. The first three immunoglobulin-like domains form a binding site for its ligand, stem cell factor (SCF), the fourth is involved in c-Kit receptor dimerization, and the function of the fifth is, as yet, unknown (3). SCF is a glycoprotein expressed in both a soluble (9) and a membrane-bound form (1, 16). SCF is mostly produced by fibroblasts and stromal, epithelial, and endothelial cells (33, 34). Binding of SCF induces homodimerization of the c-Kit receptor (2, 3, 13, 28), followed by an increase in receptor autophosphorylation activity and recruitment of the c-Kit signaling complex. Subsequent events lead to activation of a number of intracellular pathways, including the Ras-Raf-MAP kinase, Src, phosphatidylinositol 3-kinase, and the JAK/STAT cascades, to increase gene transcription, proliferation, differentiation, metabolic homeostasis, and survival (4, 5, 7, 13, 20, 25). c-Kit has been identified as a potential marker for islet progenitor cells in the early rat pancreas. c-Kit mRNA is detectable in rat pancreatic epithelial cells beginning on embryonic day 13, whereas both mRNA and protein have been observed in fetal and adult rat islets (10, 18, 19, 21). Expression of the c-Kit receptor during islet cell neogenesis is implicated in the development, function, and survival of rodent islets of Langerhans and, more importantly, in β-cell survival (18, 28). The lacZ gene has been introduced in frame into the c-Kit locus of the mouse genome to permit precise determination of endogenous c-Kit expression by X-gal staining (21). These studies showed that, during fetal life, β-cell activity occurred in insulin- and glucagon-expressing cells as well as in cells negative for these two hormones. The β-gal positive cells that lacked endocrine markers expressed pancreatic and duodenal homeobox-1 (PDX)-1 and Pax6, indicating that c-Kit is also expressed in presumptive endocrine precursor cells (21). Our recent study (31) of putative endocrine precursor cells during islet development in the pre- and postnatal rat pancreas also demonstrated that c-Kit-positive cells represent endocrine precursor cells, with the highest expression in, first, ductal and, subsequently, endocrine regions. In addition, we (26) have shown that isolated postnatal rat islet cells expressing c-Kit can be expanded in vitro, under specific conditions, to give rise to new β-cells that secrete insulin in a glucose-responsive fashion.

Although these studies demonstrate that the c-Kit receptor is important during development of rodent islets, little is known...
about the role of c-Kit in the human fetal pancreas. We (11) recently demonstrated that exogenous SCF treatment of isolated 14- to 16-wk human fetal islet-epithelial clusters enhanced islet cell differentiation and proliferation and that this process involved upregulating Akt phosphorylation in a phosphatidylinositol-3 kinase-dependent manner. As a followup, the present study used immunofluorescence, Western blot, and real-time RT-PCR approaches to examine whether c-Kit expression during human fetal islet development from 8 to 21 wk is consistent with its role as a potential endocrine progenitor cell marker. We also investigated, in cultured islet-epithelial clusters, the role of c-Kit receptor tyrosine kinase in mediating β-cell differentiation, using both anti-rat monoclonal antive ACK45 (ACK45) antibody and small interfering RNA (siRNA). Our experiments show that a significant number of highly proliferative c-Kit-expressing cells are present during human pancreatic neogenesis, often coexpressing insulin and glucagon. In addition, the level of c-Kit receptor kinase activity is associated with significant changes in PDX-1 and insulin mRNA and protein expression as well as β-cell proliferation and survival in the islet-epithelial clusters.

**MATERIALS AND METHODS**

**Pancreatic tissues.** Human fetal pancreata (8–21 wk) were collected according to protocols approved by the Health Sciences Research Ethics Board at the University of Western Ontario and the Research Ethics Board of the Royal Victoria Hospital at the McGill University Health Centre, in accordance with the Canadian Council on Health Sciences Research Involving Human Subjects guidelines. Tissues were carefully dissected and washed with phosphate-buffered saline and immediately processed for immunohistochemistry, RNA, and/or protein extraction or islet-epithelial cluster isolation (11), with at least three pancreata per age group.

