PANCREATIC-POLYPEPTIDE IN THE HUMAN PANCREAS: EXPRESSION AND QUANTITATIVE VARIATION DURING DEVELOPMENT AND IN DUCTAL ADENOCARCINOMA

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Summary: Aim: To determine the immunoreactivity of pancreatic-polypeptide (PP) during the development of the human fetal pancreas and ductal pancreatic adenocarcinoma, given that, PP positive cells were demonstrated either into its embryonic anlage or into pancreatic cancer. Methods: Tissue sections from 15 pancreatic fetal specimens, and equal number of ductal adenocarcinoma specimens, were assessed. Results: The density of positive cells in the primitive exocrine ductal epithelium and endocrine epithelium was significantly higher than the relevant density in the neoplastic pancreatic tissue of mixed (ductal – endocrine) and pure ductal type (p1=0.001, p2<0.0005, p3 =0.046 and p4<0.0005 respectively). The above values were estimated during the 10th to 12th week. There was no significant difference in the density of positive cells in the mantle zone of the islets from the 13th to the 24th week, and the neoplastic tissue of mixed (p5=0.11) and pure ductal type (p6=0.23). Conclusion: The immunostaining for PP identifies a subgroup of pancreatic ductal adenocarcinomas with a neuroendocrine component, initially considered as pure ductal tumors, and mixed ductal and neuroendocrine tumors. This pattern of expression in neoplasms recapitulates the normal pattern during the embryonal development of the organ, raising the question of therapeutic efficacy of PP and analogues as potential adjuvant treatment of pancreatic cancer.

Key words: Pancreatic-polypeptide; Fetal pancreatic tissue; Pancreatic carcinoma

Introduction

The development of the endocrine pancreas is complex and interrelated with development of the exocrine portion of the organ. It is now clear that both the exocrine and endocrine pancreas are of endodermal origin (15,16).

The evaginations of pancreatic endoderm (fifth week of gestation) into the investing mesenchyme become tubular structures which branch progressively. The primitive duct epithelium provides the stem cell population for all the secretory cells of the pancreas. It gives rise to α cells which produce glucagon, β cells which produce insulin, and δ cells which produce somatostatin during weeks 8–10. Cells (F-cells) containing pancreatic polypeptide (PP) appear somewhat later. All four different endocrine cell types can be distinguished by immunocytochemistry (8,17). Initially these endocrine cells are located in the duct walls or in buds developing from them.

Pancreatic carcinoma remains one of the most devastating neoplasms of the gastrointestinal tract. Pancreatic cancer is a malignancy that is unresponsive to conventional therapy. More than 85 % of patients have metastatic disease when they are first seen. The incidence of pancreatic cancer is 9 per 100.000 (4) and has remained steady since 1973 (20). Median survival on diagnosis is 11 months, whereas adjuvant treatment (5–fluorouracil and radiation treatment) with surgical resection (Whipple procedure) has extended life by approximately 9 months (11). A dismal prognosis is associated with pancreatic adenocarcinoma despite multimodality treatment protocols. Although total pancreatectomy in selected patients offers survival advantages in rare cases, the difference remains negligible (24). Earlier diagnosis and novel treatment modalities may help to improve survival in patients with pancreatic cancer.

The dismal prognosis of this disease may someday be improved by a better understanding of its pathogenesis. Neoplasms of the pancreas arise from ductal, acinar, stromal, or islet cells. The term carcinoma of the pancreas is customarily used only in reference to exocrine tumors and rare mixed endocrine – exocrine carcinomas. Neoplasms

including carcinomas composed primarily of endocrine cells, are collectively termed islet cell tumors. The precursors of these tumors are presumably developmentally multipotent in terms of their capacity to differentiate into various cell types producing various hormones and regulatory peptides. Whether these cells originate from the ductular epithelium or the islet cells is a matter of debate (13).

