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Morphometric studies in human pancreatic cancer argues against the etiological role of type 2 diabetes in pancreatic cancer

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Summary. Background: To understand the role of islet amyloid polypeptide (IAPP) in type 2 diabetes and pancreatic cancer (PC), we investigated the patterns of its expression and its ratio to insulin, glucagon, somatostatin and pancreatic polypeptide cells by morphometry in tissues from these two diseases in comparison to the normal pancreas. Materials and Methods: Pancreatic tissues from 11 donors (five without pancreatic disease and six with type 2 diabetes) and 11 surgical specimens from PC patients obtained from the cancer area (zone A) and the adjacent tumor-free area (zone B) were examined immunohistochemically. The size of islets, the number on ß-, α-, δ-pp- and IAPP-expressing cells and their ratios in the islets of these tissues were determined. Results: In the normal pancreas, only 50% of the ß-cells while α- and δ-cells co-expressed IAPP only sporadically. In tissues from diabetics as well as in zone A, the number of the ß-cells and the IAPP-expressing cells was reduced significantly, while the number of α- and δ-cells was increased. In zone B, however, significantly more ß-cell and IAPP-expressing cells and a significantly lower number of α-cells were found compared to those in zone A. Significant differences were also found between the specimens from type 2 diabetics and pancreatic cancer relative to the ratios of IAPP/ß-cell, IAPP/α-cells and ß-cell/δ-cells. Conclusion: The morphometric data show a decrease rather than an increase in the number of IAPP-expressing cells in PC. Differences in abnormalities in type-2 diabetics and in zone B of PC tissue strongly argue against the role of type 2 diabetes in PC. Rather, the development of diabetes in subjects prone to pancreatic cancer could be a red flag for malignancy.

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Introduction

More than 80% of pancreatic cancer (PC) patients develop impaired glucose tolerance (IGT) or frank diabetes (Schwarts et al., 1978; Cersosimo et al., 1991; Permet et al., 1993; Basso et al., 1994). The mechanism of the abnormality has remained speculative. Diabetes as the forerunner for the disease, destruction of islets by cancer cells, the production of diabetogenic substances by cancer cells and the primary affect of carcinogens on islet cells, are among the invalidated assumptions (Koopmans et al., 1991; Furnsinn et al., 1994; Permert et al., 1994; Ding et al., 1998). In recent years attention has been focused on islet amyloid peptide (IAPP), which has been found in increased levels in the plasma of type 2 diabetics and PC patients (Permert et al., 1994; Chari et al., 2001; Makimattila et al., 2001). One of the many functions of IAPP or amylin, a 37 amino acid polypeptide which is co-localized in the ß-cells and co-secreted with insulin (Khan et al., 1999; Hoppener et al., 2000), is the inhibition of insulin secretion (Wang et al., 1990a,b, 1993; Wagoner et al., 1993; Furnsinn et al., 1994) with diabetogenic effects *in vitro* and i*n vivo* (Leighton and Cooper, 1988; Frontoni et al., 1991; Koopmans et al., 1991; Kreutter et al., 1993). Although the increased plasma level of IAPP has been reported to be associated with the decreased level of its expression in pancreatic cancer tissue (Permert et al., 1994), no comparative studies exist on the distribution of IAPP in the normal pancreas, in the pancreas of type 2 diabetes and PC. Therefore, we studied the patterns of IAPP and its ratio to insulin, glucagon, somatostatin and pancreatic

polypeptide (PP) cells in the tissues of diabetic and PC patients compared with those from the pancreases of donors without pancreatic diseases.

Materials and methods

Eleven pancreases from non-obese donors and 11 surgical specimens from pancreatic cancer patients with no history of obesity were examined (Table 1). Six of the donors had type 2 diabetes (DM), one of which required insulin (case 6). The remaining cases had no history of pancreatic disease. The age and sex of the donors and patients are listed in Table 1. Six of the pancreatic cancer specimens were obtained following distal pancreatectomy and, in five patients, after the classic Whipple procedure. Two males and five females with PC had diabetes mellitus and the remaining four patients had impaired glucose homeostasis, which was

Table 1. R elative Distribution of Pancreatic Endocrine Cells within the Islets of Normal and Diseased Pancreas (mean±SD%).

