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Transcriptomes of the major human pancreatic cell types

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Abstract

Aims/hypothesis—We sought to determine the mRNA transcriptome of all major human pancreatic endocrine and exocrine cell subtypes, including human alpha, beta, duct and acinar cells. In addition, we identified the cell type-specific distribution of transcription factors, signalling ligands and their receptors.

Methods—Islet samples from healthy human donors were enzymatically dispersed to single cells and labelled with cell type-specific surface-reactive antibodies. Live endocrine and exocrine cell subpopulations were isolated by FACS and gene expression analyses were performed using microarray analysis and quantitative RT-PCR. Computational tools were used to evaluate receptor–ligand representation in these populations.

Results—Analysis of the transcriptomes of alpha, beta, large duct, small duct and acinar cells revealed previously unrecognised gene expression patterns in these cell types, including transcriptional regulators *HOPX* and *HDAC9* in the human beta cell population. The abundance of some regulatory proteins was different from that reported in mouse tissue. For example, v-maf musculoaponeurotic fibrosarcoma oncogene homologue B (avian) (*MAFB*) was detected at equal levels in adult human alpha and beta cells, but is absent from adult mouse beta cells. Analysis of ligand–receptor interactions suggested that EPH receptor–ephrin communication between exocrine and endocrine cells contributes to pancreatic function.

Conclusions/interpretation—This is the first comprehensive analysis of the transcriptomes of human exocrine and endocrine pancreatic cell types—including beta cells—and provides a useful resource for diabetes research. In addition, paracrine signalling pathways within the pancreas are shown. These results will help guide efforts to specify human beta cell fate by embryonic stem cell or induced pluripotent stem cell differentiation or genetic reprogramming.

Keywords

Alpha cell; Beta cell; Paracrine signalling; Transcription factor

Introduction

The human beta cell transcriptome has been examined in studies of whole pancreatic islets [1–4], beta cells laser-captured from frozen tissue sections [5] or transduced beta cells [6], but these observations may not reflect the nature of normal beta cells. Moreover, the transcriptomes of human exocrine pancreatic cell subtypes have not yet been reported, despite the importance of these populations for pancreatic function and their interrelationship with the endocrine pancreas. In mice, pancreatic cell subtype expression profiling has been performed using lineage marked transgenics [7, 8], but significant differences between the mouse and human pancreatic transcriptional programmes exist. Programming of pluripotent stem cells or somatic progenitors by induced transcription factor production is a promising approach for beta cell generation [9, 10], which would benefit substantially from a more complete list of factors differentially produced in human beta cells.

The importance of transcription factor fate specification is well recognised. The v-maf musculoaponeurotic fibrosarcoma oncogene homologue (avian) (MAF) family of basic leucine zipper transcription factors has been strongly implicated in the determination of islet cell fate. *Mafa*, a regulator of insulin expression, has been shown to be expressed only in beta cells in mice [11]. Conversely, although *Mafb* is expressed in both alpha and beta cells

during murine embryonic development, in adult mice it is found only in the alpha cells [12]. Among the NK-related homeobox gene family, *Nkx6-1* shows adult expression restricted to beta cells [13] like *Mafa*, whereas *Nkx2-2* is detected in alpha and beta cells [14]. The paired box (PAX) group also contains members involved in islet cell fate specification. *Pax4* is required for the development of mouse beta cells, but is absent from mature islets [15], whereas *Pax6* is found in mature alpha and beta cells [16]. Other important endocrine transcriptional regulators include *Neurog3* and *Pdx1*. Both are expressed at intervals during endocrine cell development, but only the latter is retained in adult rodent islets (specifically beta cells) [17]. The expression patterns of these murine factors are frequently used to guide efforts to reprogramme non-beta cells to a beta cell identity, as reviewed by Baeyens et al. [18], or to differentiate embryonic stem cells or induced pluripotent stem cells into beta cells, as reviewed by van Hoof et al. [19]. It is therefore important to identify the differences between human and mouse endocrine cell transcriptional regulation.

Pancreatic exocrine and endocrine cell subtypes are also distinguishable by their differential participation in paracrine signalling. Islet hormones are best known for activating cells in remote tissues to maintain glucose homeostasis, but also locally affect secretion by acinar and duct cells. The significance of pancreatic paracrine signalling is highlighted by the observation that duct-secreted cytokines can inhibit insulin secretion and contribute to immune responses in islets [20, 21]. Duct– islet association is also critical during pancreatic development, when epithelial duct cells differentiate into endocrine precursors. Understanding these relationships may hold the key to transdifferentiating duct cells to beta cells.

Here we used novel monoclonal antibodies to purify populations of live human alpha, beta, duct and acinar cells for a comprehensive study of their gene expression by microarray and quantitative RT-PCR. Transcriptional regulators known to be important for cell type specification in mouse pancreas were surveyed and compared with those in human cells. An important divergence was observed in *MAFB*, which was expressed in adult human beta cells. Several transcriptional regulators without previously reported expression restricted to pancreatic and/or exocrine cell subtype were observed, including *HOPX* (pan-islet), *HDAC9* (beta cell), *CDX2* (duct) and *BATF2* (acinar). We also evaluated potential heterotypic cell×cell interactions between endocrine and exocrine populations and identified 121 ligand–receptor pairs: 27 ligands of seven transmembrane domain (7TM) receptors, 26 ephrins (EFNs) and 14 TGF-β family ligands. Ephrins and their receptors were over-represented, suggesting an important role for these molecules.

Methods

Tissue sources and cell isolation

Human islet samples from normal human donors were provided by the National Institutes of Health Integrated Islet Distribution Program. The use of human tissue was approved by our local Institutional Ethics Committee. Clinical information regarding these samples is listed in electronic supplementary material (ESM) Table 1. Islets were collected after 100 to 700 min of cold ischaemia and cultured in CRML 1066 for 6 to 48 h prior to overnight shipment. Viable (trypan blue-excluding) cell frequency was 95% to 99%. A single cell suspension was prepared by incubation for 10 min in 0.05% (vol./vol.) HyQ Trypsin (Hyclone, Logan, UT, USA) at 37° C, with gentle dispersal by a p1000 micropipettor (Gilson, Middleton, MI, USA) every 3 min. Undispersed material was removed with a 40 μm strainer (BD Falcon, Bedford, MA, USA). Cells were then washed and resuspended in CMRL+2% (vol./vol.) FBS (Hyclone)+0.1 mg/ml trypsin/chymotrypsin inhibitor (Sigma-Aldrich, St Louis, MO, USA). For tissue section labelling, an intact human donor pancreas was provided by S.

Orloff (Oregon Health and Science University); portions of the organ were embedded in Tissue-tek cryomatrix (Sakura, Tokyo, Japan) and stored at −86°C.