Pancreatic polypeptide (PP) was discovered serendipitously nearly three decades ago (12). However, very little is known about its physiologic function or the clinical implications of elevated circulating levels of PP. Human pancreatic polypeptide (hPP) is composed of 36 amino acids. It has been localized within distinct cells in the islet of Langerhans of the pancreas, the F cells that store and secrete PP into the bloodstream (6). Histologically, F cells are abundant in the head and uncinate process of the pancreas (6,22). The release of hPP from the normal pancreas is mediated by the cholinergic nerve fibers that innervate the pancreas. Plasma levels of this linear polypeptide have been shown to increase after a meal, with increasing age, in chronic renal failure, and in patients with islet cell tumors (1,2,14,16).

Previous works describe the growth-inhibiting properties of the peptide YY (PYY), member of the Pancreatic Polypeptide Family, and its synthetic analog PYY(22–36) on human pancreatic ductal adenocarcinomas in vitro (18) and in vivo (19).

PP and its analogues have been included in experimental administration for advanced pancreatic carcinoma patients, based on their antisecretory and antiproliferative properties. However, there has been reported a stimulation effect on neoplastic growth by Ramo et al. (23).

We investigated the immunohistochemical expression of PP in a series of embryonal and neoplastic human pancreatic tissues. We tried to trace the normal expression profile of PP in tissues with different proliferative and differentiating compartments and to investigate whether PP expression in pancreatic carcinoma recapitulates the normal pattern of expression, or may occur as a result of neoplastic deregulation. We conclude that the efficacy of PP administration in pancreatic cancer is yet to be determined.

Materials and methods

Tissue Sampling

The pancreatic tissues were obtained by pancreatoduodenectomy (The Whipple procedure) for carcinoma of the pancreas. Samples from the pancreas of 15 consecutive surgical patients (nine males and six females, aged from 46 to 72 years, average 57.8(11.2) were included in the study. Two tissue samples were taken from each patient: one from the tumor and one from the resection margin. All tumors were verified as pancreatic adenocarcinomas with various degrees of differentiation. The tissues from the resection margins likewise were examined histologically and were found to be free of tumor cells.

Human embryonic (fetal) pancreatic tissue from fifteen fetuses after spontaneous abortion (10 to 12 gestational weeks: 8 samples, 13 to 24 weeks: 7 samples), were investigated.

The local hospital ethics committee approved the use of human tissue, and written informed consent was obtained from all patients.

Immunohistochemical procedure

Pancreatic-Polypeptide immunoreactivity was evaluated using the Lyophilised Polyclonal (NCL-PPp) on formalin-fixed, paraffin-embedded samples. Continuous sections of the tissue were cut into 3–(m thick slices and immunohistochemistry was performed by the avidin-biotin complex (ABC) method, using NOVOCASTRA kits. Briefly, after the sections had been dewaxed and rehydrated, they were washed in phosphate-buffered saline (PBS) and incubated for 30 min in normal goat serum to inhibit nonspecific binding. The sections were then washed in PBS and incubated with antibody against PP (NCL-PPp) overnight at 4 °C. The primary antibody was used after dilution (1:150).

PP (NCL-PPp) immunoreactivity was cytoplasmic, with only occasional and faint nuclear immunostaining. For each sample positive cells in the ducts, islets of Langerhans, aggregates or isolated cells in the pancreatic parenchyme, ware assessed by enumeration of labeled cells in each tissue compartment for a minimum of five random fields per section viewed at 40-fold magnification through a grid. Cell number was calculated per 1 mm^2 of tissue section. The counted areas were selected from random fetal and neoplastic pancreatic tissue sections, taking into account that the ratio of the exocrine pancreatic area (acinoracemose), according to the endocrine pancreatic area (islets of Langerhans) was entirely representative. Statistical analysis was undertaken using the t-test.