Case No.	age/sex	MA[1]	Loc [2]	No.of islet	size (range) [3]	ß cell (%)	αcell (%)	δ cell (%)	IAPP(%)	IAPP/ß cell (%)
NP										
1	18/F	-	NA	11	92.7 (50-140)	58.8±10.9	32.2±10.5	8.3± 2.6	40.2±10.7	68.4±11.1
2	39/M	-	NA	15	157.3 (80-250)	72.1± 7.0	22.6± 7.3	5.3± 1.9	31.4±11.7	44.1±18.9
3	52/F	-	NA	27	135.2 (80-220)	62.3± 6.8	25.7± 5.8	11.7± 4.2	32.7± 7.1	52.7±11.1
4	65/F	-	NA	10	102.0 (60-250)	68.3±14.4	22.0± 7.2	5.9± 2.5	36.1± 8.1	55.4±18.5
5	70/M	-	NA	10	127.0 (80-220)	60.7±4.5	31.2± 3.7	7.5± 2.5	33.7±10.3	56.3±16.5
average	48.8				126.4 (50-250)	64.4± 9.7	26.3± 7.8	8.5± 4.1	34.2± 9.5	54.2±16.2
DM										
1	27/F	DM	NA	28	118.2 (50-350)	44.4±10.7	39.8±12.6	15.5± 9.6	11.4± 5.7	24.8±12.1
2	46/M	DM	NA	17	143.5 (100-200)	57.1±11.6	29.8± 9.7	11.6± 7.6	12.7± 5.3	22.4± 8.6
3	55/M	DM	NA	16	74.7 (50-160)	41.3± 9.5	48.6±10.7	9.3± 4.9	21.2± 9.3	55.9±20.8
4	57/M	DM	NA	11	142.7 (80-230)	51.4±10.7	36.1± 4.1	11.9± 7.9	17.9± 6.9	31.6±15.4
5	78/F	DM	NA	10	61.0 (50-110)	52.1± 6.7	33.0± 6.5	14.5± 3.8	13.9± 7.0	35.6± 8.2
6	84/M	DM	NA	15	67.3 (50-100)	52.7±10.2	31.1± 6.8	16.3± 5.9	14.6± 5.9	28.6±12.8
average	57.8				106.1 (50-350)[b]	49.0±11.5[a]	37.0±11.5[a]	13.4± 7.7[a]	14.7± 7.4[a]	32.0±17.5[a]
zone A										
1	55/F	IGH	pb	10	151.0 (100-300)	49.4± 7.9	39.8± 7.6	10.2± 4.4	10.5± 6.3	21.3±21.2
2	70/M	IGH	pb	11	120.9 (70-250)	51.3±12.0	38.8±10.6	9.6± 3.7	7.9± 5.6	14.6± 9.4
3	73/F	IGH	pb	10	59.5 (50-100)	42.3±10.7	34.5±13.6	16.9± 5.4	2.2± 2.0	4.7± 4.0
4	77/M	IGH	pb	10	127.0 (50-240)	42.3±12.3	40.8±11.3	12.0± 7.2	9.1± 7.0	23.9±19.6
5	50/F	DM	pb	11	130.9 (60-200)	44.4±18.4	48.2± 8.4	13.2± 4.9	7.2± 2.9	18.5±12.2
6	60/F	DM	pb	16	113.4 (60-240)	55.2±17.0	35.2±14.6	9.3± 5.3	3.2± 2.7	5.7± 4.8
7	60/F	DM	ph	12	82.9 (60-150)	48.1± 9.6	39.9± 9.5	12.8± 4.9	18.2± 6.5	37.8±10.4
8	64/M	DM	ph	16	101.6 (60-230)	52.5±15.1	37.8±15.6	9.2± 3.5	15.5± 7.4	30.0±15.2
9	67/F	DM	ph	10	111.5 (50-250)	44.9±13.9	40.4±11.1	10.6± 4.5	17.5± 8.1	37.1±18.0
10	70/M	DM	ph	13	97.3 (50-140)	53.4± 9.9	32.0±12.9	11.3± 3.4	9.3± 5.4	17.9±10.4
11	75/F	DM	ph	10	140.0 (50-250)	53.0±12.4	36.9±11.4	7.7± 4.4	22.0± 9.8	42.0±16.5
average	65.5				111.3 (50-300)[c]	49.4±13.5[a]	38.3±12.3[a]	11.0± 5.1[a,f]	11.0± 8.4[a,e]	22.6±17.1[a,d]
zone B										
1	55/F	IGH	pb	14	157.9 (50-380)	52.6±11.5	36.4±10.2	11.0± 3.6	38.8± 9.6	76.0±20.2
2	70/M	IGH	pb	14	77.9 (50-100)	58.1±12.8	29.0±13.0	9.3± 4.1	31.9±10.1	57.6±17.9
3	73/F	IGH	pb	19	98.7 (50-160)	58.5±11.7	29.5±11.4	12.2± 5.7	29.7± 9.5	52.5±21.5
4	77/M	IGH	pb	10	85.0 (50-150)	60.2±11.1	24.6± 6.2	16.1±11.3	19.0± 9.3	33.5±21.1
5	50/F	DM	pb	13	93.8 (50-180)	50.6±13.9	34.5±10.6	14.2± 7.7	27.0±10.1	53.9±17.6
6	60/F	DM	pb	26	118.8 (60-230)	52.5±13.0	31.3± 9.7	15.7± 6.5	34.1±10.3	65.8±15.4
7	60/F	DM	ph	10	80.0 (70-90)	53.5±10.5	37.9±10.1	9.5± 4.5	23.5± 7.9	50.6±23.3
8	64/M	DM	ph	14	132.5 (50-380)	46.8± 6.8	39.1± 8.8	11.5± 3.7	32.1± 6.7	69.6±15.1
9	67/F	DM	ph	10	143.0 (60-250)	44.2±10.6	44.8± 8.0	11.1± 4.1	25.1± 5.2	59.7±16.9
10	70/M	DM	ph	10	71.0 (50-120)	54.0± 5.9	36.5± 4.9	9.5± 5.4	41.0± 5.3	76.5± 10.4
11	75/F	DM	ph	10	99.0 (50-180)	49.1± 9.9	41.1± 8.6	9.9± 4.6	33.4±10.6	70.5±23.5
average	65.5				107.5 (50-380)[b]	52.9±11.8[a,f,i]	34.2±10.8[a,i]	12.2± 6.2[a]	31.1±10.4[c,d,g]	61.1±21.2[c,d,g]