**Immunofluorescence and morphometric analyses.** The antibodies C-19 (rabbit polyclonal IgG; cytoplastic domain, sc-168; Santa Cruz Biotechnology, Santa Cruz, CA), CD117 (rabbit polyclonal IgG, A4502; Dako, Mississauga, ON, Canada), and c-Kit-conjugated FITC (mouse monoclonal IgG, clone YB5.88, MAB1162; Chemicon, Temecula, CA) were tested for their immunoreactivity for c-Kit protein, and they all showed the same staining pattern (Supplemental Fig. S1 and data not shown; Supplemental Material for this article is available at the AJP-Endocrinology and Metabolism web site). Because C-19 turned out to give the clearest signal in pilot studies, this antibody was used in all of the ensuing experiments. Pancreata were fixed in 4% paraformaldehyde and paraffin embedded (11). A set of six 5-μm consecutive sections were cut throughout the length of the pancreas, and the tissue sections were stained with appropriate dilutions of the following primary antibodies: mouse anti-cytokeratin 19 (CK19; Dako), guinea pig anti-human insulin (Zymed, San Francisco, CA), mouse anti-human glucagon (Sigma, St. Louis, MO), mouse anti-human Ki67 (BD Pharmingen, Mississauga, ON, Canada), and rabbit anti-PDX-1 (gift from Dr. Christopher V. Wright; University of Vanderbilt, Nashville, TN), as described previously (11). Negative controls included the omission of the primary antibodies, and the specificity of the antibody against c-Kit (C-19) was determined by preabsorption with a c-Kit-blocking peptide (1:100, sc-168P; Santa Cruz Biotechnology); these tests resulted in negative staining reaction (Supplemental Fig. S1C). To identify the expression of c-Kit in cells in both ductal and islet cluster regions, dual immunostaining for c-Kit and epithelial or endocrine cell markers was performed. Fluorescent secondary antibodies were obtained from Jackson Immunoresearch Laboratories (West Grove, PA).

The percentage of cells expressing c-Kit was determined by cell counting. In brief, 12 random fields from the head, middle, and tail of the pancreata per pancreatic section were chosen, with a minimum of three pancreata per age group, and ≥1,000 cells per ductal region and 500 cells per endocrine region per pancreata were counted, except at 8–10 wk of age when all cells were counted. Data are expressed as the percentage of c-Kit-expressing cells over the total number of CK19- or insulin- and glucagon-positive cells, representing ductal and islet regions, respectively (11). To determine the percentage of c-Kit-expressing cells coexpressing with insulin and glucagon, as well as the Ki67 cell proliferation labeling index of c-Kit expressing cells, the double-labeled cells were counted and expressed as a percentage of the total number of insulin-, glucagon-, or c-Kit-positive cells counted.

**Protein extraction and Western blotting.** Pancreatic tissues were homogenized in Nonidet-P40 lysis buffer, as described previously (27). Twenty-five micrograms of pancreatic lysate proteins from each age group were separated by 7.5% SDS-PAGE and transferred to a nitrocellulose membrane (PerkinElmer Life Science, Boston, MA). A CCRF-HSB-2 whole cell lysate (sc-2265; Santa Cruz Biotechnology) was used as a positive control for c-Kit. The membranes were washed in Tris-buffered saline containing 0.1% Tween-20 and blocked with 5% nonfat dry milk overnight at 4°C. Immunoblotting was performed with anti-c-Kit (C-19; Santa Cruz Biotechnology) and anti-c-Kit phosphoY730 (Abcam, Cambridge, MA) antibodies. Proteins were detected by ECL Plus reagents (PerkinElmer Life Science) and exposed to BioMax MR Film (Kodak, Rochester, NY). The loading control was calnexin (BD Biosciences, Mississauga, ON, Canada). Densitometric quantification of bands at subsaturating levels was performed using the Syngene gel analysis software (Syngene, Cambridge, UK) and normalized to calnexin (27).