Indirect immunofluorescent staining

Adult human pancreas cryosections (5 μm) were cut with a CM1950 cryostat (Leica Biosystems, Nussloch, Germany) and dehydrated in acetone for 10 min at −20°C. Non-specific labelling was blocked with 2% (vol./vol.) goat serum (Hyclone). To produce primary antibody, the hybridoma lines listed in ESM Table 2 were grown to super-confluence in 50 ml DMEM+10% (vol./vol.) FBS. Antibody-containing supernatant fractions were collected, tested for reactivity and stored at 4°C. Primary labelling used hybridoma supernatant fractions diluted 1:50 in DPBS for 30 min; secondary labelling was with 1:200 dilution of DyLight488-conjugated anti-mouse IgM (μ chain) and Cy3-conjugated anti-mouse IgG (1+2a+3) (Jackson ImmunoResearch, West Grove, PA, USA) for 20 min. Anti-EPH receptor (EPH) B2 (R&D Systems, Minneapolis, MN, USA) and EFNB3 (LifeSpan BioSciences, Seattle, WA, USA) were diluted 1:100 and detected with the same secondary antibodies. Polyclonal rabbit anti-keratin 19 (KRT19), a gift from X. Wang (Shanghai Institutes for Biological Sciences), was used at 1:400 and detected with Cy3-conjugated anti-rabbit IgG (Jackson ImmunoResearch). Nuclei were labelled with Hoechst 33342 (Molecular Probes, Eugene, OR, USA). An Axioskop 2 plus (Carl Zeiss, Jena, Germany) was used for imaging.

Flow cytometry

Dissociated islet cells were incubated for 30 min at 4°C in hybridoma supernatant fraction diluted 1:50 in CMRL+2% (vol./vol.) FBS+0.1 mg/ml trypsin/chymotrypsin inhibitor. This dilution permitted consistent and specific cell type labelling over a broad range of cell concentrations (0.1×10^6–5×10^6 cells/ml). Cells were then washed with cold CMRL and resuspended in a 1:200 dilution of secondary antibodies (PE-conjugated anti-mouse IgM [μ chain] and Dylight488-conjugated anti-mouse IgG [1+2a+3]; Jackson ImmunoResearch). Purified human pancreas islet (-specific antibody) (HPi2) and human pancreas alpha (-specific antibody) (HPa2) antibodies (Novus Biologicals, Littleton, CO, USA) diluted at 1:200 were tested and found to be equally effective as hybridoma supernatant fraction. Dead cells were marked with propidium iodide (10 μg/ml). Cells were analysed with a FACScalibur or sorted by an inFlux V-GS (BD Biosciences, San Jose, CA, USA for both) at 15 psi using a 100 μm nozzle. The forward scatter (FSC): pulse width gating excluded cell doublets from sorts. Data were analysed using FlowJo (Treestar, Ashland, OR, USA).

RNA isolation and quantitative RT-PCR

Cells were sorted into Trizol Liquid Sample (Invitrogen, Carlsbad, CA, USA). First strand cDNA synthesis and quantitative RT-PCR reactions were performed as previously described [22]. Primer sequences are listed in ESM Table 3. Gene expression levels are reported as the difference between baseline-corrected, curve-fitted cycle thresholds for the gene of interest minus the average cycle thresholds of the housekeeping genes Lamin A/C and 18S rRNA. Statistical mean and SD were obtained with Microsoft Excel.

Western blotting

Cell lysates were generated by resuspension of flash-frozen pellets of FACS-sorted populations in SDS-PAGE loading buffer and separated by 12% to 20% gradient Bis-Tris SDS-PAGE (Bio-Rad, Hercules, CA, USA). After semi-dry electrophoretic transfer, membrane-bound proteins were labelled with antibodies recognising histone deacetylase 9 (HDAC9), HOP homeobox (HOPX) (Santa Cruz Biotechnology, Santa Cruz, CA, USA for both) or β-actin (Cell Signaling Technology, Danvers, MA, USA), and visualised by

electrochemiluminescence (Perkin Elmer, Waltham, MA, USA). Two patient samples were used, in which $>10^5$ alpha and beta cells were available (one man, 30 years old, type O, BMI 32.3 kg/m^2; one woman, 50 years old, type unknown, BMI 35.6 kg/m^2).

Microarray analyses

cDNA was synthesized from RNA (isolated as described above) and amplified (WT-Ovation Pico; NuGEN Technologies, San Carlos, CA, USA). Amplified cDNA (2 μg) was labelled using BioPrime (Invitrogen) with Cy3/Cy5-labelled nucleotides (GE Amersham Biosciences, Piscataway, NJ, USA). Of the four biological replicates, two were labelled with Cy3 and the others with Cy5; reciprocal labelling controlled for dye bias. We amplified 12 whole-islet cDNA preparations, then pooled and divided them into portions to create four reference samples. Labelled samples were hybridised overnight to the 4×44 Whole Human Genome Array (Agilent, Santa Clara, CA, USA) and scanned with a DNA microarray scanner (G2565B; Agilent). Data were normalised by the print-tip loess method using linear models for microarray data in R [23]. Subsequently, 1000 consistent probes were selected with a maximum of 1.2-fold change across all comparisons. Final expression ratios were generated using loess normalisation. Differentially expressed genes were called using the Significance Analysis of Microarrays one class response package [24] with a false discovery rate (FDR) of 20%. Log_2 expression ratios for probes were quantile-normalised and converted to a ratio for each gene (the median of the median of all the values of each probe for that gene). Differentially expressed genes (5038) were identified using PaGE [25] (95% CI). Replicate results were hierarchically clustered using TIGR (www.tm4.org/mev.html, accessed 1 February 2011) [26, 27]. K-means clustering using Pearson correlation was performed to identify patterns for Ingenuity analysis (Ingenuity Systems, Redwood City, CA, USA).

Results

Isolation of human pancreatic endocrine and exocrine subpopulations

To determine the gene expression profiles of the major human pancreatic cell types, we used the collection of cell surface-labelling monoclonal antibodies [22] listed in ESM Table 2. As shown in Fig. 1a–c, dual labelling with the pan-islet marker HPi2 and the alpha cell marker HPa2 allowed separation of alpha (HPi2$^+$HPa2$^+$) and beta (HPi2$^+$HPa2$^-$) cells. To mark exocrine, rather than endocrine cell subpopulations, a three-antibody combination containing the acinar marker human pancreas exocrine (-specific antibody) (HPx1), pan-duct marker HPd3 and the large duct-specific antibody HPd1 was employed. Figure 1d–g shows cells from the same human islet sample sorted with this exocrine marking combination; acinar (HPx1$^+$), large duct (HPd1$^+$HPd3$^+$) and small duct (HPd1$^-$HPd3$^+$) populations are indicated. These example plots show flow cytometric analysis of a sample of relatively low purity (<50% islets), but viable acinar and duct cells were recoverable from all samples. Antibody-labelled endocrine and exocrine subpopulations are illustrated in intact pancreatic tissue in Fig. 1h–j. As expected, HPa2$^+$ alpha cells comprised a subset of HPi2$^+$ islet cells (Fig. 1h), HPx1$^+$ acinar cells were distinct from HPd3+ ducts (Fig. 1i) and large ducts (HPd1$^+$) were seen to be a subset of cells labelled by pan-duct marker HPd3 (Fig. 1j). The pan-duct labelling specificity of HPd3 was also confirmed by co-labelling for keratin 19 (KRT19) [28]; a complete overlap was observed (Fig. 1k).

