

DOES NEOPLASTIC CHOLECYSTOKININ EXPRESSION REFLECT THE EMBRYONAL PATTERN OF THE PROTEIN? A STUDY IN HUMAN PANCREAS

Demetrio Tamiolakis¹, Ioannis Venizelos², Constantine Simopoulos³, Maria Lambropoulou⁴, Athanasia Kotini⁵, Theodoros Jivannakis⁶, George Alexiadis⁴, Panagiotis Boglou⁴, and Nikolas Papadopoulos⁴

General Hospital of Chania, Greece: Department of Cytology¹; Ippokration Hospital of Salonica, Greece: Department of Pathology²; Democritus University of Thrace, Greece: Department of Experimental Surgery³, Department of Histology-Embryology⁴; Department of Medical Physics⁵; General Hospital of Drama, Greece: Department of Pathology⁶

Summary: Aim: To determine the immunoreactivity of cholecystokinin (CCK) during the development of the human fetal pancreas and pancreatic adenocarcinoma, given that, CCK positive cells were demonstrated either in its embryonic anlage or in pancreatic cancer. In order to obtain possible parallels in the expression pattern of neoplastic cells in adults (well – moderately – poorly differentiated), we investigated the pattern of CCK expression in the pancreatic tissue during the various stages of development and compared these with the proliferation of tissue assessed by proliferating cell nuclear antigen (PCNA) immunohistochemistry. Experimental design: Tissue sections from 15 pancreatic fetal specimens, and equal number of ductal adenocarcinoma specimens, were assessed using immunohistochemical methods for CCK. Results: The density of positive cells in the primitive exocrine ductal walls and outgrowing buds was significantly higher than the relevant density in the neoplastic pancreatic tissue of mixed (ductal – endocrine) and pure ductal type ($p_1=0.004$, $p_2<0.0005$, $p_3<0.0005$ and $p_4=0.023$ respectively). The above values were estimated from 20th to 22nd weeks of gestation. There was no significant difference in the density of positive cells in the islet cell epithelium from 25–30 weeks, and the neoplastic tissue of mixed ($p_5=0.10$) and pure ductal type ($p_6=0.15$). Conclusions: The immunostaining for CCK identifies a subgroup of pancreatic ductal adenocarcinomas with a neuroendocrine component (initially considered as pure ductal tumors), and mixed ductal-endocrine tumors. This pattern of expression in neoplasms recapitulates the normal pattern during the embryonal development of the organ, and may be important for the development of new therapeutic approaches with eventual clinical utility.

Key words: Cholecystokinin; Fetal pancreatic tissue; Pancreatic carcinoma

Introduction

The cholecystokinin (CCK) is a gastrointestinal polypeptide hormone, closely related chemically to gastrin. It was supposed primarily to be implicated in the control of pancreatic and biliary secretion, gall-bladder contraction, and gut motility (5,25,17,26), but recent studies have pointed out a key role of it in the central nervous system as well, perhaps as a neurotransmitter or neurohormone (3,4,5, 25,29). The peripheral actions of CCK are mediated by a receptor subtype termed as CCK-A, while the central actions are mediated by a receptor subtype termed as CCK-B, for which the minimum agonist ligand required is CCK-4, i.e., Try-Met-Asp-Rhe-NH₂, (33), which is identical to tetragastrin (G-4). A third receptor subtype, which appears to be closely related to CCK-B subtype, is the stomach gastrin receptor (1).

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 and has remained steady since 1973 (23). 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 (16). 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 (32). Earlier diagnosis and novel treatment modalities may help to improve survival in patients with pancreatic cancer.

One day the dismal prognosis of this disease may 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 (18).

Previous reports examining the type and distribution of CCK receptors in animal pancreas have yielded results which may not be directly applicable to humans. CCK-A receptors have been observed on both normal and neoplastic rat pancreas (10,27). In some cases of rat pancreatic adenocarcinoma, these receptors are overexpressed (34). Furthermore, in some nonhuman pancreatic cancer models, expression of CCK-B receptors has been identified on malignant tissue but not on normal pancreas (34). The relationship between different animal models of pancreatic cancer and humans remains controversial (10,20). The majority of human pancreatic adenocarcinomas appear to be of ductal cell origin, while pancreatic malignancy in most animal models arises in acinar cells. While it is possible that among different species there is significant variation in the cell of origin for pancreatic adenocarcinoma, there is also substantial evidence to support the possibility of a pancreatic stem cell capable of differentiation into ductal or acinar cell types (15). Furthermore, transdifferentiation from acinar to ductal cell phenotype has been described as a potential link between exocrine pancreatic cell types (9,10). Thus, pancreatic tumor development in animals and humans could originate in the same cell type, with variable subsequent differentiation.