**RT-PCR and real-time RT-PCR.** Total RNA was extracted immediately after the pancreatic tissue collection using TRIzol reagent (Invitrogen, Burlington, ON, Canada). For each RT reaction, 2 μg of DNA-free RNA was used with oligo(dT) primers and Superscript reverse transcriptase (Invitrogen). The PCR primers used included c-Kit, forward: 5′-CAG GCA ACG TTG ACT ATC AGT-3′, reverse: 5′-ATT CTA AGA CTT GGA ATA ATC-3′ (288 bp); PDX-1, forward: 5′-GAG CTG GCT GTC ATG TTG AAC T-3′, reverse: 5′-GTT TGG GAA CCA GAT CCT GTT GTC-3′ (57 bp); insulin, forward: 5′-TCA CAC CGT CTG GAA GCT C-3′, reverse: 5′-ACA ATG GAG CTG TGC TGC-3′ (179 bp); and 18S, forward: 5′-GTA ACC CGT TGA ACC CCA TTC-3′, reverse: 5′-CCA TCC AAT CGG TAG TAG CG-3′ (151 bp). Real-time PCR analyses of c-Kit, PDX-1, and insulin were performed with 0.1 μg cDNA using the iQ SYBR Green Supermix kit in Chromo4 real-time PCR (Bio-Rad Laboratories, Mississauga, ON, Canada). Data were normalized to the 18S rRNA subunit with at least four repeats per experimental group (27). The relative gene expression was calculated using the Gene Expression Macro version 1.1 analysis software (Bio-Rad) (24). Controls involved omitting reverse transcription, cDNA, or DNA polymerase and showed no reaction bands.

**Treatment of cell cultures with anti-ACK45 antibody.** Human fetal pancreata (14–16 wk of fetal age) were digested with a collagenase V solution (1 mg/ml; Sigma). Resulting islet-epithelial clusters, which contained mostly undifferentiated epithelial cells and 2–10% endocrine cells (11), were plated onto 12-well plates (Corning/VWR, Toronto, ON, Canada) and cultured for 48 h with either rat monoclonal anti-ACK45 (3 μg/ml; antibody binds to the c-Kit extracellular domain, although the precise epitope is not known; Pharmingen) or with rat IgG2b isotype (IgG, 3 μg/ml; Pharmingen) as a control, using DMEM-F-12 and 5% fetal bovine serum (Invitrogen) medium with 10% human fetal islet isolations per experimental group. Cells were collected and processed for immunofluorescent staining and protein, as described above. RNA was extracted from cells using the RNequeous–4PCR kit (Ambion, Austin, TX) according to the manufacturer’s instructions (26).

**Transfection of cell cultures with c-Kit(h) siRNA.** Freshly isolated islet-epithelial clusters from 14 to 16 wk of human fetal pancreata, after a 1-h equilibration incubation in antibiotic-free medium, were
transiently transfected for 30 h with 60 nM c-Kit(h) siRNA (sc-29225) or control siRNA (sc-36869, proprietary sequence) commercially produced by Santa Cruz Biotechnology, using an siRNA transfection kit (Santa Cruz Biotechnology) with five independent human fetal islet preparations per experimental group (27). A pool of four sequences for human c-Kit siRNA (Santa Cruz) was as follows: 1) CCAGAGACAUCAAGAAUAtt (sense), UCAUUCUUGAUGUCUCUGGtt (antisense); 2) CCUCAGUGCUACUCUCAAtt (sense), UUUGAGUUCAGACUCAGGtt (antisense); 3) GAAGCUUGCACUAUUAAGtt (sense), UCUUAUAAAGUGUCACUUCtt (antisense); 4) GCUCUAGAAUUCGAUGCUAtt (sense), UCAAAUGCCAUUCUGAAGCtt (antisense). Seventy-two hours after transfection, islet-epithelial clusters were harvested and assessed for the expression of c-Kit, PDX-1, and insulin mRNA by quantitative RT-PCR as well as protein by Western blotting. Transfection efficiency was monitored using fluorescein-conjugated control siRNA (Santa Cruz Biotechnology), with ~60% of the islet-epithelial cluster cells being transfected, as described previously (27).