1, metabolic abnormalities (DM, diabetes mellitus; IGH, impaired glucose homeostasis); 2, tumor location (pb, pancreatic body; ph, pancreatic head); 3, average diameter of each islet (*μ*m); zone A, tumor area frompancreatic cancer cases; zone B, tumor free area from pancreatic cancer cases; M, male; F, female; NA, not applicable; a, significant difference (p<0.001) compare to NP; b, significant difference (p<0.01) compare to NP; c, significant difference (p<0.05) compare to NP; d, significant difference (p<0.001) compare to DM e, significant difference (p<0.01) compare to DM; f, significant difference (p<0.05) compare to DM; g, significant difference (p<0.001) compare to NP; h, significant difference (p<0.01) compare to DM; i, significant difference (p<0.05) compare to zone A

determined according to WHO criteria. Six of the specimens were from the head and six were from the body of the pancreas. Morphologically, all of the tumors were moderately differentiated adenocarcinomas. All tissues were fixed in buffered formalin, processed for histology according to conventional methods and cut in 30 serial sections.

Tissues from at least five different areas were obtained from the donors and pancreatic cancer specimens. The sampling sites were matched across the groups and all were from the glucagons-rich area. From the pancreatic cancer cases, the area occupied by tumor (zone A) and the tumor-free area (zone B), at least 10 mm away from the tumor, were examined. One section of each sample was stained with hematoxylin and eosin and evaluated histopathologically for their suitability for the study by adhering to the following criteria: 1) well preserved tissue with no evidence for autolysis, 2) sample diameter of at least 1.0x1.0 cm, 3) adequate number of intact islets (at least five islets per section), and 4) a lack of cancer cells, inflammation and fibrosis in tissues from zone B.

Immunohistochemistry

Immunostaining was carried out using an avidin-biotin-peroxidase complex (ABC) method with mouse monoclonal anti-IAPP antibody, Clone R10/99 (LAB VISION, Fremont, CA), rabbit polyclonal IAPP antibody (Abcam Inc, Cambridge, MA), mouse monoclonal anti-insulin antibody and polyclonal antibodies against glucagon, somatostatin and pancreatic polypeptide (Zymed Laboratories, San Francisco, CA). For IAPP, immunohistochemical staining antigen retrieval was performed by incubating the sections with 0.1 N citrate buffer (pH 6.2) at 100° for 10 min. Several methods were used for the visualization of islet hormones. The consecutive serial sections were processed with individual antibodies and microphotographs were taken from each section. Photo micrographs of the sections stained with IAPP and another antibody (insulin, glucagon, somatostatin or PP) were superimposed in Adobe Photoshop layers using different opacity for photos taken from the serial sections of the same specimen stained with different antibody. The stained cells with each antibody were counted. This technique allowed us to identify cells that co-expressed both antibodies (Fig. 1).

In addition, slides were also processed by a multi-labeling method (Pour et al., 1994) to demonstrate the ratio of immunoreactive cells in each islet and for the detection of cells co-stained with IAPP and other antibodies. One of the antibodies was processed either with Histomark red® (Kirkegaard & Perry Laboratories, Gaithersburg, MD) to obtain a red color, with Histomark blue®, giving a blue color, or with DAB, producing a brown color (Kirkegaard & Perry Laboratories, Gaithersburg, MD). The second antibody was processed with a different color than the first antibody (Figure 1). The reverse color combination was used in a series of other serial sections. For example, in the first set, ß-cells were stained red and IAPP blue. In the second set, ß-cells were stained in blue and IAPP in red. Co-expression of the hormones was judged by the change of color hue (for example, brownish red (red and brown) or purple (red and blue)). Also, in the multi-labeling method, insulin was represented in red and IAPP in blue in one slide. In the subsequent slide, insulin was represented in blue and IAPP in red. This method allowed us to more accurately differentiate the cell type and determine their numbers and their ratio within each islet. For proper evaluation of the color intensity, no counterstaining was performed. Negative control slides were processed similarly except that either no primary antibody was used (for monoclonal antibody) or a non-immunized serum was used instead of the primary antibody (for polyclonal antibody). In tissues from the diabetics, only islets unaffected or slightly affected by amyloid deposits were evaluated. To examine whether fibrosis in pancreatic tissue determines the size of islets and the number of the endocrine cells, we compared the islets in the fibrotic areas and in uninvolved parenchyma.

Evaluation criteria

Depending on the number of islets in each of the 20 serially sectioned tissues a minimum of five intact islets was evaluated. Islets less than 50 μm in diameter, showing amyloid deposition, atrophy, fragmentation and invasion by cancer cells were excluded from the evaluation. Photomicrographs were taken from the islets of each section, the number of the cells immunoreactive with each antibody was counted, and the percentage of stained cells within each islet was registered. PP cells were very low in most specimens that did not contain the pp-rich area of the pancreas. Even in one case from the pp-rich area the typical diffuse patterns of the pp-cells did not allow for accurate counting.