This study demonstrated and compared the CCK expression in human fetal pancreas from 20-22 and 25-30 weeks of development, and pancreatic adenocarcinoma. We attempted to track the normal expression fashion of CCK in tissues with different proliferative and differentiating regions and to search whether CCK expression in pancreatic cancer simulates the normal model of display, or may result from neoplastic disarray. We conclude that the efficacy of CCK 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 (eight males and seven females, aged from 48 to 74 years, average 57.8 ± 11.2) were included in the stu-

dy. Two tissue samples were taken from each patient: one from the tumor and one from the resection margin to serve as a negative control. All tumors were verified as pancreatic adenocarcinomas with various degrees of differentiation. The carcinomas were graded according to their cytologic appearance as well differentiated (grade 1, 5 cases), moderately differentiated (grade 2, 8 cases), and poorly differentiated (grade 3, 2 cases).

In order to obtain possible parallels in the expression pattern of neoplastic cells in adults (well - moderately - poorly differentiated), we investigated the pattern of CCK expression in the pancreatic tissue during the various stages of development and compared these with the proliferation of tissue assessed by proliferating cell nuclear antigen (PCNA) immunohistochemistry. 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 involuntary abortion (20 to 22 gestational weeks 8 samples: 3 cases due to infectious factors, 2 cases to endocrine factors, and 3 cases to placental abnormalities. 25 to 30 weeks 7 samples: 5 cases due to leiomyoma, and 2 cases to endometriosis - adenomyosis), was investigated.

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

Immunohistochemical procedure

Cholecystokinin (CCK) immunoreactivity was evaluated using the lyophilised Polyclonal (NCL-CCK-8p) on formalin-fixed, paraffin-embedded samples. Serial sections of the tissue were cut into 3- μ m thick slices and immunohistochemistry was performed by the avidin-biotin complex (ABC) method, using NOVOCASRA 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 non-specific binding. The sections were then washed in PBS and incubated with antibody against CCK (NCL-CCK-8p) overnight at 4 °C. The primary antibody was used after dilution (1:150).

CCK (NCL-CCK-8p) 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 parenchyma, were 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 mm² 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 (acinar/acemose), according to the endocrine pancreatic area (islets of Langerhans) was entirely representative. Statistical analysis was obtained using the t-test.

Results

Embryonal pancreatic tissue

20 - to 22 - week - old human embryos: During this period of development, enteroendocrine cells demonstrated a strong positive immunoreactivity for CCK (NCL-CCK-8p), initially in the primitive exocrine duct epithelium amongst the undifferentiated columnar ductal cells (density of CCK positive cells = mean of cells/mm² of tissue SEM = 37.1±1.5) (Fig. 1) or forming small aggregates (buds) in the surrounding the ductal structures, loose mesenchymal tissue (density of CCK positive cells = mean of cells/mm² of tissue ± SEM = 21.6±0.8) (Fig. 2).

From the 25th to the 30th week of gestation, a strong positive immunostaining for CCK (NCL-CCK-8p) was observed to APUD cells of the islet cortex epithelium (density of CCK positive cells = mean of cells/mm² of tissue ± SEM = 28.1±1.3). Expression of CCK in the pancreatic tissue from pregnancies up to 20-30 weeks varied considerably

both within and among specimens and no-age specific pattern could be detected. Neither did cells in any location (walls - buds - cortex) showed any particularly strong difference in staining intensity.

Neoplastic pancreatic tissue

CCK was demonstrated in ten out of fifteen pancreatic adenocarcinomas. The five CCK negative pancreatic adenocarcinomas were of mucinous type. CCK 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 CCK positive cells was 30.4±1.2 cells/mm² (Fig. 3); in the remaining four cases diagnosed as typical ductal adenocarcinoma the density of CCK positive cells was 25.4±1.6 cells/mm² (Fig 4).

There was a statistically significant difference in the expression of CCK in the ductlike structures of the primitive exocrine embryonal pancreatic tissue from the 20th to

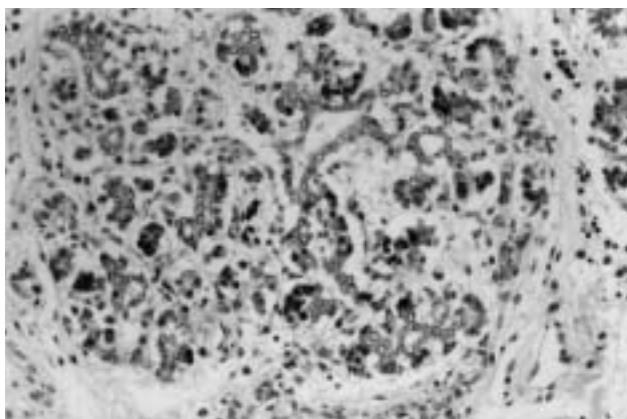


Fig. 1: CCK expression in the primitive exocrine ductal epithelium. NCL-CCK-8p X100.

