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Distribution of insulin mRNA transcripts within the human body

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ABSTRACT

Here we sought evidence for the existence of insulin mRNA-producing cells outside the human pancreas. Commercially available complementary DNA (cDNA) arrays prepared from 72 different types of adult human tissues were screened by PCR for transcripts encoding insulin, and other classic pancreatic hormones. Insulin mRNA transcripts were detected by standard PCR in the pancreas, stomach, pylorus region of the stomach, and the duodenum; and additionally by nested PCR in the jejunum, ileum and cecum, but not in other body tissues including the brain and colon. Most of these tissues also variably expressed mRNA transcripts for amylase α2B, amylin, glucagon, somatostatin, and pancreatic polypeptide. In summary, using sensitive PCR methods we have provided evidence for the presence of rare insulin mRNA-expressing cells within the stomach, small intestine, and cecum. Their role at these sites may be to support classical enteroendocrine cells as sentinels to sense and monitor gastric contents passing into and through the bowel.

1. Introduction

Pancreatic islet β-cells are dispersed within small islands of endocrine tissue, namely the islets of Langerhans [1–3]. They evolved to continuously monitor blood glucose levels and respond to hyperglycemia by secreting insulin and other hormones. Pancreatic β-cells are destroyed in type 1 diabetes due to uncontrolled autoimmunity, resulting in hyperglycemia and other life-threatening complications [4].

The pancreas appeared late in evolution, being restricted to vertebrates [1–3]. In insects the brain supplies hormones including insulin [2,3,5,6]. In protochordates, four types of endocrine cells expressing insulin, glucagon, somatostatin and pancreatic polypeptide are scattered unaggregated within the gut mucosa together with exocrine cells [2,3,7]. The Atlantic hagfish appears to represent an evolutionary link. It has a primitive islet-like organ which buds out from the bile duct and contains insulin- and somatostatin-producing cells [2,3,8]. The latter cells are numerous in the bile duct mucosa, whereas glucagon-producing cells and pancreatic polypeptide (PP)-cells are only present in the gut mucosa together with small numbers of somatostatin-producing cells. Higher vertebrates, such as sharks and rays (plagiostomian cartilaginous fish), possess a primitive pancreas that has endocrine islets containing all four classical hormone-producing cell types [2,3,9]. The luminal cells fold to form the exocrine tissue of the ductular pancreas. The classical hormone-producing cell types, with the exception of insulin-producing cells, are still also present within the gut mucosa. In teleost bony fish, the four classical types of endocrine cells are organized into anatomically discrete islets called Brockmann bodies (principal islets) [3,10]. Again, with the exception of insulin, the pancreatic endocrine polypeptides are also found in cells dispersed throughout the GI tract.

The gut in humans is the largest endocrine system, secreting more than 30 GI peptide hormones, including gastrin, ghrelin, cholecystokinin, serotonin, glucose-dependent insulinotropic peptide, glucagon-like peptides, and peptide YY, that play key roles in regulating glucose metabolism and energy homeostasis [11,12]. There are at least 15 types of enteroendocrine cells, scattered as single cells throughout the intestinal tract. They are mostly located within the intestinal crypts and villi where they comprise ~1% of the epithelial cell population. They act as sensors of changes to the contents of the gut lumen, function and food intake, and respond by releasing peptide hormones [12]. Despite a common endodermal origin, gut endocrine cells express numerous peptide hormones not expressed by pancreatic cells, and have very different developmental fates. Unexpectedly, fetal and adult ablation of the transcription factor Foxo1 in enteroendocrine progenitors gives rise to cells with lineage and functional features of insulin-producing, glucose-responsive cells, suggesting that

Abbreviations: GI, gastrointestinal; PP, pancreatic polypeptide.

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Table 1
Oligonucleotide primers.