Measurement of islet size:

Microscopically, the length and width of 5-50 randomly selected intact islets (islets with regular defined boundaries without fragmentation or destruction) were measured by a micro-scale using a Zeiss Axiomat microscope (Zeiss, Germany). The average size was considered to be the representative value for that pancreas (Area: $\mu m^2 = \pi$ x length a /2 x length b /2).

Statistical evaluation

The results are presented as mean±SD. Significance in the differences between the groups of cases was tested by analysis of variance (ANOVA) with a Bonferroni

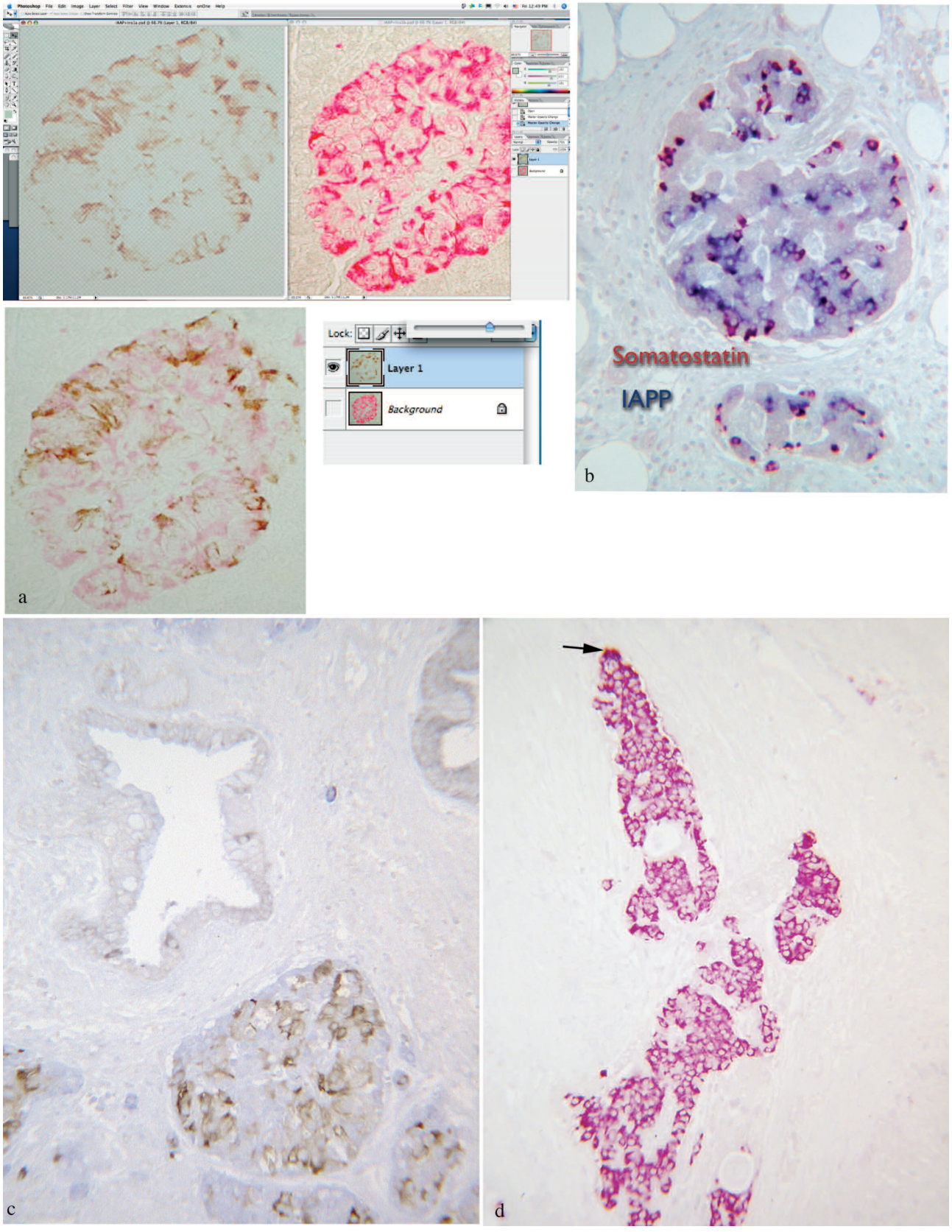

Fig. 1. Preparation of immunostained cells for comparative cell counting. **a.** Sections stained with IAPP and another antibody (insulin, glucagon, somatostatin or PP) were superimposed in Adobe Photoshop and the stained cells in each layer of Photoshop were counted. This technique allowed us to identify cells that co-expressed both antibodies. **b.** In parallel, sections were also processed simultaneously with two antibodies as described in the text. **c.** In pancreatic cancer, a great variation was seen in the expression of IAPP. While in some islets a large number could be identified in the same size of islets or even islets of larger size **(d)**, none or only a few IAPP cells were found (arrow). Note the presence of IAPP in a malignant gland (c, center field). ABC method, x 25.

correction for pair-wise.