In situ cell death detection (terminal deoxynucleotidyl transferase-mediated nick end labeling). For the cell death assays, cells from both anti-ACK45- or c-Kit(h) siRNA-treated groups were embedded in 2% agarose and fixed in 4% paraformaldehyde, followed by paraffin embedding. Five-micrometer sections were deparaffinized, pretreated with 0.1% trypsin, and incubated with the terminal deoxynucleotidyl transferase-mediated nick end labeling (TUNEL) reaction mixture (Roche) for 60 min at 37°C, as described previously (27, 32). The sections were subsequently stained with guinea pig anti-human insulin labeled with tetramethylrhodamine isothiocyanate (TRITC). The percentages of total TUNEL-positive islet-epithelial cluster cells and TUNEL-positive β-cells were determined rel-

Fig. 1. Expression of c-Kit in the human fetal pancreas. A: morphometric analyses of c-Kit-immunoreactive cells in duct and endocrine regions of the developing human pancreas. Data are expressed as means ± SE (***duct, #endocrine region; ***P < 0.001 and #P < 0.04 vs. respective 8- to 12-wk group, n = 5-13 pancreata/age group). B: semi quantitative Western blot analyses of c-Kit and phospho-c-Kit in the human fetal pancreatic tissue. Representative blots are shown. Data are normalized to calnexin and expressed as means ± SE (**P < 0.01 vs. 8 wk and #P < 0.04 vs. 14 wk, n = 4–5 pancreata/age group), with CCRF-HSB-2 as a positive control. C: real-time RT-PCR analysis of c-Kit mRNA expression in human fetal pancreatic tissue. Data are normalized to 18S rRNA subunit and expressed as means ± SE (n = 6 pancreata/age group).
ative to the total number of cells counted per experimental group (27).

Statistical analysis. Data are expressed as means ± SE. Statistical significance was determined using either a paired student’s t-test or one-way ANOVA followed by the post hoc least significant difference group comparison test. Differences were considered to be statistically significant when \( P < 0.05 \).

RESULTS

Expression pattern of c-Kit receptor tyrosine kinase in the developing human pancreas. In our previous study (11), we observed c-Kit expression in ductal (CK19 positive) and islet endocrine (insulin positive, glucagon positive) cells in 14- to 16-wk fetal pancreata. Since then we have investigated the ontogenic pattern of c-Kit expression from early to midgestation. c-Kit-positive cells were detected at the earliest stage of pancreatic development studied (8 wk), not only within the ductal epithelium but also in small islet clusters near the ducts. Morphometric analysis of the expression pattern revealed that there was a significant decline in the proportion of c-Kit-positive cells located in the ductal regions of the developing pancreas from 8 to 21 wk (\( P < 0.001 \), Fig. 1A). Expression of c-Kit in endocrine cells was detectable from as soon as single endocrine (insulin positive, glucagon positive) cells formed from the ducts (8 wk). A significant increase in the number of c-Kit-positive cells in islet cell clusters was observed at 14–16 wk (\( P < 0.04 \)), followed by a slight decline by 19–21 wk of fetal age (Fig. 1A). These observations were supported by Western blot analyses of intact pancreata, which showed a 2.7-fold increase in total c-Kit protein from 8 to 16 wk (\( P < 0.01 \)) and a slight decline from 16 to 20 wk (Fig. 1B), whereas the phosphorylated (Tyr730) c-Kit levels showed a slight decrease from 8 to 20 wk that was not significant (Fig. 1B).