Gene	GenBank Accession No.	Forward	Reverse
Oligonucleotide primers used for standard and multiplex PCR amplification			
Amylase α2B	NM_020978.3	AATAAAGGGTTGGAGCCTCTGTG	CATCCACATAAATACGAACCCCAAC
Amylin	NM_000415	AGCAATGGGCATCCTGAAGCTG	TCGTTCTTAAACCTGTGCCACTGA
Glucagon	NM_002054	AGCACACTACCAGAAGACAGC	TGGCATGCAAAGCAATGTGG
Insulin	NM_000207	GCCATCAAGCAGATCACTGTCC	GCTGCGTCTAGTTGCAGTAGTTC
Neurogenin-3	NM_020999.2	TTGCGCCGGTAGAAAGGATG	CTCAAGCAGGCGGAAAAGGT
Pancreatic PP	NM_002722.3	TCTGGACTCCGGATGGCTG	CGCTGCTCATGGAGTCGTAG
Somatostatin	NM_001048.3 NM_002046	CACTCTCCAGCTCGGCTTTC	CTTGGCCAGTTCCTGCTTCC GTA

Gene	Forward	Reverse
Oligonucleotide primers used for nested PCR amplification		
Amylase α2B	AATAAAGGGTTGGAGCCTCTGTG	CATTTGGTGGAGAGACCTGAACC
Amylin	CACCCATTGAAAGTCATCAGGTGG	TCGTTCTTAAACCTGTGCCACTGA
Insulin	AGCCTTTGTGAACCAACACC	AGCTGGTAGAGGGAGCAGATG

PP, polypeptide.

pancreatic and gut endocrine cells may be more closely related than previously appreciated [13].

Here we sought evidence for the existence of insulin mRNA-producing cells in tissues outside the pancreas as a reflection of our evolutionary history. We provide evidence that mRNA transcripts encoding insulin are expressed outside the pancreas within the intestinal tract, particularly within the stomach, stomach pylorus, duodenum, small intestine, and cecum, but not in other body tissues including the brain and colon.

2. Materials and methods

2.1. Tissue cDNAs

Panels of cDNAs from 48 different types of human tissues (TissueScan Human Normal, Cat# HMRT102), and from 24 regions of the human brain (TissueScan Human Brain, Cat# HBRT101) were obtained from OriGene (Rockville, MD, USA). Integrity of the panels was confirmed by PCR of GAPDH transcripts using a GAPDH Real-time Primer Mix from OriGene. The cDNAs were used unamplified, or were amplified using the Illustra GenomiPhi V2 DNA Amplification Kit (GE Healthcare), and then aliquoted into MicroAmp Optical 96-Well Reaction Plates (Cat#: 4316813, Life Technologies), and stored at −20 °C. Panels of cDNAs prepared from adult human duodenum, jejunum, ileum, cecum, and placenta (Cat# C8234517), and from adult human pyloric stomach (Cat# C1234253-10) were obtained from BioChain (Newark, CA, USA) and used unamplified.

2.2. Polymerase chain reaction (PCR)

Standard PCR with Taq polymerase and multiplex PCR amplifications with AmpliTaq Gold were performed for 35 cycles in a CG1-96 Palm-Cycler (Corbett Research) DNA thermal cycler. Nested PCR was performed for 30 cycles, using a 1 in 20 dilution of the PCR products as the template DNAs. All primers, as listed in Table 1, were synthesized by Invitrogen or Integrated DNA Technologies. PCR products were electrophoresed on 2% agarose gels, stained with SYBR Safe DNA gel stain (Invitrogen), and visualized using a LAS-3000 scanner (FujiFilm Corporation).

3. Results

3.1. Insulin mRNA transcripts are expressed within the adult human GI tract

Complementary DNAs in two panels (Origene), prepared from the total RNA of 72 different human tissues (including 24 regions of the brain) and amplified using the Illustra GenomiPhi V2 DNA Amplification kit, were screened by PCR for the presence of cDNA encoding insulin. As shown in Fig. 1A and Supplementary Fig. 1, the appropriately sized amplicon of 369 bp, indicative of insulin mRNA transcripts, was obtained only from the pancreas. We anticipated that extrapancreatic cells expressing insulin would be rare, and hence only detectable using the more sensitive technique of nested PCR. Thus, products of the first round of PCR were subjected to a subsequent round of amplification using a nested set of insulin-specific primers (Table 1). Nested PCR produced the expected 246 bp amplicon, indicative of insulin mRNA expression, from the pancreas, duodenum and stomach, but not from the other tissues (Fig. 1B, and data not shown). Insulin transcripts were notably absent from the colon.