Results

Embryonal pancreatic tissue (10 – to 12 – week – old human embryos). During this period of development, endocrine cells (F – cells) demonstrated a strong positive immunoreactivity for PP (NCL-PPp), initially in the primitive exocrine duct epithelium (density of PP positive cells = mean of cells/mm^2 of tissue ± SEM = 37.9 ± 1.6) (Fig. 1) or forming small aggregates (buds) in the surrounding the ductal structures, loose mesenchymal tissue (density of PP positive cells = mean of cells/mm^2 of tissue ± SEM = = 22.3 ± 1.1) (Fig. 2). From the thirteenth to the twenty – fourth week of gestation, period that coincides with the formation of the islets of Langerhans, a strong positive immunostaining for PP (NCL-PPp) was observed to the endocrine cells (F – cells) in the islet cortex epithelium (density of PP positive cells = mean of cells/mm^2 of tissue ± SEM = 27.4 ± 1.3) (Fig. 3).

Neoplastic pancreatic tissue. PP was demonstrated in ten out of fifteen pancreatic adenocarcinomas. The five PP
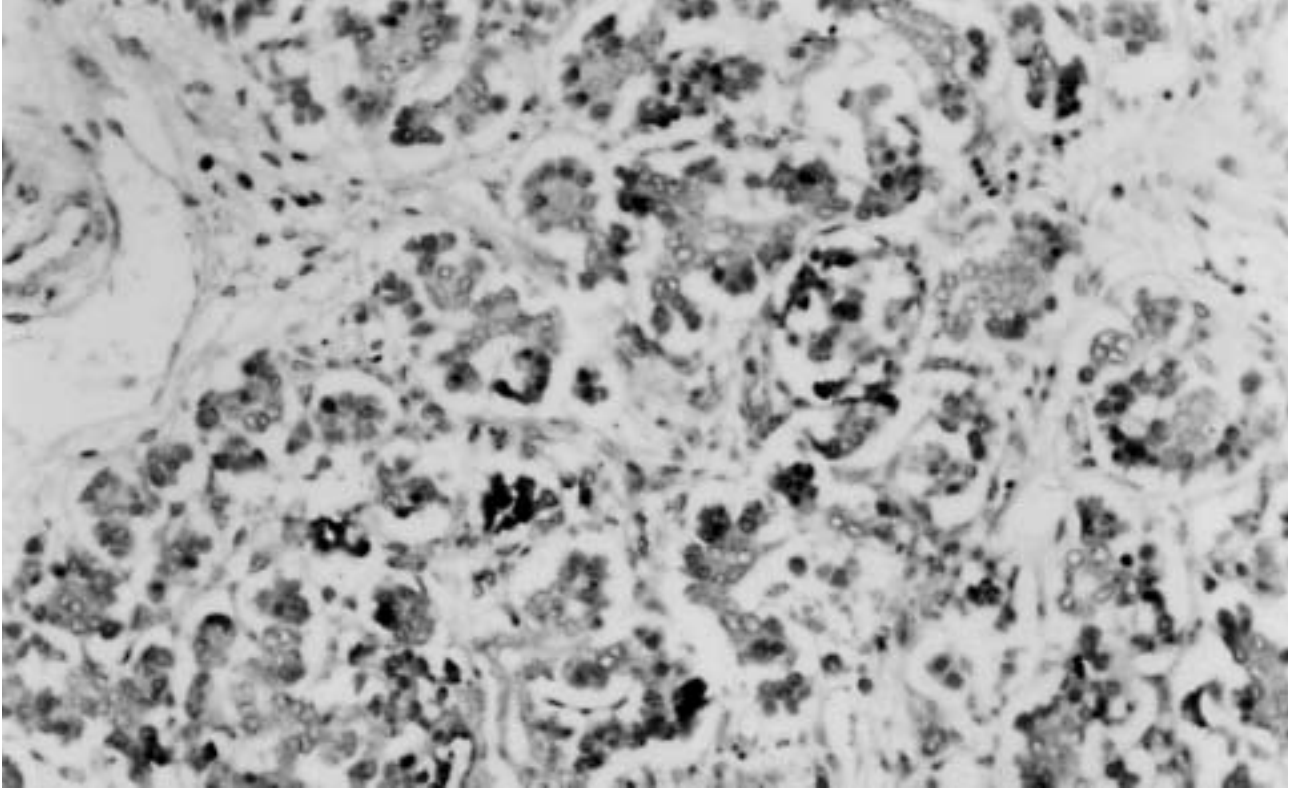
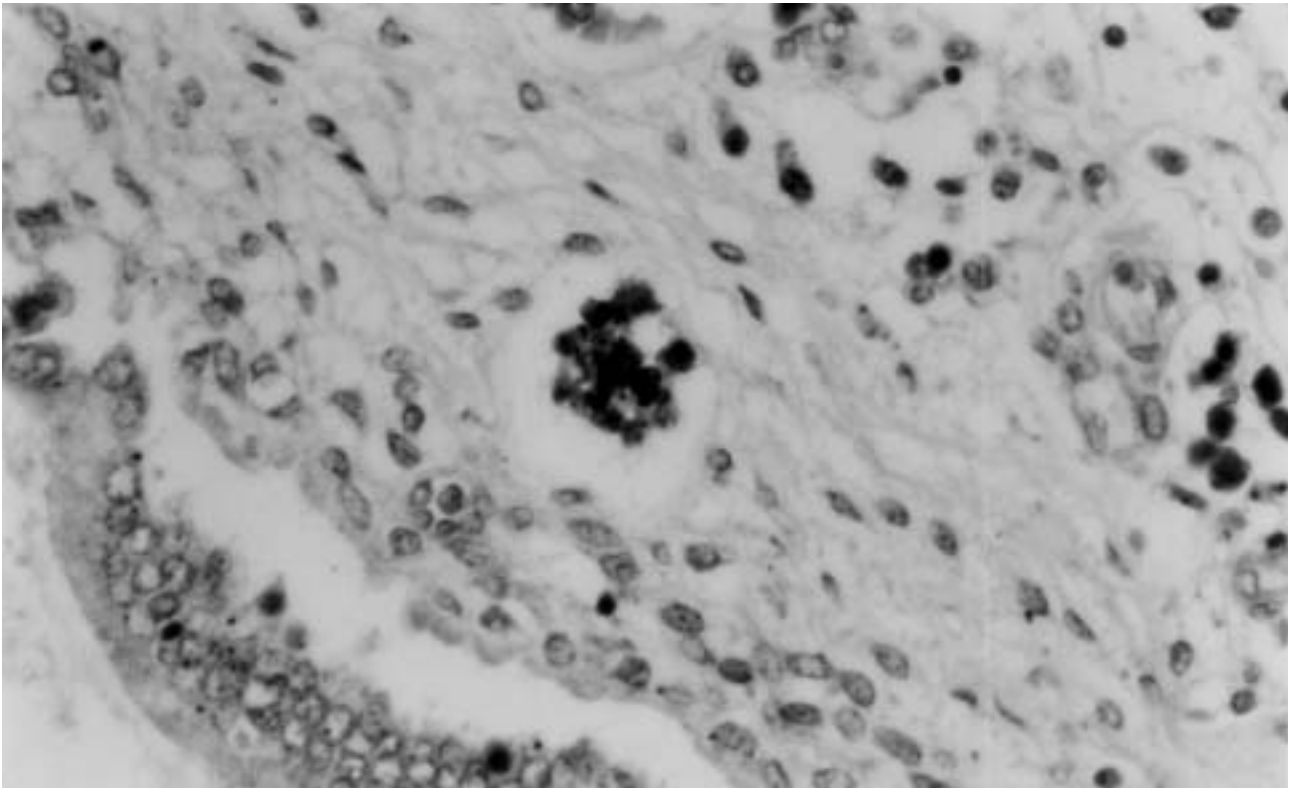**Fig. 1**: Pancreatic polypeptide expression in the primitive exocrine ductal epithelium. NCL-PPp X200.