Pancreatic cell type purity assessment

Figure 1l shows the relative levels of mRNA encoding the prototypical cell type markers insulin, glucagon, somatostatin, trypsin and KRT19, as measured in the seven samples listed in ESM Table 1. The HPi2$^+$HPa2$^-$ beta cell fraction contained 115-fold more insulin transcripts than any of the other populations, indicating that any beta cell contamination of

the other populations was low. However, the presence of the majority of the somatostatin mRNA in this fraction indicated that delta cells were also $HPi2^{+}HPa2^{-}$. For comparison, $HPi2^{+}HPa2^{+}$ alpha cells contained 45-fold more glucagon, the $HPx1^{+}$ acinar population had 396-fold more trypsin and the duct fractions ($HPd3^{+}HPd1^{+/-}$) had 25-fold and 21.2-fold more *KRT19* mRNA than their counterparts. Thus, our sorting scheme permitted separation and molecular analysis of these populations.

Microarray analyses of pancreatic cell subpopulations

Having verified the purity of these populations, we assessed their global mRNA expression profile by microarray analysis using four biological replicates per population. These data are available through the ArrayExpress database (www.ebi.ac.uk/arrayexpress/, accessed 1 July 2011; accession numbers E-MTAB-463 and E-MTAB-465), and gene lists can be found at http://137.53.250.24/grompelab/ (accessed 1 July 2011). Cluster analysis of the global gene expression profiles was performed using five sorted cell populations and focusing on 5,038 genes that were differentially expressed between any pair of cell types. As shown in Fig. 2, the two endocrine populations are closely related, but a group of genes was expressed differently in alpha and beta cells. The duct and acinar populations appear on a separate branch of the tree. Large and small ducts are nearly identical, but unique blocks are found in duct and acinar populations. K-means clustering revealed five major expression patterns: endocrine (1,086 genes), beta cell-specific (528), non-endocrine (845), acinar-specific (925) and duct-specific (457). Genes expressed specifically in endocrine cells (or beta cells only) were significantly enriched for diabetes ($p=5\times 10^{-11}$) and neurological disorder ($p=1\times 10^{-20}$) categories according to Ingenuity analysis, indicating an association of neurological secretion pathways with endocrine cells. The top pathway in the acinar-specific genes was protein synthesis ($p=6\times 10^{-17}$). Several pathways were strongly enriched among genes specific to ducts, namely: cell migration ($p=3\times 10^{-15}$), cell-to-cell signalling, especially adhesion ($p=6\times {}^{-14}$), cell death ($p=7\times 10^{-14}$) and tumorigenesis ($p=9\times 10^{-11}$).

Comparative gene expression in beta vs alpha cells

To assess the major differences in gene expression between alpha and beta cells, we performed two-colour microarray analyses. These experiments permitted a direct comparison of these highly related endocrine populations using cells subjected to identical handling (islet isolation from the same individual, followed by simultaneous enzymatic dispersal, antibody labelling and FACS). The most strongly differentially expressed genes in human beta and alpha cells are listed in Tables 1 and 2. Genes with strong beta cell-specific expression included known factors, e.g. *HADH* [29], *IL1B* [30], *IAPP* [31] and *PTGS2* [32], as well as several novel genes. Alpha cell-selective genes have been less well studied, but transcription factors shown to contribute to alpha cell fate specification (*IRX2*, *ARX*, see below) were strongly upregulated in this population. To measure whether delta cell-selective genes might have been misidentified as beta cell-selective, ten of the top candidates were examined in cells enriched by Newport Green dye positivity rather than antibody selection. As illustrated in ESM Table 4, this method provided weaker beta cell enrichment, but substantially reduced delta cell contamination (NG+ cells were only twofold enriched for somatostatin). Other than *DCX*, each of the genes examined was also enriched in the NG+ population and therefore beta cell-selective. Certain known beta cell-selective genes showed differential expression, but did not meet statistical significance (e.g. *PDX1*, which was ninefold enriched in beta cells, but with >10% *FDR*) or were not detected by the array (e.g. *MAFA*), suggesting that low abundance transcripts such as transcription factors were being missed. Because these include important regulators of pancreatic cell fate, we specifically examined transcriptional regulators by quantitative RT-PCR to augment the microarray results.

Cell type-restricted expression of transcriptional regulators

Of the islet samples listed in ESM Table 1, five were used to examine specific transcription factor expression by quantitative RT-PCR. Several known regulators of mouse endocrine pancreatic cell fate were surveyed (Fig. 3a) in human alpha and beta cell mRNA. Among these we found that the expression of *PDX1* and *MAFA* was restricted to beta cells and that of *IRX2* and *ARX* to alpha cells, as expected from prior data obtained in rodents. *MAFB*, *NKX2-2*, *NKX6-1*, *PAX6* and *HNF4A* expression was detected in both cell types, whereas *PAX4* and *NEUROG3* mRNA was not detected in either population (data not shown). Of these results, the most notable was that *MAFB* is expressed in human beta cells at a level comparable to that in alpha cells (whereas adult mouse beta cells lack *Mafb*) [12]. We also report the first detection of *IRX2* in adult alpha cells; prior observations were confined to fetal mouse tissue [33].

Microarray analysis also revealed differential expression of regulators not previously described in pancreatic islets. Figure 3b shows quantitative RT-PCR confirmation that expression of *HOPX*, *TFCP2L1* and *HDAC9* in the human pancreas is restricted to cell types. *HOPX* mRNA was detected in alpha and beta cells, but was absent from any exocrine population. *TFCP2L1* was expressed only by beta cells within the islet, but its mRNA was also detectable (at a lower level) in acinar cells. *HDAC9* was detected at much higher levels in beta cells than in any other pancreatic cell population. As illustrated in Fig. 3c–e, western blotting indicated that the mRNA beta cell-selective and pan-islet expression patterns were also reflected at the protein level for HDAC9 and HOPX, respectively.

We also identified a few transcription factors that were differentially expressed in exocrine pancreatic populations (Fig. 3f). These included genes with an alpha and acinar cell-specific transcript distribution (*TCF25*), a pan-exocrine expression pattern (*SOX9* and *GSC*), duct-specific gene activation (*CDX2*) and acinar-specific expression (*ONECUT1* and *BATF2*). Of these, only *SOX9* and *ONECUT1* had previously been reported to mark pancreatic cell lineages; the proteins encoded by these, SRY-box containing gene 9 and one cut domain, family member 1, are markers of developing ducts and islets in mice [34, 35].