To corroborate the above findings, cDNAs prepared from adult human duodenum, jejunum, ileum, cecum, placenta, and pylorus of the stomach were obtained from a different donor and supplier (BioChain), and screened by standard PCR for the presence of insulin cDNA. Insulin mRNA transcripts were detected in the duodenum, with very weak expression in the pylorus region of the stomach, whereas there was no detectable expression in the cecum, ileum, jejunum and placenta (Fig. 2A). However, nested PCR of this same set of cDNAs revealed weak expression in the cecum, ileum, and jejunum (Fig. 2B), suggesting that insulin mRNA transcripts are expressed at a low level throughout the adult human small intestine and as distal as the cecum.

3.2. Expression of mRNAs transcripts for other pancreatic markers in GI tract tissues

Unamplified OriGene and BioChain tissue cDNAs were screened by multiplex PCR for the expression of insulin transcripts, and transcripts encoding other pancreatic markers, in order to determine whether insulin transcripts were potentially expressed by pancreatic-like rather than gut-like endocrine cells. The markers examined included amylase α2B (pancreatic exocrine marker), amylin (pancreatic β cell marker), glucagon (pancreatic α cell marker), somatostatin (pancreatic δ, gut, brain and stomach cell marker), and pancreatic polypeptide (pancreatic PP cell marker). Multiplex PCR revealed that amylase α2B mRNA transcripts were expressed in the cecum, ileum, placenta and stomach pylorus; glucagon mRNA transcripts were expressed in the cecum, duodenum, jejunum, small intestine (very weak), pancreas, and stomach; somatostatin mRNA transcripts were expressed by all tissues except peripheral blood lymphocytes (PBL); and pancreatic polypeptide mRNA transcripts were expressed by the duodenum, pancreas,