Fig. 2: Pancreatic polypeptide expression in the primitive exocrine ductal buds. NCL-PPp X200.
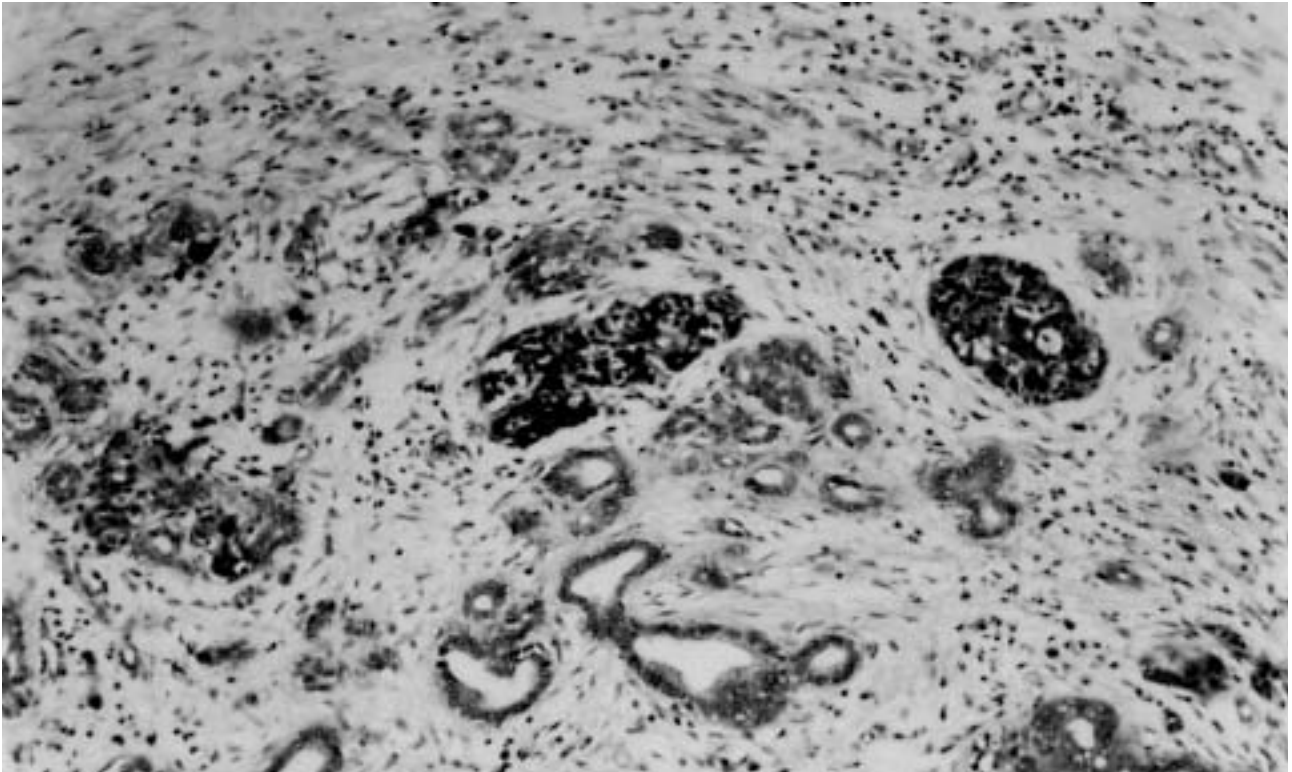
Fig. 3: Pancreatic polypeptide expression in neoplastic