Ki-67 immunofluorescence staining was used to determine the fraction of c-Kit positive cells in the ducts and islets that were in a proliferative state from early to midgestation (Fig. 2A). c-Kit-positive cells were seen to be highly proliferative at 8–12 wk. At the later developmental stages, a significant reduction in the percentage of proliferative c-Kit-positive cells in both ductal (\( P < 0.001 \)) and islet cluster (\( P < 0.03 \)) regions was observed (Fig. 2B).

Double immunofluorescence staining revealed that the majority of c-Kit-positive cells in the early stage pancreata co-stained with CK19, an epithelial cell marker, as we have previously reported for 14- to 16-wk pancreata (11). It was also apparent that many single insulin- or glucagon-positive cells or endocrine cells in small islet clusters co-stained with c-Kit (Fig. 3A). A high proportion of c-Kit and insulin co-staining was observed at 10 and 16 wk (22 ± 1.4 and 23 ± 1.8%, respectively), with a slight decrease in the coexpression pattern by week 20 (16 ± 0.4%, \( P < 0.01 \), vs. 10 and 16 wk; Fig. 3B). A similar coexpression pattern of c-Kit with glucagon was observed: 27 ± 3.5% at 10 wk, 25 ± 1% at 16 wk, and a reduced coexpression at 20 wk (18 ± 1%, \( P < 0.02 \), vs. 10 and 16 wk; Fig. 3B).

Fig. 2. Proliferation of c-Kit-positive pancreatic cells in the developing human pancreas. A: representative images of costaining for c-Kit (FITC, green) and Ki67 (tetramethylrhodamine isothiocyanate (TRITC), red) in a 16-wk human fetal pancreas. Arrows, proliferative c-Kit-positive cells. *Nonspecific staining. Original magnifications, \( \times400 \); insets, \( \times630 \). B: proliferation of c-Kit-positive cells significantly declines over time in both ductal and endocrine regions. Data are expressed as means ± SE (\( n = 5–9 \) pancreata/age group; #endocrine region, \( P < 0.03 \) vs. respective 8- to 12-wk group; **duct, \( P < 0.001 \)).
Activation of c-Kit receptor tyrosine kinase increases PDX-1 and insulin expression. Our recent study determined that activation of c-Kit receptor tyrosine kinase activity in 14- to 16-wk human fetal islets using exogenous SCF treatment was associated with increased numbers of insulin-expressing cells and that this process involved increased phosphorylation of Akt (11). To examine whether the c-Kit receptor tyrosine kinase activity could also be influenced using an anti-c-Kit antibody, islet-epithelial clusters were pretreated with either ACK45 or IgG and cultured for 48 h. Western blot analysis showed a significant increase in c-Kit receptor phosphorylation in the ACK45 treatment group compared with the IgG group (P < 0.02; Fig. 4A), demonstrating that this antibody stimulates the c-Kit receptor. The anti-ACK45-treated group also showed a significant increase in the number of cells expressing PDX-1 (P < 0.01) and insulin (P < 0.05) at the protein level and insulin (P < 0.03) at the mRNA level, compared with the IgG control (Fig. 4B). This was associated with an increase in the proliferation capacity as determined by Ki67 labeling (P < 0.003; Fig. 4C). In addition, ACK45 treatment resulted in a decrease in the number of cells undergoing apoptosis, with twofold fewer TUNEL-positive cells, as shown in Fig. 4D.
There was also a significant reduction in the total number of apoptotic insulin-positive cells in the ACK45 group (11±1.5% vs. 23±2% of IgG group, *P*<0.001; Fig. 4D).