Results

Normal pancreas

In each tissue sample between 10 and 27 intact islets could be examined. The size of islets varied considerably in each tissue and was on average about 126 μm (Table 1). Only about 50% of the ß–cells co-expressed IAPP with a strong staining intensity (Fig. 1a) and with an IAPP to insulin ratio of 1:2 (Table 1). IAPP immunoreactivity was also seen sporadically in α- and δ-cells. No differences were found between the reactivity of the monoclonal and polyclonal IAPP antibodies. The ratios of different types of islet cells are summarized in Table 2.

The normal pancreases served as controls.

Pancreas of diabetes cases

In all six cases, the size of the intact islets was significantly smaller than in the controls (Fig. 2). They were generally ill-defined and, especially in case #6, fragmented. In all of the cases, marked proliferation and hypertrophy of centroacinar cells occasionally intermingled with a single or a small group of cells immunoreactive with insulin, glucagon and somatostatin, were present in many areas of the exocrine pancreas. In these areas, only some of the ß-cells co-expressed IAPP. All of the islets in case #6 presented amyloid deposition reactive with Congo red but only marginally with anti-IAPP. No differences were found between the reactivity of the monoclonal and polyclonal IAPP antibodies.

The number of the ß-cells per islet ranged between 41% and 57% and was significantly lower than in the normal pancreas (Table 1). On the contrary, the number of α- and δ-cells was significantly higher than in the controls. Compared with the normal pancreases, only about 15% of the ß-cells co-expressed IAPP (P<0.001) with the same staining intensity as the controls. The ß-cell to IAPP ratio (3.3:1.0) was also significantly different from the controls (1.9:1.0; Table 2). More δ-cells co-expressed IAPP than in the controls.

Pancreatic cancer

Between 10 and 16 islets per tissue could be evaluated. The average size of the islets in both zones was comparable to that of the diabetics and significantly smaller than in the controls (Fig. 2). In zone A, the number of the ß-cells per islet (in average 49%), α-cells (38%) and δ-cells (11%) did not differ from those in the

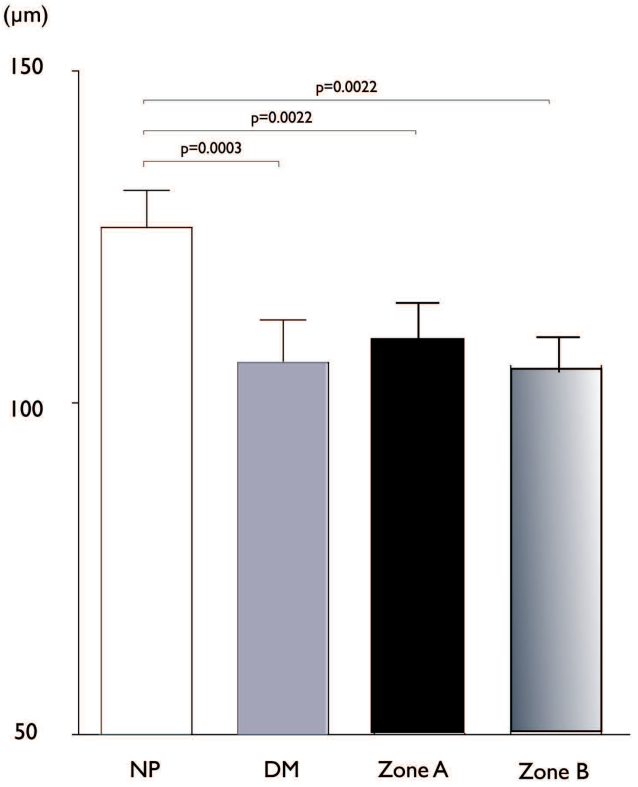

Fig. 2. The size of islet in the normal and diseased Pancreases. NP, normal pancreas; DM, pancreas from diabetics; Zone A, tumor area; Zone B, tumor free area.

Table 2. Ratios of pancreatic endocrine cells within the islets of normal and diseased pancreases.

	ß cell/α cell	ß cell/δ cell	IAPP/ß cell	α cell/δ cell	IAPP/α cell	IAPP/δ cell
NP	2.79	9.94	0.54	3.91	1.45	5.15
DM	1.56[a]	5.54[a]	0.32[a]	4.15	0.48[a]	1.71[a]
zone A	1.65[a]	5.88[a]	0.23[a,b]	4.33[c]	0.35[a,b]	1.30[a]
zone B	1.89[a,c,e]	6.02[a]	0.61[b,d]	3.78[e]	1.06[a,b,d]	3.38[a,b,d]

NP, normal pancreas; DM, pancreas from diabetes cases; zone A, tumor area from pancreatic cancer cases; zone B, tumor free area from pancreatic cancer cases; a, significant difference (p<0.001) compare to NP; b,significant difference (p<0.001) compare to DM; c, significant difference (p<0.05) compare to DM; d,significant difference (p<0.001) compare to zone A; e, significant difference (p<0.01) compare to zone A

diabetic cases but their average number was significantly lower (ß-cells) or higher (α-and δ-cells) than in the controls ($p<0.001$). In this zone the number of IAPP-expressing cells and the IAPP to insulin ratio was significantly lower than in diabetics as was the number of δ-cells (Tables 1 and 2). In zone B, however, the decrease in the ß-cells and the increase in the α-cells was significantly less than in zone A. Also, significantly more IAPP-expressing ß-cells were found in this zone than in zone A and and slightly but not significantly more than the controls.