Heterotypic cell×cell interaction in the human pancreas

To evaluate signalling interactions between different human pancreatic cell subpopulations, we conducted differential expression analysis using PaGE with a 20% FDR for ligand- and receptor-encoding genes for each pair of cell types (each cell type vs each of the remaining four cell types). Table 3 lists the matches compared with a curated human receptor–ligand database (Human Plasma Membrane Receptome [HPMR], www.receptome.org/HPMR/, accessed 1 February 2011). To specifically evaluate interactions between islet hormones and duct-resident hormone receptors, we compared our RNA-based observations for these populations with those of Bertelli and Bendayan [36], who recently published a protein-based survey of secondary islet hormones reported to affect duct and/or acinar secretion (Table 4). Substantial agreement was observed.

Predicted heterotypic ligand–receptor pairs

For ten pancreatic cell-pairs and two directions (ligand–receptor), 121 ligand–receptor pairs were identified (Table 3, ESM Table 5). These included 62 non-redundant relationships previously reported in non-pancreatic tissues or tumours. Others have been described previously in the pancreas, such as EPHA4+ EFNA5, vascular endothelial growth factor +neuropilin, angiotensinogen+AGT receptor and betacellulin+EGF receptor. We identified 89 pairs of endocrine ligands (60 beta, 29 alpha) and exocrine receptors, and 24 pairs of endocrine receptors and exocrine ligands. For the remaining eight pairs, ligand and receptor

were in the same compartment. ESM Fig. 1 illustrates these interactions; the detailed relationships are listed in ESM Table 6.

Ligand–receptor family composition

To obtain an overview of 121 ligand–receptor pairs, the distribution of ligands among the families was categorised in HPMR. The ligand distribution among families was uneven, with ligands of 7TM receptors, EFN and TGF-beta family ligands being the most frequent (ESM Table 6). This was mirrored in the corresponding receptor subfamilies (ESM Table 6). After accounting for ligand family representation, EFNs and EPH receptors were substantially over-represented. This is consistent with a recent publication that examined intra-islet paracrine signalling and identified EFN family gene expression [37].

From in vivo and/or in vitro protein×protein interaction, 199 ligand–receptor pairs from the Human Protein Reference Database (HPRD) were inferred (Table 3, ESM Table 5). Table 5, ESM Table 7 and ESM Fig. 1 show comparisons of these results with the HPMR observations described above. Of the additional protein×protein interactions identified only in the HPRD database, most belong to the TGF-β and fibroblast growth factor families.

The expression of EPH receptor–EFN gene families revealed distinct usage of protein isoforms by cell types. EFNB3 and EFNA5 were upregulated in endocrine (alpha and beta) cells, whereas EFNB2 and EFNA1 were produced at higher levels in the exocrine compartment (large, small duct and acinar). For the EPH receptors, each exocrine cell type had at least two genes upregulated, but none were differentially activated in alpha or beta cells (Table 5). This pattern suggests that communication between exocrine and endocrine cells occurs and can be mediated by EPH receptor–EFN signalling. ESM Fig. 2 illustrates the distribution of receptor–ligand pair EFNB3 and EPHB2 protein in pancreas tissue. Consistent with the RNA-based observations, these proteins were observed primarily in islets or ducts, respectively.

Discussion

In this study, we generated the first gene expression atlas of adult human pancreatic alpha, beta, duct and acinar cells. The expression of known transcriptional regulators was similar—but not identical—to that in mouse islets, and novel transcriptional regulators were discovered in islet and exocrine cells. In addition, a computational ligand–receptor survey revealed potential paracrine regulatory circuits, including several EFN family members. These observations extend the understanding of human pancreatic cell regulation and should aid future studies of cell fate determination and function.

Transcriptional regulator expression in endocrine and exocrine cells

Although MAFB is often described as an alpha cell marker in adult pancreatic tissue [12], several exceptions have been observed. In the islets of adult *Meriones* jirds (gerbils), MAFB is found in alpha and beta cells [38]; even in mice, MAFB is detectable in the beta cells of adolescents and pregnant adults [39]. Coupled with our observation that *MAFB* is expressed in adult human beta cells, this suggests that the pattern observed in adult mice is the exception rather than the rule. MAFB has not been regarded as a useful reprogramming agent for the derivation of beta cells; our findings suggest that this should be re-evaluated. Iroquois homeobox genes like *IRX2* are transcription factors with developmental roles in broad pattern specification [40]. In one report, *Irx2* was shown to be restricted to alpha cells in the developing mouse pancreas [33], suggesting a potential role for the specification of this lineage. Since *IRX2* is also restricted to alpha cells in adult humans, it could be a target for inhibition to suppress polyhormonal fates and promote beta cell identity. The deletion of

Arx, expression of which is also restricted to alpha cells in the adult human islet, increases the ratio of beta:alpha and/or delta:alpha cells in knockout mice [41].

We observed cell type-selective expression of several transcriptional regulators not previously reported in the pancreas; three of these had particularly noteworthy cell type specificities. The transcriptional regulator *HOPX* contains a homeobox domain, but lacks the DNA binding motif normally associated with HOX genes [42]. In developing cardiac and pulmonary tissue, *HOPX* acts downstream of *NK2-1* and *NK2-5* (respectively) [43]. Its expression in adult human islet cells, which also express NK family genes, suggests that *HOPX* might contribute to the specification or maintenance of endocrine cell identity. Beta cell-specific expression of *HDAC9* is of particular interest. HDAC9 is a transcriptional co-repressor characterised principally as a neuronal development factor [44]. However, it was recently implicated as a fasting-induced upstream transcription factor-1 inhibitor and an important element of a novel insulin-dependent metabolic regulatory system [45]. Beta cell-specific *HDAC9* expression in the pancreas suggests that it could be a useful reprogramming agent and enforcer of beta cell identity. Information on *BATF2* is extremely limited, but our observation of highly acinar cell-specific expression is potentially important. The reprogramming of acinar cells to a beta cell-like state has been demonstrated [46]; if this process could be enhanced by suppression of factors like BATF2, it could become a significant therapeutic approach.