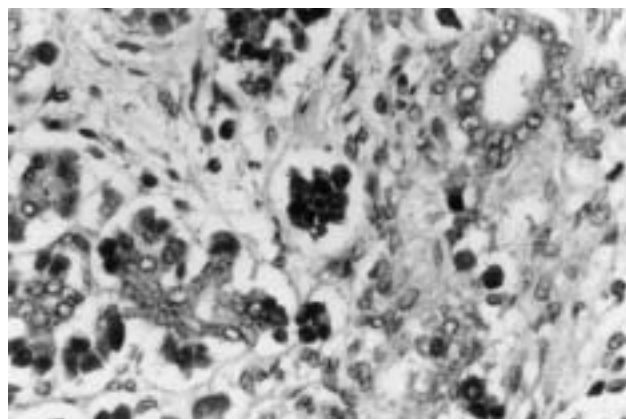


Fig. 2: CCK expression in the primitive exocrine ductal buds. NCL-CCK-8p X200.

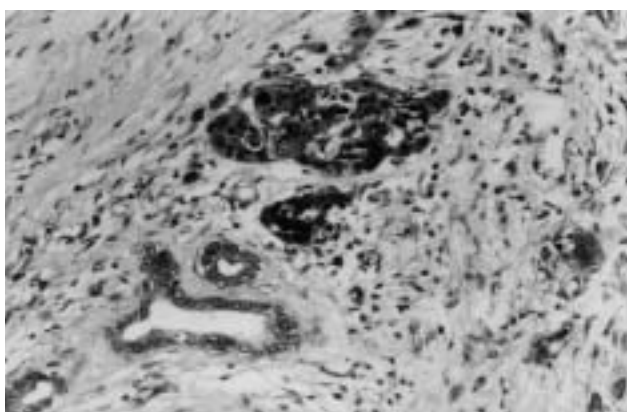


Fig. 3: CCK expression in neoplastic pancreatic tissue with recapitulation of the relevant expression of the antigen in the primitive embryonal pancreatic anlage. NCL-CCK-8p X200.

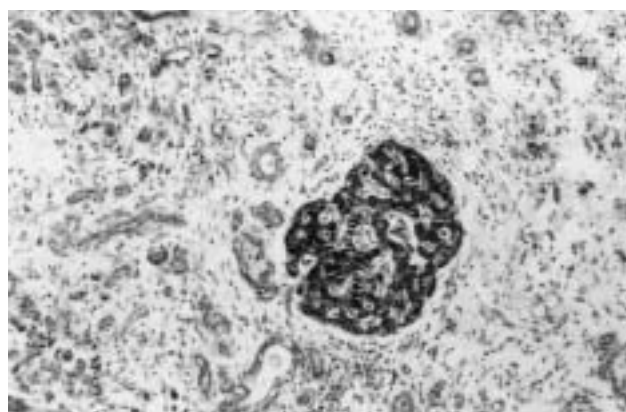


Fig. 4: CCK expression in pancreatic adenocarcinoma of pure ductal type NCL-CCK-8p X100.

Tab. 1: Reactivity of CCK (NCL-CCK-8p) in human embryonal and neoplastic pancreatic tissue.

Pancreatic tissue	Number of cases	Density of CCK positive cells (average cells/mm ² of tissue \pm SEM)
Embryonal (20–22 weeks)	8	
Primitive exocrine duct walls		37.1 \pm 1.5
Primitive exocrine ductal buds		21.6 \pm 0.8
Embryonal (25–30 weeks)	7	
Islet cortex epithelium		28.1 \pm 1.3
Neoplastic tissue	10	
Mixed ductal-endocrine carcinoma	6	30.4 \pm 1.2
Pure ductal carcinoma	4	25.4 \pm 1.6

the 22th gestational week, over the neoplastic pancreatic tissue of mixed type ($p_1=0.004$) and pure ductal type ($p_2<0.0005$).

There was also a statistically significant difference in the expression of CCK in the buds surrounding the ductal structures of the primitive exocrine embryonal pancreas from the 20th to 22th over the neoplastic pancreatic tissue of mixed type ($p_3<0.0005$) and pure ductal type ($p_4=0.023$).

No statistically significant difference was observed in the expression of CCK in the islet cortex tissue from the 25th to the 30th week, in comparison with the neoplastic tissue of mixed type ($p_5=0.10$) and pure ductal type ($p_6=0.15$).