In zone A the number of IAPP-expressing cells varied considerably between the islets. While in some areas about 70% of the ß-cells co-expressed IAPP, in another area the same or even larger islets did not show any immunoreactivity to anti-IAPP (Fig. 1c,d). In all cases, various numbers of ß-cells with or without IAPP could be found in the malignant glands (Fig. 1). No differences were found between the islets. In zone B, however, although the number of the ß-cells was also reduced, more of these cells co-expressed IAPP in a pattern comparable with the normal pancreas (Fig. 3). There were no differences in the size of islets and the number of ß-cells between islets in intact tissue and in areas of fibrosis (Fig. 4).

The ratios of the endocrine cells in all cases are listed in Table 2. The ratio of ß-cells to α-cells and of IAPP to ß-cells was similarly low in tissues from type-2 diabetics, zone A and zone B (Table 2). In zone B, however, these ratios were higher than in zone A, indicating a higher number of ß-cells and IAPP-expressing cells in zone B. Although the ratio of IAPP to α-cells was lower in type 2 diabetics and zone A, it was significantly higher in zone B compared to zone A, again pointing to a greater number of IAPP-expressing cells in zone B comparable with the control data.

Table 3. Relative distribution of pancreatic endocrine cells within the islets of Syrian golden hamster (mean±SD%).

	size (μm)	ß cell (%)	α cell (%)	δ cell (%)	IAPP(%)	IAPP:Insulin
NP	120.9±38.0	81.2±6.7	13.9±6.2	4.9±1.2	45.6±10.9	1.0:1.8
BOP	123.2±49.1	74.5±3.7[a]	20.7±2.5[a]	4.8±1.6	41.3±5.3	1.0:1.8
zone A	91.4±42.8[a,b]	70.4±8.1[a]	21.1±5.8[a]	8.1±3.8[a,b]	27.0±5.9[a,b]	1.0:2.6[a]
zone B	103.1±41.5	70.6±7.9[a]	21.4±6.3[a]	8.0±2.2[a,b]	31.6±2.7[a,b]	1.0:2.2[a,b]
zone C	120.5±39.6[c]	80.0±6.8[b,c,d]	14.5±6.0[b,c,d]	5.5±16.6	36.2±6.9[a,c]	1.0:2.2[a,b]

NP, normal hamster pancreas; BOP, BOP treated hamster pancreas; zone A, cancer area; zone B, around tumor area; zone C, tumor free area. a, significantly different from NP, ($p<0.05$); b, significantly different from BOP, ($p<0.05$); c, significantly different from zone A, ($p<0.05$); d, significantly different from zone B, ($p<0.05$).

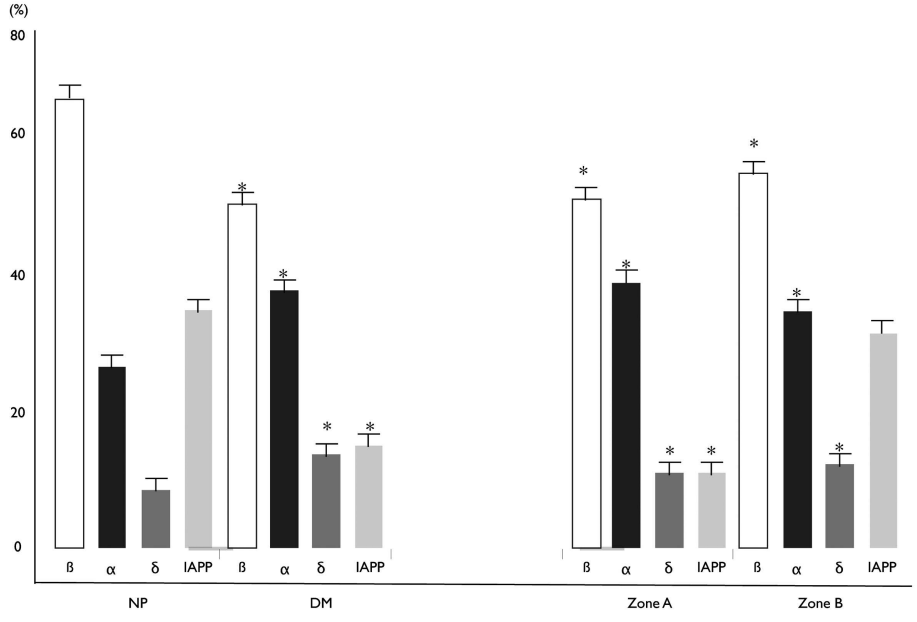

Fig. 3. Relative distribution of the endocrine cells within the islets of the normal and diseased Pancreases. NP, normal pancreas; DM, type 2 diabetics; Zone A, tumor area; Zone B, tumor free area; *,**, significant difference ($p<0.0001$, $P<0.005$) compared to NP.

No differences in the data were found between tissues from diabetic cases and those with IGT in zone A. In zone B, however, the number of the ß-cells was significantly higher and that of the α-cells was lower in IGT than those in diabetic cases (Figure 5); the number of IAPP cells was similar in both cases.