Heterotypic cell type interactions: the roles of EPH receptor–EFN signalling in the pancreas

The ligand and receptor in EPH receptor–EFN signalling are cell surface localised; binding between these proteins requires cell–cell contact and triggers a forward signalling pathway in the receptor cell and a reverse signalling pathway in the ligand cell [47]. EPH receptor–EFN signalling has been implicated in several processes, including tissue development, cancer and glucose homeostasis. In the latter, EPH receptor–EFN signalling regulates insulin secretion, with EPH receptor signalling suppressing insulin secretion, whereas EFN signalling activates it; the balance correlates with blood sugar level [48]. Islet hormone regulation of duct cell bicarbonate fluid secretion is physiologically important [49]. However, the topographical association between islet and duct cells in the pancreas implies that cell organisation during epithelial branching morphogenesis and capillary formation is important for pancreatic development. EPH receptor–EFN signalling is important for regulating this process during neural development and may play a similar role during pancreatic tissue organisation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

EFN	Ephrin
EPH	Ephrin receptor
FDR	False discovery rate
FSC	Forward scatter
HPa2	Human pancreas alpha (-specific antibody)
HPi2	Human pancreas islet (-specific antibody)
HPx1	Human pancreas exocrine (-specific antibody)
HPd1/HPd3	Human pancreas duct (-specific antibody)
HPMR	Human plasma membrane receptome
HPRD	Human protein reference database
7TM	Seven transmembrane domain

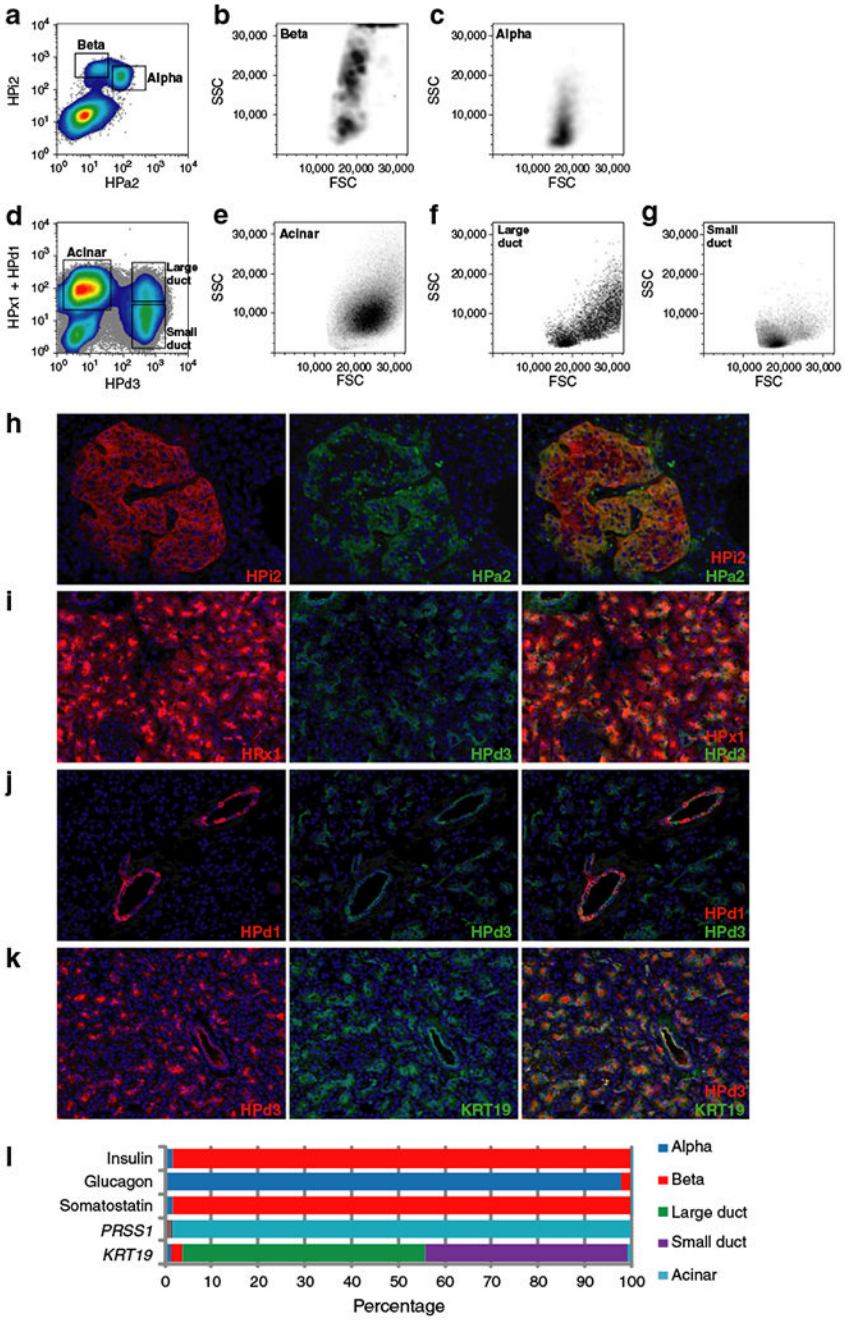

Fig. 1.

The isolation of subsets of live human pancreatic endocrine and exocrine cells. **a** The FACS-detected fluorescence of pancreatic islet cells co-labelled with endocrine cell markers HPi2 and HPa2, with back-scattering visualisation of (**b**) beta and (**c**) alpha cells to reveal their size/granularity (FSC/side scatter [SSC]) characteristics. **d** As above (**a**), but co-labelled with exocrine markers HPx1, HPd1 and HPd3, and with back-scattering visualisation of the populations defined as acinar (**e**), large duct (**f**) and small duct (**g**). **h** Dual immunofluorescent labelling of adult human pancreatic cryosections simultaneously labelled with the same (**a**, **b**) groups of endocrine or (**i**, **j**) exocrine cell type-specific antibodies,

respectively. **k** The comprehensive duct labelling of HPd3 is demonstrated by co-labelling with KRT19. **l** The relative levels of markers of beta (insulin), alpha (glucagon), delta (somatostatin), acinar (*PRSS1*) or duct (*KRT19*) cell identity. Quantitative RT-PCR results obtained from FACS-isolated populations were calculated as ΔC_t values relative to the mean of Lamin A/C and 18S rRNA. The total signal detected for each marker gene is indicated as a percentage