pancreatic tissue with recapitulation of the relevant expression of the antigen in the primitive embryonal pancreatic anlage. NCL-PPp X100.
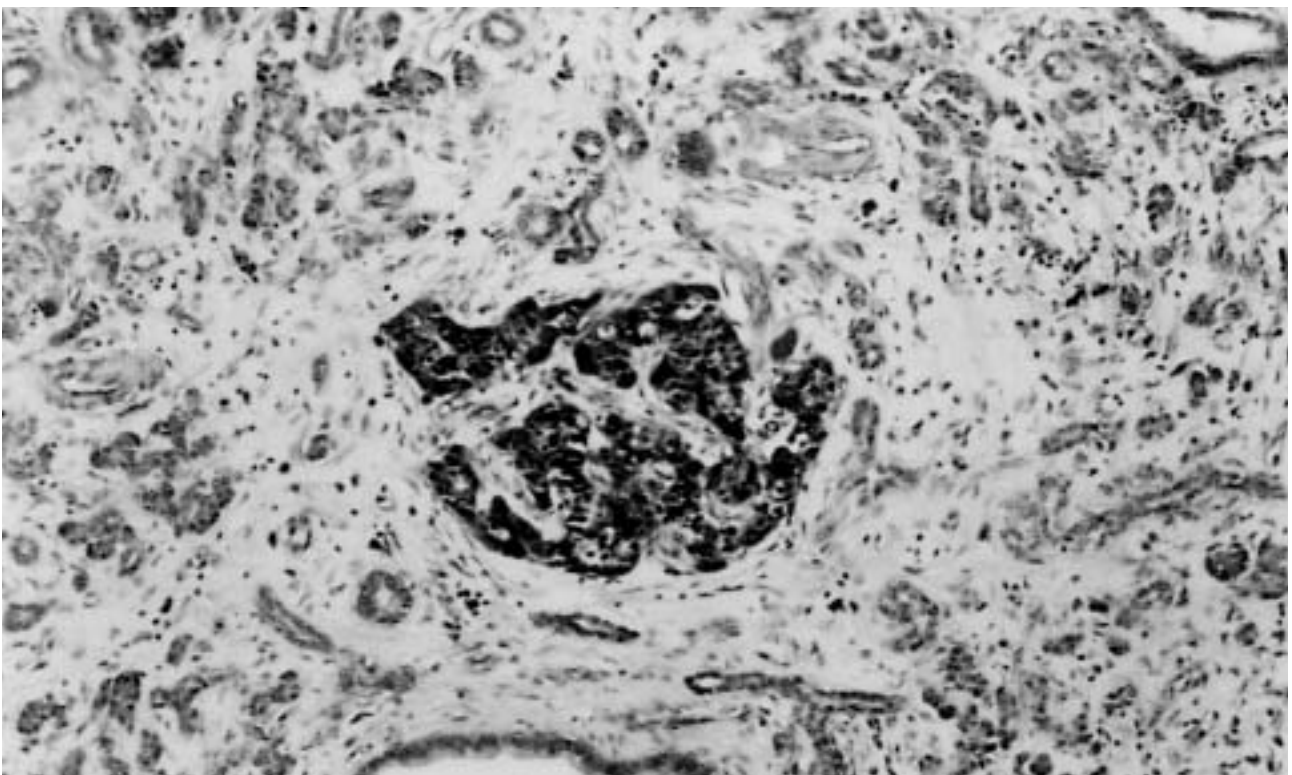