Discussion

Reasons for the development of IGT in most PC patients are not well understood. Based on recent studies, diabetes occurs shortly before the clinical manifestation of PC, therefore, the role of type 2 diabetes as a predisposing factor appears doubtful. The possibility that the destruction of pancreatic islets by the invasive cancer is the underlying cause has been ruled out (Fogar et al., 1993). The increased level of plasma IAPP in diabetic PC patients (Chari et al., 2001; Permert et al., 1994) has been suggested to be the ultimate cause of glucose metabolic abnormality, because IAPP has been implicated in the development of peripheral insulin resistance and type 2 diabetes (Leighton and Cooper, 1988; Frontoni et al., 1991; Koopmans et al., 1991; Kreutter et al., 1993; Wang et al., 1993, 1999a,b; Wagoner et al., 1993; Furnsins et al., 1994). The elevated plasma IAPP level in patients with neuroendocrine tumors associated with altered glucose homeostasis (Stridsberg et al., 1995) supports this notion. Since plasma IAPP concentrations decrease after surgery, as does the severity of diabetes (Permert et al., 1994), it was assumed that cancer cells somehow stimulate overt IAPP release (Ding et al., 1998; Wang et al., 1999; Permert et al., 2001). Experiments with human and experimental pancreatic cancer cells in vitro demonstrated a hyper secretion of IAPP relative to insulin on a molar basis (Ding et al., 1998; Wang et al., 1999a,b; Permert et al., 2001).

To understand the role of IAPP in PC, we compared the patterns of pancreatic endocrine cells in the pancreases of fairly age-and gender-matched donors with type 2 diabetes and PC as well as donors without pancreatic diseases. We used non-obese donor pancreases to avoid bias by nutrition, hospitalization and treatment. Although the number of cases is small, the similarities in all aspects of the data, including gender and age in each group, justify the generalization of the findings. The study was aimed at evaluating the expression of IAPP in these tissues. On one hand, we examined whether the patterns differ between type 2 diabetics and PC. On the other hand, we examined the patterns between tissues from cancer and non-cancer areas and between PC with and without diabetes (IGT).

Confirming our previous human studies (Schmied et al., 2000; Pour et al., 2001), a significant reduction of ß-cells and an increase in α-cells and δ-cells were found in the islets of PC cases and were comparable with type 2 diabetic cases and another similar study in type 2 diabetics (Saito et al., 1979).

In the normal specimens, only about 50% of the ß-cells co-expressed IAPP and there were remarkable variations in the number of IAPP-expressing cells between the islets of the same size and within the same specimen. This was especially true in the samples from zone A, where some islets were free of IAPP and others presented numbers that exceeded the normal range. This

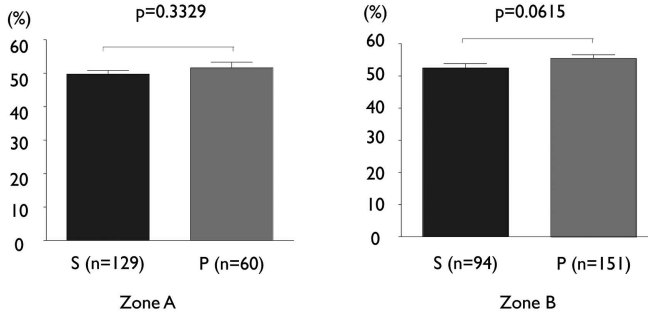

Fig.4. Comparison of the ß-cell number (in%) between sclerotic region (S) and within the parenchyma (P).

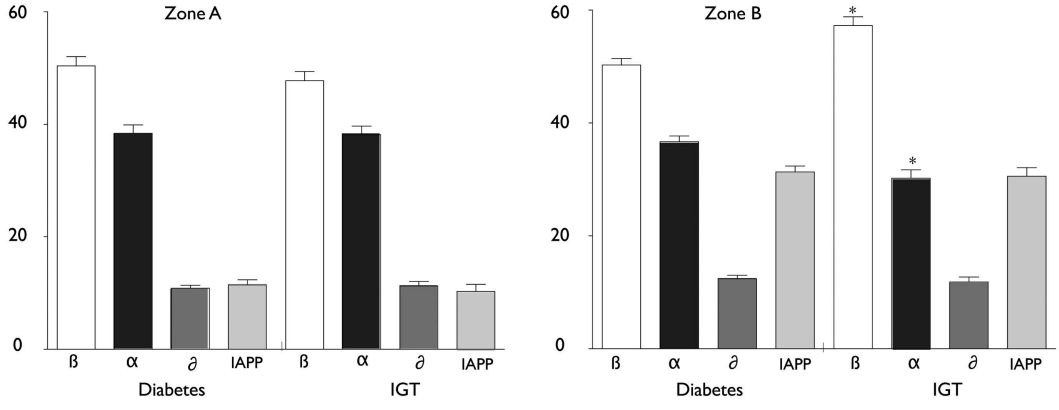

Fig. 5. Relative distribution of islet cells in pancreatic cancer patients with type 2 diabetes and those with impaired glucose tolerance (IGT). Zone A, cancer area; Zone B, tumor free area; IGT, patients with impaired glucose tolerance; *, significant difference ($p<0.001$) compared to diabetes cases.

pattern, which was also found in the extract from human pancreatic tumors (Permert et al., 1994), was unrelated to the size of islets and the degree of fibrosis and inflammation surrounding the islets. Hence, it appears that there are subsets of ß-cells, those containing IAPP and those lacking it. Based on the study in the human fetal pancreas, ß-cell maturation seems to be associated with islet IAPP expression (In 't Veld et al., 1992), therefore, IAPP-expressing cells are either immature or mature ß-cells. Also, IAPP may be involved, among its other functions, in the ß-cell maturation process.