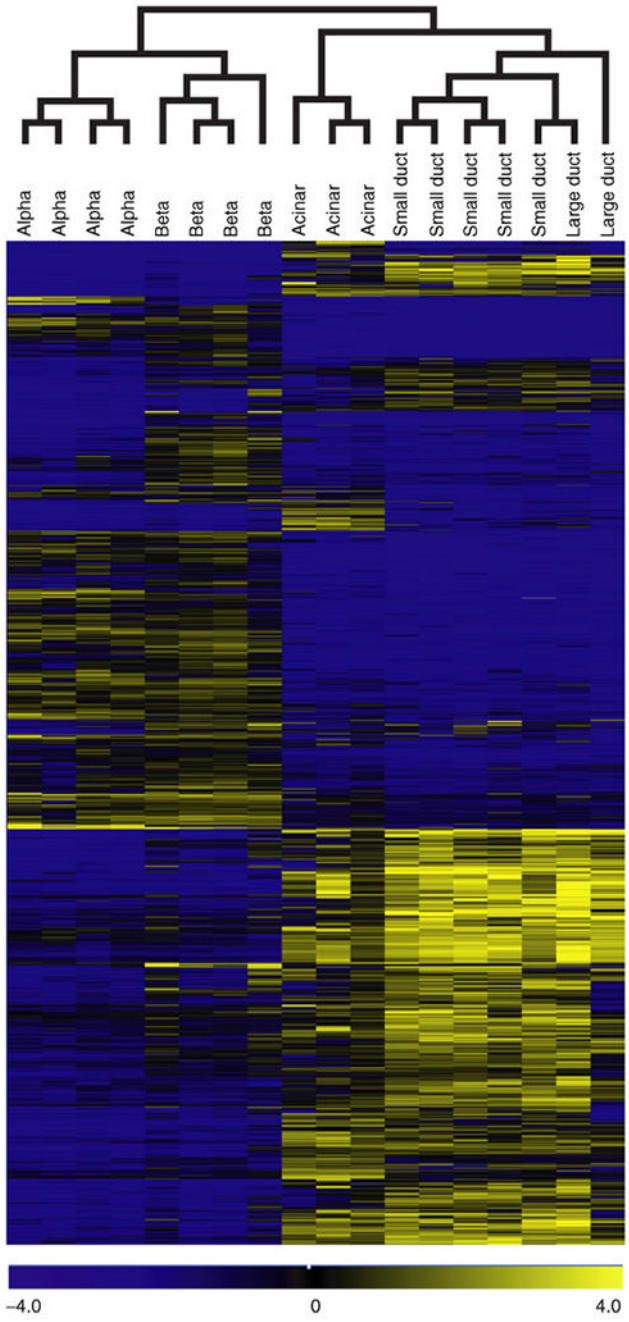

Fig. 2.

Hierarchical clustering of microarray-assessed gene expression in human pancreatic endocrine and exocrine cell subpopulations. We used PaGE algorithm to identify 5,038 differentially expressed genes (in any pair-wise comparison; 95% CI), and clustered them hierarchically using TIGR Multiexperiment Viewer

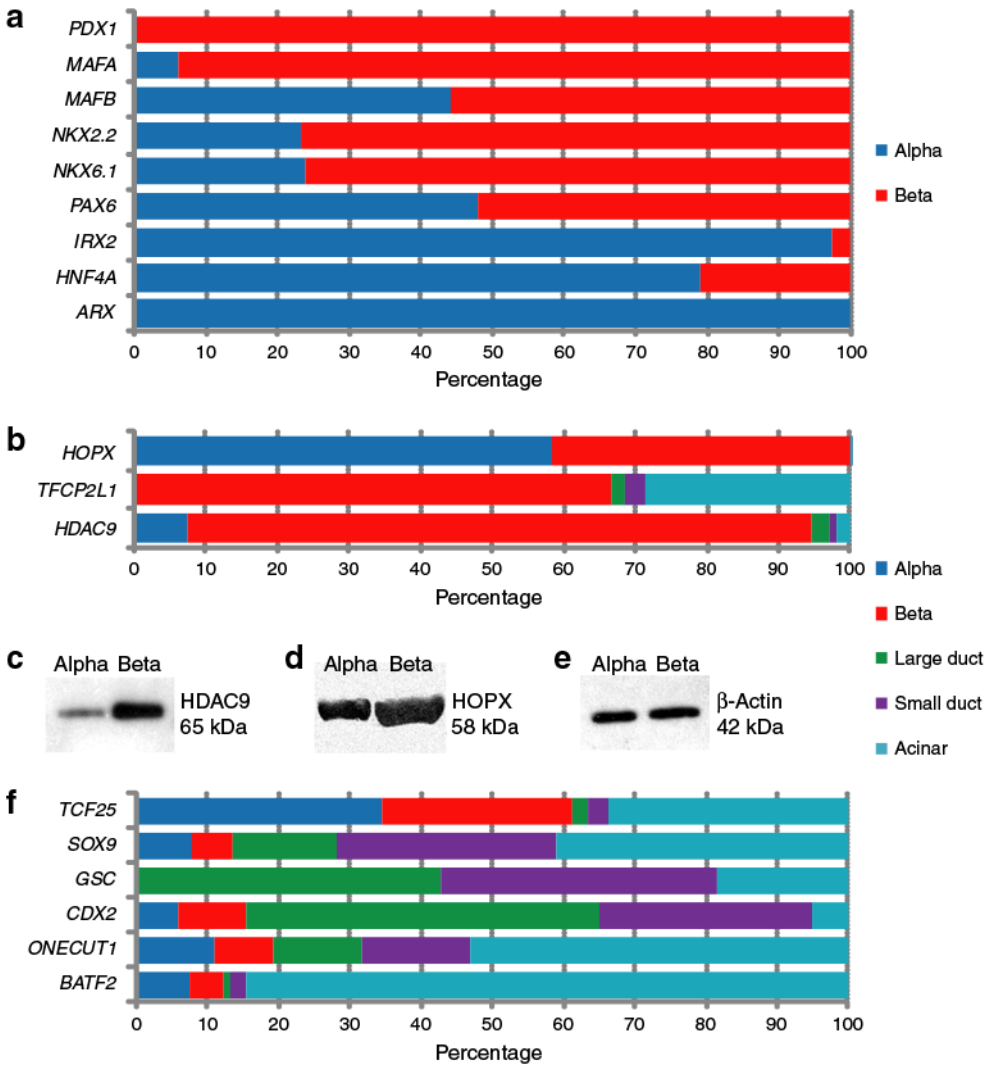

Fig. 3.

Gene expression distribution of known and novel transcriptional regulators in pancreatic cell subpopulations. **a, b, f** Quantitative RT-PCR results obtained from RNA isolated from FACS-isolated human pancreatic cell populations were calculated as ΔC_t values relative to the mean of housekeeping genes Lamin A/C and 18S rRNA. The total signal detected for each gene in each pancreatic cell subpopulation is indicated in per cent. **c**–**e** Protein levels of HDAC9, HOPX and β-actin were determined by SDS-PAGE of lysates obtained from sorted populations and by western blotting

Table 1

Genes with >20-fold elevated expression in sorted human beta cells relative to alpha cells