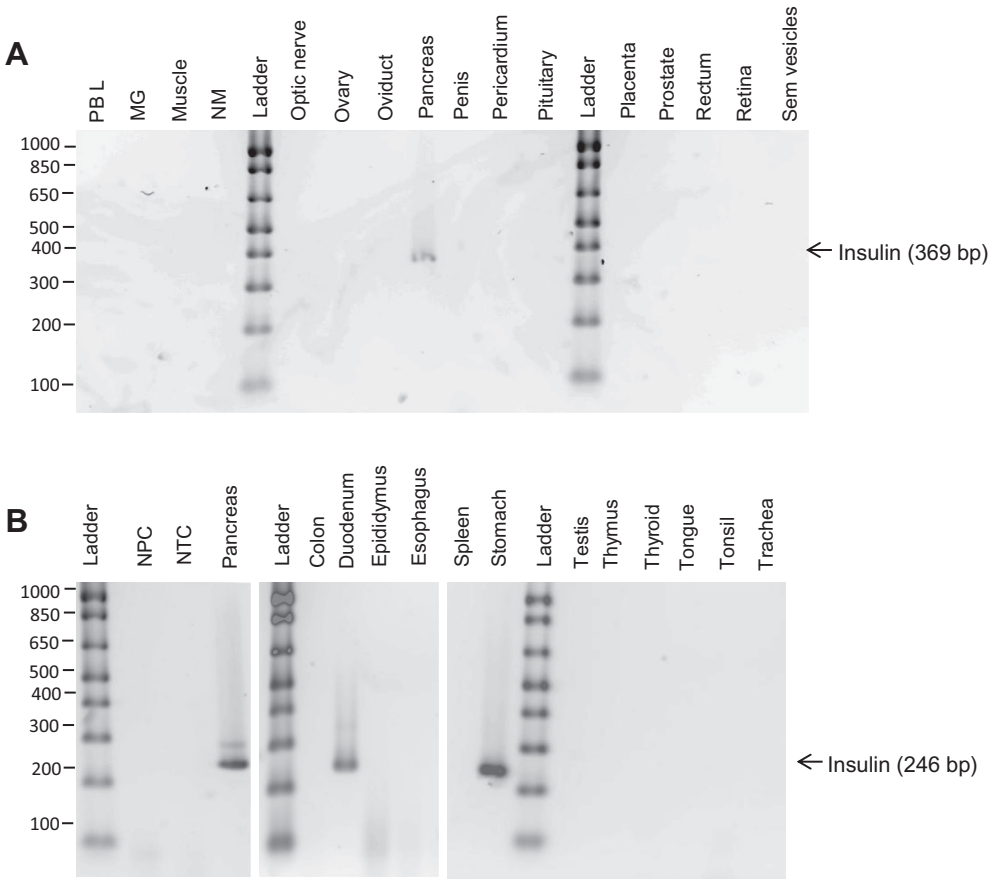

Fig. 1. Insulin mRNA is expressed in the human pancreas, duodenum and stomach. (A) Complementary DNAs prepared from 72 different human tissues (OriGene) were subjected to standard PCR with insulin-specific primers, to produce an amplicon of 369 bp. The PCR results for a subselection of tissues are shown, including the pancreas, which was the only tissue that displayed expression of insulin. Refer to Supplementary Fig. 1 for the other tissues analyzed. (B) The PCR products in (A) were subsequently subjected to nested PCR, with an internal set of insulin-specific primers, to produce an amplicon of 246 bp. Only those tissue cDNAs which generated the amplicon are shown, together with a small selection of tissues that did not produce the amplicon. The positions of the insulin amplicons are marked with arrows in the right-hand margin. The ladder size markers are in bp. MG, mammary gland; NM, nasal mucosa; NPC, no primer control; NTC, no template control; PBL, peripheral blood lymphocytes; Sem vesicles, seminal vesicles.

stomach (very weak) and stomach pylorus (Fig. 3A). An amplicon representing amylin was not detected for any of the tissues.

Subsequent nested PCR of selected multiplex PCR products revealed that amylase α2B mRNA transcripts were expressed in all tissues examined, as well as placenta and PBL (Fig. 3B), and amylin mRNA transcripts were expressed in the duodenum, pancreas, stomach and pyloric stomach (Fig. 3C). The expression of amylase α2B transcripts by PBL and placenta appears unusual, however, Seyama et al. reported that amylase α2B transcripts are widely expressed by many different cell types and tissues, including T and B cell lymphomas, and normal leukocytes [14]. A summary of the results from both the multiplex and nested PCRs is shown in Table 2.

4. Discussion

Here we have provided evidence that insulin mRNA transcripts outside the pancreas are restricted to the human GI tract, particularly within the stomach, stomach pylorus, and duodenum, and in lesser amounts within the small intestine and cecum. The coexpression of other pancreatic hormones at these sites suggests that insulin is expressed by pancreatic-like rather than strictly gut-like enteroendocrine cells, albeit this notion has yet to be formally demonstrated. There was a notable absence of insulin transcripts within the colon and brain. Extrapancreatic islets of Langerhans expressing all four classic pancreatic hormones were reported to be commonly present in the duodenal mucosa of the adult rat, and lost after streptozotocin-induced development of diabetes, leaving just glucagon-expressing cells [15,16]. Kendzierski et al. reported that insulin transcripts and protein were expressed by glandular cells of the rat stomach and colon, but were absent from the small intestine [17]. It was suggested that insulin expressed at these sites has an autocrine or paracrine role in controlling cell division, the secretion of other peptides, or motility and absorption.