If IAPP was responsible for type 2 diabetes, diabetes in PC patients, then an increase in the number of IAPP-expressing ß-cells was expected. On the contrary, significantly less ß-cells co-expressing IAPP were found in these tissues, indicating that the reduction in the number of the ß-cells is due to the loss of IAPP-expressing ß-cells. This loss was significantly greater in zone A than in the type 2 diabetic cases. These findings, which are consistent with a previous study on PC tissue extracts (Permert et al., 1994), could imply that the loss of IAPP in zone A is compensated by its increase in zone B. This loss may also be responsible for the increased level of IAPP in PC patients. The data also suggest that changes in the number of α-, δ, ß-cells are controlled independently from that of IAPP cells (Madsen et al., 1991; Mulder et al., 1995, 1999a,b, 2000; Ahren and Gutniak, 1997; Ahren et al., 1997) in humans. The possibility that IAPP is discharged independently from the ß-cells in the disease state via a constitutive secretory pathway, a non-storage, bulk-flow pathway, as was shown in the neonatal rat ß-cell (Verchere et al., 2000), thus explaining the apparent loss of IAPP, needs investigation. Regrettably, we did not have data relative to the plasma level of IAPP for a better understanding of the issue. Nevertheless, the differing data between diabetic cases and zone B of PC, including the islet amyloidosis and IAPP expression independent of the age of the subjects, implies that diabetes in PC is not a longstanding abnormality. Also, if diabetes in cancer patients were a preceding disease, alteration in zone B would have been comparable with that in the type 2 diabetic cases.

Noteworthy is the patterns on the endocrine cells between diabetic (DM) and IGT cases (Fig. 5). Although in zone A the data between these two subgroups were identical, in zone B the number of the ß-cells was significantly higher and that of the α-cells lower in IGT compared to DM cases. The data also implies that: 1) the alteration of the cells is associated with the opposite response of the α-cells and 2) changes in ß- and α-cells rather than IAPP dictate the development of diabetes. In fact, recent clinical studies have shown that elevated levels of plasma IAPP is not specific for pancreatic cancer and occurs in several disorders, including chronic pancreatitis, other GI malignancies and biliary obstruction (Brand et al., 2002). Also, an increase in IAPP is not a consistent finding in PC patients (Chari et al., 2001).

Differences in the expression of IAPP between zone A and zone B strongly suggests the role of cancer cells in the expression of IAPP, possibly by substances released from cancer. These differences affect the islets by a paracrine pathway as shown in *in vitro* experiments (Koopmans et al., 1991; Furnsinn et al., 1994; Permert et al., 1994; Ding et al., 1998). The effect of cancer in altering islet cell composition of the islets has also been shown in another independent carcinogenesis experiment (Asano et al., 1991). Presently, there is data, which supports this possibility. Several peptides corresponding with the N-terminal of the S100 has recently been detected in human PC cells (Rosty et al., 2002; Iacobuzio-Donahue et al., 2003; Missiaglia et al., 2004; Shen et al., 2004; Basso et al., 2006; Zervos et al., 2006). Among these proteins the S100A8 appears to be more relevant to the metabolic alteration in PC as it occurs in tissues from diabetic but not in non-diabetic PC patients, whereas other members of the family seem to be engaged in poor differentiation (Rosty et al., 2002). Also the cytokine-like FAM3B gene, also known as pancreatic derived factor, has been found to be associated with diabetes in PC (Souza, 2006). Consequently, it appears that several factors are involved in the development of diabetes in PC. Although not to the same extent as in zone A, there were also changes of ß-and α-cells in zone B. This complicates the theory that the diabetogenic substances released by cancer cells (in zone A) affects the endocrine tissue in a paracrine mode, because in that case no alteration is expected in the B zone.

In conclusion, the significant differences found in the patterns of pancreatic islet cells, amyloid deposition, and IAPP expression clearly exclude the possibility of preexisting diabetes in PC patients and confirms the claim that diabetes in PC patients is a recent event and presents a cancer-associated abnormality (Permert et al., 1993; Basso et al., 2005). Experimental studies have shown that abnormality in glucose metabolism coincide with the first microscopic appearance of malignant pancreatic lesions (Ahren and Andren-Sandberg, 1993; Andren-Sandberg, 1996) and, in clinical investigation, the impairment of glucose metabolism has been recorded as the lone abnormality in small pancreatic cancer. Consequently, if IGT or diabetes were proven to be caused by cancer cell-related substances, then the development of IGT in subjects prone PC should be considered as a red flag for PC.

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