Gene	Transcript	Description	FC	FDR	AdjP
SST[a]	NM_001048	Somatostatin	107.8	0	4.4×10^{-3}
CLIC6	NM_053277	Chloride intracellular channel 6	76.6	0	5.2×10^{-3}
BM678403	BM678403	cDNA clone UI-E-EO0-ahw-c-09-0-UI 3′	70.1	0	4.4×10^{-3}
CB305813	CB305813	cDNA clone UI-CF-EN1-aeg-d-07-0-UI 3′	65.1	0	3.4×10^{-3}
TFCP2L1	NM_014553	Transcription factor CP2-like 1	56.3	0	3.4×10^{-3}
ITIH5	NM_030569	Inter-alpha (globulin) inhibitor H5	56.1	0	9.1×10^{-3}
DGKB	NM_004080	Diacylglycerol kinase, beta 90 kDa	54.9	0	1.0×10^{-2}
INS	NM_000207	Insulin	45.9	0	1.8×10^{-2}
HADH	NM_005327	Hydroxyacyl-coenzyme A dehydrogenase	43.6	0	8.0×10^{-3}
SOD3	NM_003102	Superoxide dismutase 3, extracellular	42.2	0	3.4×10^{-3}
HLA-DQB1	NM_002123	MHC class II HLA-DQ-beta	40.9	0	2.1×10^{-2}
C9orf135	NM_001010940	Chromosome 9 open reading frame 135	37.9	0	4.4×10^{-3}
DLK1	NM_003836	Delta-like 1 H. log (Drosophila)	37.9	0	1.8×10^{-2}
BX438895	BX438895	cDNA clone IMAGE:785925 3′	34.5	0	1.8×10^{-2}
SSPN	NM_005086	Sarcospan (Kras oncogene-associated gene)	34.1	0	7.1×10^{-3}
PTPRK	NM_002844	Protein tyrosine phosphatase, receptor type, K	33.8	0	4.7×10^{-3}
ASB9	NM_001031739	Ankyrin repeat and SOCS box-containing 9	32.2	0	5.2×10^{-3}
DCX[a]	NM_000555	Doublecortex; lissencephaly, X-linked	30.8	0	4.9×10^{-3}
IAPP	NM_000415	Islet amyloid polypeptide	30.6	0	1.0×10^{-2}
RBP4	NM_006744	Retinol binding protein 4, plasma	30.4	0	5.2×10^{-3}
PTGS2	NM_000963	Prostaglandin-endoperoxide synthase 2	29.6	0	1.3×10^{-2}
SCD5	NM_001037582	Stearoyl-CoA desaturase 5	29.6	0	5.2×10^{-3}
DAPL1	NM_001017920	Similar to death-associated protein	29.3	0	3.4×10^{-3}
RGS16	NM_002928	Regulator of G-protein signalling 16	28.9	0	4.3×10^{-3}
PLCH2	NM_014638	Phospholipase C, eta 2	28.8	0	7.8×10^{-3}
BG818013	BG818013	cDNA clone IMAGE:4915128 5'	28.5	0	6.6×10^{-3}
LOC284033[b]	AK095052	cDNA FLJ37733 fis, clone BRHIP2020827	28.5	0	9.3×10^{-3}

Gene	Transcript	Description	FC	FDR	AdjP
TNS1	NM_022648	Tensin 1	27.2	0	4.7×10^{-3}
GLT25D2	NM_015101	Glycosyltransferase 25 domain containing 2	27.2	0	6.4×10^{-3}
CALD1	NM_033138	Caldesmon 1, transcript variant 1	27.0	0	4.7×10^{-3}
PRPH	NM_006262	Peripherin	26.3	0	6.0×10^{-3}
IL1B	NM_000576	Interleukin 1, beta	26.0	0.15	3.0×10^{-2}
TSHZ2	ENST00371497	Teashirt H.log 2 (Zinc finger protein 218)	25.0	0	1.7×10^{-2}
SGIP1	NM_032291	SH3-domain GRB2-like interacting protein 1	24.6	0	3.4×10^{-3}
ESR1	NM_000125	Oestrogen receptor 1	24.4	0	4.3×10^{-3}
CDH22	NM_021248	Cadherin-like 22	24.2	0	8.6×10^{-3}
IGF2	NM_000612	Insulin-like growth factor 2 (somatomedin A)	23.7	0	3.4×10^{-3}
ANTXR1	NM_032208	Anthrax toxin receptor 1	23.6	0	9.6×10^{-3}
THC2656690	THC2656690	NA	23.6	0	9.5×10^{-3}
BC063022	BC063022	cDNA clone IMAGE:5246259, partial cds	23.6	0	1.1×10^{-2}
IGSF11	NM_152538	Immunoglobulin superfamily, member 11	23.4	0	4.7×10^{-3}
SFRP1	NM_003012	Secreted frizzled-related protein 1	23.1	0.25	4.5×10^{-2}
XYLT1	NM_022166	Xylosyltransferase I	23.0	0.09	2.3×10^{-2}
ADCYAP1	NM_001099733	Pituitary adenylate cyclase-activating polypeptide	22.8	0.09	2.2×10^{-2}
EPDR1	NM_017549	Ependymin related protein 1 (zebrafish)	22.8	0	4.2×10^{-3}
FBN2	NM_001999	Fibrillin 2 (congenital contractural arachnodactyly)	22.8	0	1.8×10^{-2}
PCDH7	NM_002589	BH-protocadherin, transcript variant a	22.4	0	7.9×10^{-3}
SLC17A6	NM_020346	Solute carrier family 17, member 6	22.2	0	1.7×10^{-2}
CNGA3	NM_001298	Cyclic nucleotide gated channel alpha 3	21.6	0	4.7×10^{-3}
BC104421	BC104421	cDNA clone IMAGE:40004940	21.6	0	8.0×10^{-3}
CAPN13	NM_144575	Calpain 13	20.6	0	3.4×10^{-3}

Expression levels are the means derived using four runs of two-colour pairwise binding of FACS-sorted alpha and beta cell cDNA. Genes with an FDR of ≥5%, duplicate probe results and probes called as ‘marginal’ were all excluded

[a] Attributed to delta cell contamination (see below);

[b] Also known as SHISA6

AdjP, adjusted *p* value; FC, fold change

Table 2

Genes with ≥20-fold elevated expression in sorted human alpha cells relative to beta cells