A knockdown in c-Kit mRNA results in a downregulation of β-cell differentiation. To examine the effects of suppressing c-Kit receptor tyrosine kinase expression in islet-epithelial clusters, we used specific human c-Kit siRNAs. After the transfection and 72 h of culture, a significant downregulation of c-Kit protein expression was observed through both Western blot and immunostaining analyses (19±2% of c-Kit-positive cells in the islet-epithelial cluster of c-Kit siRNA-treated group vs. 34±1.7% in controls, *P*<0.001; Fig. 5, A and B). Knockdown of c-Kit receptor tyrosine kinase was associated with a decrease in the percentage of PDX-1 and insulin-positive cells in the islet-epithelial clusters [54% (*P*<0.01) and 36% (*P*<0.03) decreases relative to controls, respectively; Fig. 5B], which was correlated with a significant increase in islet and β-cell death (*P*<0.05; Fig. 5C) and a significant decrease in cell proliferation rate (*P*<0.05; Fig. 5D). Decreased PDX-1, insulin, and c-Kit protein levels in the c-Kit siRNA-treated group were correlated with a three- to fourfold downregulation in their mRNA expression in the clusters (*P*<0.04, *P*<0.04, and *P*<0.003, respectively; Fig. 5E).

**DISCUSSION**

This study demonstrates for the first time that c-Kit is expressed in a dynamic, temporally-regulated fashion in the developing human fetal pancreas from early to midgestation. Our findings are consistent with previous investigations (21, 26, 28, 31) using animal models that suggest that c-Kit is a marker for β-cell progenitors. Furthermore, we have shown that enhancement or downregulation of c-Kit receptor tyrosine kinase activity in isolated human fetal islet-epithelial cell clusters results in the respective increase or reduction of PDX-1 and insulin expression at both mRNA and protein levels, indicating that the c-Kit receptor tyrosine kinase is...
Fig. 5. c-Kit small interfering RNA (siRNA) transfection. A: transfection of islet-epithelial clusters with c-Kit(h) siRNA resulted in decreases in c-Kit protein, as determined by immunofluorescent staining and Western blot. The number of immunoreactive c-Kit, pancreatic and duodenal homebox-1 (PDX-1), and insulin-expressing cells (B), TUNEL-positive cells (C), and Ki67 labeling index in the islet-epithelial clusters (D). Data are expressed as means ± SE (***P < 0.006, **P < 0.02, and *P < 0.05 relative to control siRNA, n = 3–5). E: PDX-1, insulin, and c-Kit mRNA expression (reverse transcriptase omitted) by quantitative RT-PCR analysis. Data are normalized to 18S rRNA subunit and expressed as means ± SE (*P < 0.04, **P < 0.003 vs. control, n = 4).
playing an important role in modulating several aspects of islet biology during human fetal pancreatic development.

To begin to elucidate the function of the c-Kit receptor in human fetal β-cell development, we first examined when and where c-Kit expression occurs from early to midgestation, using immunohistochemical, morphometric, protein, and RNA analyses. The percentage of c-Kit-positive cells located within the ducts, where they coexpress with cytokeratin 19, declines significantly from 8 to 21 wk (Fig. 1A). This is consistent with a population of c-Kit-positive progenitors located in the ducts that is differentiating into other cell types. c-Kit-positive cells increase in the islet clusters from 8 to 16 wk, with a subsequent decline at 21 wk (Fig. 1A). This parallels the finding of a relatively high number of cells coexpressing c-Kit and insulin or glucagon in 16-wk pancreata, with a decline at 20 wk (Fig. 3). These declines may be explained by the differentiation of the precursor endocrine cells into their mature endocrine state, as suggested by our previous study of rodent islet-derived c-Kit-positive epithelial cells (31).

If c-Kit is a marker for progenitor cells in the pancreas, a high proliferative capacity of these cells should be seen in the early stages of development. We have demonstrated, through Ki67 incorporation studies, that proliferating c-Kit-positive cells reside not only within the ductal epithelium, a site of β-cell neogenesis, but also within the newly formed islet clusters of the human fetal pancreas (Fig. 2). Their proliferative activities are highest at the earliest stage examined, 8–12 wk, and then decrease significantly by 19–21 wk, suggesting that they contribute to more differentiated, less rapidly dividing, cell types as the human fetal pancreas develops more mature islet structures.