The density of PCNA positive cells was $0-4\pm 1.2$ cells/mm² in the primitive exocrine ductal walls; $0-7\pm 1.2$ cells/mm² in the primitive exocrine ductal buds; $0-6\pm 1.3$ cells/mm² in the islet cortex epithelium; 26.6 ± 1.3 cells/mm² in the mixed ductal endocrine carcinoma and 32.1 ± 1.7 cells/mm² in the pure ductal carcinoma. So PCNA and CCK immunoreactivity showed a clear inverse relationship in the two types of carcinoma. On the contrary, a negative relationship was found between the two antigens during the embryonic pancreatic tissue development.

Discussion

CCK, a peptide hormone produced in the upper small intestine, is known to stimulate the secretion and growth of the normal exocrine pancreas (28). Many studies have been conducted to examine the role of CCK in the development of pancreatic adenocarcinoma and promotion of its growth once established.

There is considerable evidence supporting a central role for cholecystokinin (CCK) in human pancreatic cancer (11). The influence of endogenous hormones is well-described for several human malignancies, including breast, ovary, and prostate. Generally, the hormones implicated are important in both the health and disease of their target organ. CCK is an important mediator in the growth of the normal pancreas (21,27). Animal studies in which exogenous CCK was administered or in which endogenous CCK levels were manipulated documented pancreatic hyperplasia, dysplasia, and the production of frank malignancies

(8). Similar studies after the induction of pancreatic tumors suggest that CCK administration accelerates the growth of malignant tissue compared to uninvolved tissue (6,13). In human cancer cell lines and xenografted human tumors, CCK promotes the growth of pancreatic adenocarcinoma (14,31). The identification of a specific molecular marker for pancreatic cancer could be of substantial diagnostic and therapeutic benefit.

The role of CCK-A or -B receptors in human pancreatic carcinogenesis remains unclear. The growth of several human neoplastic cell lines derived from pancreas (31), colon (12), and lung (22) is stimulated by CCK or gastrin in vitro. Functional CCK-B receptors have been demonstrated on human biopsy specimens of small cell lung (22) and colon cancers (12), implicating a potential role for CCK or gastrin in human carcinogenesis (30). The demonstration of novel CCK-A receptor expression specifically on ductal cells in pancreatic adenocarcinomas is intriguing. This unique expression could reflect the presumed ductal origin of human pancreatic tumors. CCK-A receptors may be expressed at very low levels in ductal cells in normal human pancreas, below the level of detection by current techniques (24). Tumorigenesis may expand the population of ductal cells, increasing the expression of CCK-A receptors. In addition, CCK-A receptors on ductal cells may play a direct role in mediating the process of pancreatic tumorigenesis.

Alternatively, these studies are consistent with the hypothesis that CCK-A receptors may be a feature only of the developing human pancreas. CCK-A receptors might predominate during human fetal pancreatic development, but their expression would be unnecessary in normal adult pancreas. It has been suggested that CCK mediates normal pancreatic growth and development (27). Previous studies in the calf have demonstrated the expression of CCK-A receptors during fetal development, but with a predominance of CCK-B receptors in the normal adult bovine pancreas (19). Expression of novel antigens by pancreatic neoplastic cells, including fetal markers, has been described (7). Accordingly, expression of CCK-A receptors in pancreatic adenocarcinomas may reflect the reexpression of fetal markers during tumorigenesis. The expression of CCK-A receptors by human fetal pancreas is currently under examination in this laboratory.

Although there is a substantial body of literature supporting a role for CCK in the modulation of pancreatic cancer growth, our unpublished preliminary data do not support this hypothesis.

The aim of our paper pointed towards the CCK expression in embryonic and neoplastic pancreata. In the embryo, CCK was expressed in distinguished growth steps implying a differentiation – related role. Our data reveal the behavioral potentialities 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 employ progenitor cells, which if properly triggered differentiate into endocrine cells such as the cells expressing CCK.

The incidence of cancer is increased either by the influence of a carcinogen with a direct impact on DNA or by an increase in cell proliferation (2). The increased cell proliferation may also be related to the decreased cell proliferation. Therefore, the decrease of CCK may indicate cellular dedifferentiation. Results from our examination of carcinomas, revealed low CCK expression in less-differentiated tissues with a high cell turnover.

In conclusion, CCK immunoreactivity appears to show a negative correlation with cell proliferation and differentiation in these developing, normal adult and pathologically transformed adult pancreatic tissues. Within more heterogeneous groups such as mixed carcinomas, further studies will be needed to determine whether there are criteria that may help in estimating the potential for malignancy in the altered tissues.

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Submitted January 2004.

Accepted February 2004.

*Nikolaos Papadopoulos,
Assoc. Professor in Histology-Embryology,
Democritus University of Thrace,
Dragana, 68 100 Alexandroupolis,
Greece.
e-mail: npapad@med.duth.gr*