Gene	Transcript	Description	FC	FDR	AdjP
GCG	NM_002054	Glucagon	111.1	0	1.5×10^{-2}
POPDC3	NM_022361	Popeye domain containing 3	83.3	0	1.4×10^{-2}
LOXL4	NM_032211	Lysyl oxidase-like 4	83.3	0	4.3×10^{-3}
GRIN3A	NM_133445	Glutamate receptor, ionotropic, N-methyl-D-aspartate 3A	66.7	0	3.4×10^{-3}
STK32B	NM_018401	Serine/threonine kinase 32B	58.8	0	5.4×10^{-3}
SPINK4	NM_014471	Serine peptidase inhibitor, Kazal type 4	55.6	0	3.4×10^{-3}
CRH	NM_000756	Corticotropin releasing hormone	43.5	0	1.2×10^{-2}
THC2690347	THC2690347	Seven TM receptor protein 224, partial	41.7	0	1.7×10^{-2}
GC	NM_000583	Group-specific component (vitamin D binding protein)	38.5	0	5.0×10^{-3}
FA P	NM_004460	Fibroblast activation protein, alpha	33.3	0.25	4.1×10^{-2}
GJA3	NM_021954	Gap junction protein, alpha 3, 46 kDa (connexin 46)	31.3	0	5.0×10^{-3}
MUCL1	NM_058173	Small breast epithelial mucin	29.4	0	3.4×10^{-3}
AI492422	AI492422	cDNA clone IMAGE:2131746 3'	29.4	0	1.3×10^{-2}
C20orf39[a]	NM_024893	Chromosome 20 open reading frame 39	28.6	0.07	1.9×10^{-2}
VIM	NM_003380	Vimentin	27.0	0	8.9×10^{-3}
STC2	NM_003714	Stanniocalcin 2	27.0	0	4.4×10^{-3}
PTPRT	NM_133170	Protein tyrosine phosphatase, receptor, T transcript variant 1	27.0	0	6.0×10^{-3}
MUC13	NM_033049	Mucin 13, cell surface associated	25.6	0	4.5×10^{-3}
BQ286187	BQ286187	cDNA clone IMAGE:5782164 5′	25.6	0	4.4×10^{-3}
IRX2	AY335940	Iroquois-class homeodomain protein IRX-2	25.0	0	9.3×10^{-3}
THC2496213	THC2496213	cDNA clone IMAGE:2735726 3′	23.8	0	6.8×10^{-3}
RP11-35N6.1[b]	NM_207299	Plasticity related gene 3, transcript variant 1	23.8	0	1.0×10^{-2}
F10	NM_000504	Coagulation factor X	22.7	0	5.2×10^{-3}
ARX	NM_139058	Aristaless related homeobox	22.2	0	4.7×10^{-3}
BVES	NM_147147	Blood vessel epicardial substance, transcript variant 5	21.7	0.22	3.4×10^{-2}
NPNT	NM_001033047	Nephronectin	20.8	0	8.9×10^{-3}
C11orf41	NM_012194	G2 protein mRNA, partial cds	20.8	0.32	4.2×10^{-2}

Gene	Transcript	Description	FC	FDR	AdjP
FXYD5	NM_144779	FXYD domain containing ion transport regulator 5	20.0	0	3.4×10^{-3}
BQ777622	BQ777622	cDNA clone IMAGE:6032433 3′	20.0	0	9.4×10^{-3}

Expression levels are the means derived using four runs of two-colour pairwise binding of FACS-sorted alpha and beta cell cDNA. Genes with an FDR of ≥5%, duplicate probe results and probes called as ‘marginal’ were all excluded

[a] *C20orf39*, also known as *SYNDIG1*;

[b] *RP11-35N6.1*, also known as *LPPR1*

AdjP, adjusted *p* value; FC, fold change

Table 3

Ligand–receptor pairs supported by HPMR and by HPRD protein × protein interaction prediction

Population with ligand	Population with receptor	HPMR (*n*)[a]	HPRD (*n*)[a]
Acinar	Alpha	5	5
Acinar	Beta		1
Acinar	Small duct		3
Alpha	Acinar	8	16
Alpha	Beta	1	2
Alpha	Large duct	9	15
Alpha	Small duct	12	18
Beta	Acinar	18	28
Beta	Alpha	2	2
Beta	Large duct	22	29
Beta	Small duct	20	32
Large duct	Acinar	1	1
Large duct	Alpha	8	8
Large duct	Beta	3	7
Small duct	Acinar	4	5
Small duct	Alpha	5	15
Small duct	Beta	3	12

[a]Total number of pairs: HPMR 121, HPRD 199

Table 4

Expression pattern of known islet hormone and ductal receptor genes

Hormone precursor	Expression pattern	Hormonelocalisation[a]	Receptor	Expression pattern
IAPP	↑ in beta, alpha	Beta+delta		
ADM	↑ in small duct, beta	PP	*RAMP2*	↑ in alpha
CALCA	↑ in acinar, alpha	Subpopulation of delta		
INS		Beta		
CHGA	CHGB ↑ in alpha, beta	Alpha+beta		
SCG2	↑ in alpha, beta	Alpha+beta		
GHRL	n.d.e.	Epsilon		
CORT	↑ in alpha, beta	Single islet cells	*SSTR3*	↑ in large duct, acinar, small duct
HCRT		Alpha+beta	*HCRTR1*	↑ in acinar
RETN	n.d.e.	Beta		
UCN	↑ in large duct, alpha	Beta		
CRH	↑ in alpha, beta	Alpha	*CRHR*[b]	↑ in large duct
PYY	↑ in large duct	Alpha, some PP	*PPYR1*	↑ in acinar, large/small duct
			NPYR5[c]	↑ in large/small duct
INS	↑ in beta, alpha		*INSR*	↑ in acinar, large duct
INSL3/5	↑ in large/small duct			
GCG	↑ in alpha, beta		*GCGR*	↑ in large duct
SST	↑ in beta		*SSTR1*	↑ in alpha and beta
			SSTR2	↑ in alpha
			SSTR3	↑ in large duct, acinar, small duct
			SSTR4	↑ in large/small duct
			GLP1R	↑ in beta

Vertical arrow symbols (↑) denote increased expression.

[a] As reported by Bertelli and Bendayan [36];

[b] CRHR, also known as CRHR1;

[c] NPYR5, also known as NPY5R

IAPP, islet amyloid polypeptide; n.d., not determined; n.d.e., no differential expression; PP, polypeptide

Table 5

EPH receptor–EFN pairs predicted in inter-tissue (exocrine–endocrine) and intra-tissue (alpha–beta) cell type pairs

Population with ligand	Population with receptor	Ligand	Recepto
Acinar	Alpha	EFNA1	EPHA4
Small duct	Alpha	EFNA1	EPHA4
Acinar	Alpha	EFNA2	EPHA4
Alpha	Small duct	EFNA5	EPHA2
Alpha	Small duct	EFNA5	EPHB2
Beta	Large duct	EFNA5	EPHA2
Beta	Small duct	EFNA5	EPHA2
Beta	Alpha	EFNA5	EPHA4
Beta	Large duct	EFNA5	EPHA4
Beta	Large duct	EFNA5	EPHA5
Beta	Acinar	EFNA5	EPHA8
Beta	Large duct	EFNA5	EPHA8
Beta	Small duct	EFNA5	EPHA8
Beta	Acinar	EFNA5	EPHB2
Beta	Large duct	EFNA5	EPHB2
Beta	Small duct	EFNA5	EPHB2
Small duct	Alpha	EFNB2	EPHA4
Alpha	Large duct	EFNB3	EPHB2
Alpha	Small duct	EFNB3	EPHB2
Alpha	Large duct	EFNB3	EPHB3
Beta	Large duct	EFNB3	EPHA4
Beta	Acinar	EFNB3	EPHB2
Beta	Large duct	EFNB3	EPHB2
Beta	Small duct	EFNB3	EPHB2
Beta	Large duct	EFNB3	EPHB3
Beta	Small duct	EFNB3	EPHB3