In general, these results on the distribution and expression of c-Kit in the human fetal pancreatic ductal cells are consistent with the previous observations of Oberg-Welsh et al. (18), as well as our own (31), in the rat. However, it is important to note that c-Kit expression within cells of newly formed small islet clusters is unique to the human and suggests an important role for this receptor during human islet cell development (21, 28).

Studies in the rat and human fetal pancreas (11, 19) have determined that SCF acts as a paracrine factor, through activation of the c-Kit receptor, to promote the differentiation of islet precursor cells and the proliferation of β-cells. To further examine the functional role of the c-Kit receptor in developing islets, we used a rat-anti-mouse c-Kit (ACK45) antibody (20). The human fetal pancreatic cells treated with a 3 μg/ml dose of ACK45 antibody showed an increased phosphorylation of c-Kit receptor tyrosine kinase that was paralleled by a significant increase in cell proliferation, a significant increase in the number of cells positive for PDX-1 and insulin, enhanced PDX-1 and insulin mRNA and protein expression, and reduced islet-epithelial cluster cell as well as β-cell death. Thus, ACK45 showed similar regulatory effects on β-cell differentiation and survival as SCF (11).

The mechanism by which the anti-ACK45 antibody led to an increase in islet-epithelial cluster differentiation remains speculative, since most antireceptor antibodies block rather than stimulate receptor function. In support of our observations, it has been reported that bone marrow B cells treated with an alternative anti-c-Kit antibody (20), anti-ACK2, did not show inhibition of B cell neogenesis as expected, and instead, there was increased proliferation of precursor B cells (22). In addition, Moss et. al. (17) have described that, when black mice were treated with a low dose of ACK2, the expected depigmentation did not occur. Thus, these anti-c-Kit antibodies may function differently depending on the cell or tissue type under investigation or the dosage used.

We recently demonstrated the efficiency of gene silencing in primary rat neonatal and human fetal islets using a nonadenoviral transient transfection of β1 integrin siRNA (27, 32). In the present study, we have shown the effectiveness of using siRNA transfection to decrease c-Kit expression in human fetal islet-epithelial clusters; there was a significant decrease in islet c-Kit receptor mRNA (67%) and protein (42%) associated with a reduction in islet PDX-1 and insulin mRNA levels as well as the number of PDX-1 and insulin-expressing cells. We also demonstrated that decreasing c-Kit receptor expression in islet-epithelial clusters is associated with an increase in the total number of islet cells as well as specific β-cells undergoing apoptosis and decreased fetal islet-epithelial cell proliferation. These data are in line with the previously described role for the c-Kit receptor (1, 6, 8, 15) in offering protection from cell death through activation of antiapoptotic genes such as Bcl-2 or inactivation of proapoptotic genes such as Bad. These data also support our hypothesis that activation of c-Kit receptor tyrosine kinase in human fetal pancreatic cells is a crucial regulatory activity during pancreatic endocrine neogenesis and islet cell survival.

In summary, the spatial/temporal expression pattern of c-Kit in the developing human fetal pancreas is consistent with its role as a putative endocrine cell precursor. The coexpression of c-Kit with insulin and glucagon suggests that there is a transition of c-Kit-positive cells into endocrine cells, although there is no direct evidence as yet to show that the endocrine cell populations forming from these particular c-Kit-positive cells are functional (e.g., will release insulin and glucagon in response to the appropriate stimuli). This is, however, an important first step toward confirming the role of c-Kit as a marker for β-cell progenitors in the human. Identifying such factors will be critical for developing new islet cell-based therapies for the treatment of β-cell destruction in insulin-dependent diabetics.

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