Extrapancreatic islets of Langerhans have been reported to exist in humans as a hallmark of a condition termed heterotopic pancreas, affecting 0.55–13.7% of adult humans [18,19]. The most common sites of involvement are the upper portions of the GI tract, particularly the stomach, but also the duodenum, and proximal jejunum. In the present study, insulin mRNA transcripts were found to be present throughout the small intestine and cecum rather than being solely localized to the upper region of the GI tract indicating that they are unlikely to result from heterotopic pancreas. Nevertheless, insulin mRNA expression predominated in the upper GI tract, raising the possibility that heterotopic pancreas may arise from the outgrowth of insulin-expressing cells that are naturally dispersed within the stomach, duodenum, and less frequently, within more distal regions of the bowel. Single β-cell units are located in or along the pancreatic ductules from which they appear to bud. It is possible that such cells might be dispersed via the duct to the proximal duodenum and pyloric stomach [20].

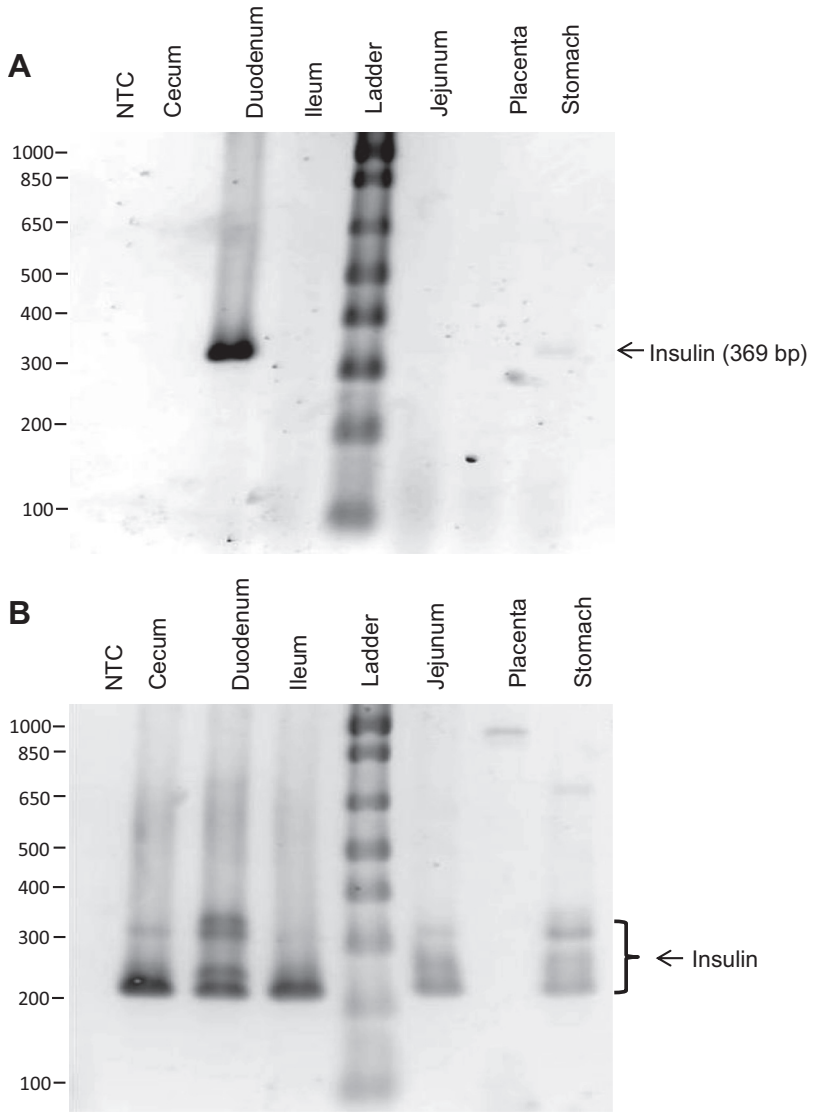

Fig. 2. Insulin mRNA is expressed throughout the stomach, small intestine and cecum of a different adult human donor. (A) Complementary DNAs prepared from different regions of the GI tract of a different donor, obtained from a different supplier (BioChain), were subjected to standard PCR with insulin-specific primers to produce the 369 bp amplicon. (B) The PCR products in (A) were subsequently subjected to nested PCR with an internal set of insulin-specific primers to produce the 246 bp amplicon. The higher molecular weight bands result from carryover of PCR primers from the first round of PCR amplification. The positions of the insulin amplicons are marked with arrows in the right-hand margin. The ladder size markers are in bp. NTC, no template control.