Fig. 4: Pancreatic polypeptide expression in pancreatic adenocarcinoma of pure ductal type NCL-PPp X100.

negative pancreatic adenocarcinomas were of mucinous type. PP positive cells constituted the majority of neoplastic cells in the ductlike structures or small cords of the tumor. Especially, in six cases diagnosed as mixed ductal – endocrine carcinoma, the density of PP positive cells was 29.8 ± 1.2 cells/mm^2 (Fig. 4); in the remaining four cases diagnosed as pure ductal adenocarcinoma the density of PP positive cells was 25.5 ± 1.4 cells/mm^2 (Fig. 5).

There was a statistically significant difference in the expression of PP in the ductlike structures between the primitive exocrine embryonal pancreatic tissue from the 10th to the 12th gestational week, and the neoplastic pancreatic tissue of mixed type (p1=0.001) and pure ductal type (p2<0.0005).

There was also a statistically significant difference in the expression of PP in the buds surrounding the ductal structures between the primitive exocrine embryonal pancreas from the 10th to 12th week, and the neoplastic pancreatic tissue of mixed type (p3=0.046) and pure ductal type (p4<0.0005).

No statistically significant difference was observed in the expression of PP in the islet cortex tissue from the 13th to the 24th week, in comparison with the neoplastic tissue of mixed type (p5=0.11) and pure ductal type (p6=0.23).

Tab. 1: Reactivity of Pancreatic polypeptide (NCL-PPp) in human embryonal and neoplastic pancreatic tissue.

Pancreatic tissue	Number of cases	Density of PP positive cells (average cells/mm^2 of tissue ± SEM)
Embryonal (10–12 weeks)	8	
Primitive exocrine duct walls		37.9 ± 1.6
Primitive exocrine ductal buds		22.3 ± 1.1
Embryonal (13–24 weeks)	7	
Islet cortex epithelium		27.4 ± 1.3
Neoplastic tissue	10	
Mixed ductal-endocrine carcinoma	6	29.8 ± 1.2
Pure ductal carcinoma	4	25.5 ± 1.4

Discussion

The prognosis of patients with exocrine pancreatic cancers remains very poor. Only 36,1 % of patients surgically treated, however, with a 5–year postoperative survival rate of less than 20 % (21). Therefore, new therapeutic approaches for the treatment of exocrine pancreatic cancers must be developed. In the past two decades, the employment of certain gastrointestinal hormones, growth factors, and steroids has been reported in new approaches to control exocrine pancreatic cancers (10).

Pancreatic polypeptide, first isolated by Kimmel and colleagues in 1968, is a 36–amino acid peptide secreted from the F-cells, which are most prominently found in the periphery of the islets in the head of the pancreas (9). Pancreatic polypeptide binds to specific receptors and inhibits exocrine pancreatic secretion of enzyme, bicarbonate, and water and decreases pancreatic blood flow (7). In 1980 Tatemoto and Mutt (25) isolated peptide YY (PYY), and neuropeptide Y (NPY), also 36–amino acid peptides sharing about 50 % homology with pancreatic polypeptide and having similar actions. PYY and its synthetic analog BIM-43004–1 have been shown to cause significant reduction in growth of the human ductal pancreatic cancer cell line MIA PaCa-2 in vitro (18). This analog of PYY was subsequently shown to bind to receptors on these pancreatic cancer cells, decrease intracellular cAMP levels, and suppress tumor growth in vivo (19). By contrast, another study reported increased incorporation of ^{3}H-thymidine in MIA PaCa-2 cells and two other cell lines, one human (Capan-2) and the other a hamster pancreatic adenocarcinoma (H2T), after exposure to NPY and PYY (23). Fisher et al (5) examined the effect of pancreatic polypeptide on the growth of the Capan-2 and H2T cell lines and examined the cells for pancreatic polypeptide receptors using competitive binding assays with ^{125}I-PP. Dose-dependent inhibition of tumor cell proliferation was observed when the H2T cells were cultured with increasing concentrations of pancreatic polypeptide from 10^{-10} to 10^{-7} M. However, no growth effect was detected with Capan-2. Neither cell line could be shown to have pancreatic polypeptide receptors by competitive binding studies. Pancreatic polypeptide, as well as other members of this family of related peptides, may exert an inhibitory effect on pancreatic ductal adenocarcinoma cells. Although Fisher et al were unable to demonstrate receptors in their preliminary unpublished work, others have shown that specific receptors appear to be involved in the mechanism of growth inhibition.

The purpose of our article pointed towards the PP expression in embryonic and neoplastic pancreata. In the fetus, PP was expressed in selected developmental phases suggesting a differentiation – related role. Our data reveal the dynamic behavior of the glandular epithelium in the neoplastic pancreas as well, thus indicating that the human epithelial cells in the branching ducts of the neoplastic pancreas may serve as stem cells, which if appropriately induced may differentiate into endocrine cells such as the F-cells expressing PP. Further studies are warranted to determine the usefulness of this peptide and its analogs, either alone or in combination with chemotherapy and radiation, in the adjuvant treatment of pancreatic cancer.

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