Enteroendocrine cells which are dispersed as single cells among mucosal cells of the GI tract constitute the largest amount of endocrine tissue in the human body [11,12]. They secrete several hormones including gastrin, ghrelin, somatostatin, cholecystokinin, serotonin, glucose-dependent insulinotropic peptide, glucagon-like peptides and peptide YY. They have been described as chemoreceptors which sense the luminal contents and adjust function and food intake accordingly [12]. Extrapancreatic cells expressing pancreatic hormones may serve a similar function. Enteroendocrine cells and pancreatic endocrine cells share a common endodermal origin [21], and they are both derived from Neurog3-expressing progenitors [22,23]. Whereas pancreatic endocrine progenitors are rare in the adult pancreas, enteroendocrine progenitors are constantly produced from gut stem cells in order to continually replenish the enteroendocrine cell population. Talchai et al. have shown in mice that continued expression of the transcription factor, Foxo1, is required to repress the expression and secretion of bioactive insulin by Neurog3+ enteroendocrine progenitors [13]. In accord, inhibition of Foxo1 generates functional insulin-producing cells in human gut organoid cultures [24]. Whether there is any natural interconversion of enteroendocrine cells into pancreatic-like endocrine cells remains to be examined.

Insulin mRNA and insulin-producing cells appear in multiple organs in diabetic mice and rats, which is not the case in non-diabetic animals [25]. Nevertheless, insulin-positive cells were generated in extrapancreatic tissues in nondiabetic mice rendered hyperglycemic by glucose injections [25]. Most of the extrapancreatic proinsulin-producing cells were shown to originate from the bone marrow. Thus, extrapancreatic cells expressing pancreatic hormones may form at sites in the body which are naturally prone to becoming hyperglycemic, such as sites within the GI tract where carbohydrates are broken down. After carbohydrates have passed through the stomach and into the small intestine, key digestive enzymes are secreted from the pancreas and the small intestine where most digestion and absorption occurs.

In summary, using sensitive PCR methods we have provided evidence that rare insulin mRNA-expressing cells exist within the stomach, small intestine and cecum of the adult human GI tract.

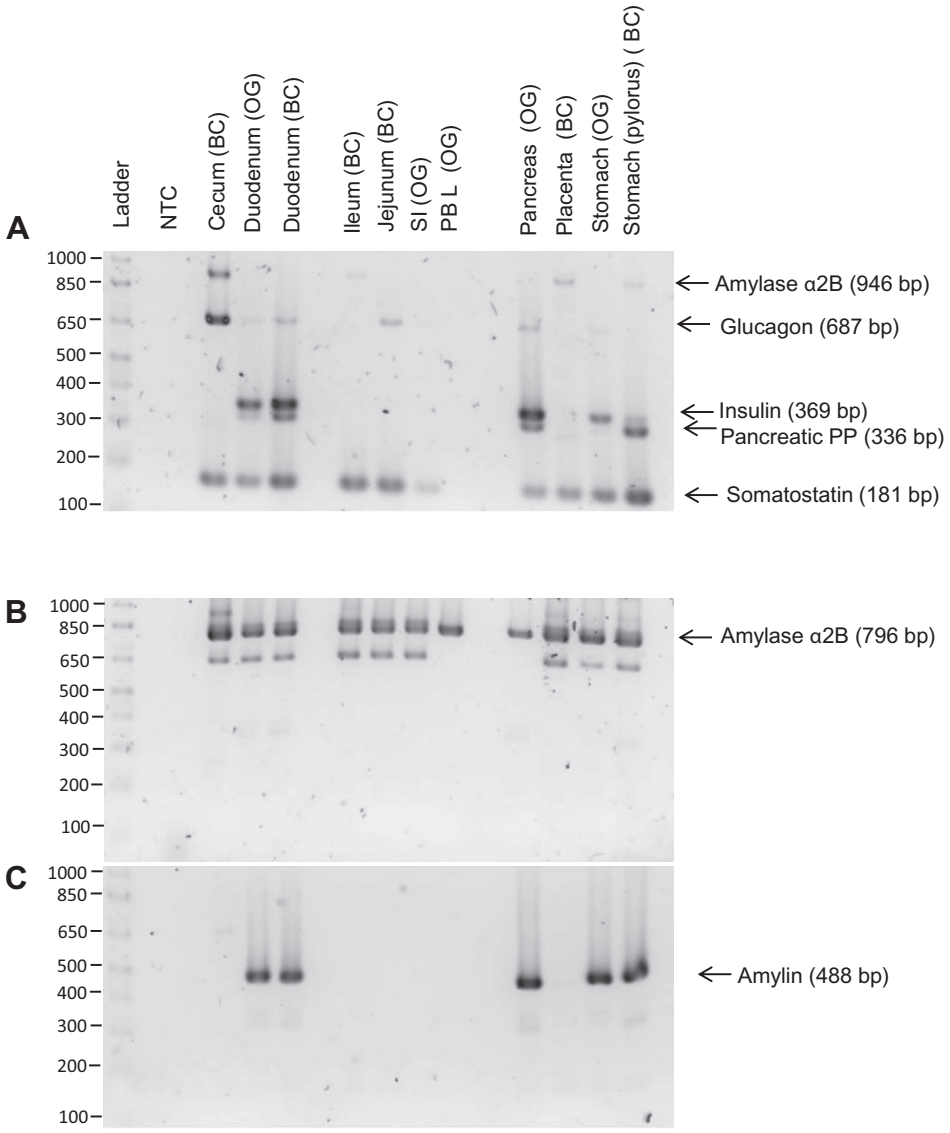

Fig. 3. GI expression of RNA transcripts encoding other pancreatic markers. (A) The cDNAs from GI tissues and placenta, and PBL (control), were subjected to multiplex PCR using primers specific to pancreatic markers, including amylase α2B, amylin, glucagon, insulin, pancreatic polypeptide, and the more widely spread marker somatostatin. The positions of the marker-specific amplicons are indicated. An amylin amplicon was not detected (not shown). (B, C) The PCR products from (A) were subjected to nested PCR using primers specific to amylase α2B (B), and amylin (C), respectively. BC, BioChain; NTC, no template control; OG, OriGene; PP, polypeptide; SI, small intestine.

Table 2
Summary of tissue expression of mRNA transcripts encoding pancreatic markers.

Tissue (Source)	Amylase (α2B)#	Amylin#	Glucagon	Insulin#	Pancreatic PP	SST
Pancreas (OG)	+∗	+∗	+	+	+	+
Stomach (OG)	+∗	+∗	+	+		+
Stomach pylorus (BC)	+	+∗		+	+	+
Duodenum (OG)	+∗	+∗	+	+	+	+
Duodenum (BC)	+∗	+∗	+	+	+	+
Small Intestine (OG)	+∗		+			+
Jejunum (BC)	+∗		+	+∗		+
Ileum (BC)	+			+∗		+
Cecum (BC)	+		+	+∗		+
PB lymphocytes (OG)	+∗					
Placenta (BC)	+					+

+, expression was detected; #, nested PCR was undertaken; ∗, expression was detected by nested PCR , but not by first round PCR.
BC, BioChain; OG, OriGene; PB, peripheral blood; PP, polypeptide; SST, somatostatin.

Whether such cells are derived from the dispersal of ductal β-cells, from the bone marrow in response to regional hyperglycemia, or from interconversion of classical enteroendocrine cells remains to be investigated. Insulin mRNA-producing cells may support the populations of enteroendocrine cells as sentinels to sense and monitor gastric contents passing into and through the bowel.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.07.140.

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