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Correlation between Proliferating Index and Prognostic Factors in Papillary Cystic Tumors of the Pancreas

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Fifteen cases of papillary cystic tumor of the pancreas (PCTP) were studied (14 female patients, one male patient; mean age: 23.5 years). Most tumors developed in the head of the pancreas as a well circumscribed large mass. The tumor had a mean diameter of 6.7cm(range; 2 to 15 cm). Histopathologically abundant delicate papillary fragments, monomorphic tumor cells and degenerative changes of the solid area of the tumor were characteristic. All but two cases had completely circumscribed capsules. Two cases had duodenal invasion; one of all cases had cul de sac metastasis. Compared with 12 non-aggressive tumors, the aggressive cases had larger tumor size (more than 9 cm) with a thicker capsule (more than 2 mm). In studies to investigate the prognostic index using nucleolar organizing region (NOR), proliferating cell nuclear antigen (PCNA) and flow cytometry as well as nuclear grade and mitotic index, we could not find the useful parameter to detect the malignant potential of PCTP. In the flow cytometric analysis of cellular DNA contents, two invasive cases and the only one case of the male patient among the non-aggressive group were aneuploid. In conclusion, although it is hard to predict the prognosis by microscopic findings only, those with a thick capsule and aneuploidy tend to be related to malignant potential.

Key Words: *Papillary cystic tumor, Malignant potential, Capsule, NORs, PCNA, Flow cytometry*

INTRODUCTION

Papillary cystic tumor of the pancreas (PCTP) is rare, usually affecting adolescent girls or young women. Although the biologic behaviour of these tumors generally pursues a favorable course, a few malignant cases have recently been reported (Compagno et al., 1979; Benhamin and Wright, 1980; Warren, 1985; Kaufman et al., 1986; Rustin et al., 1986; Todani et al., 1988; Hernadez-Maldonada et al., 1989; Yamaguchi et al., 1989; Cappellari et al., 1990; Matsunou et al., 1990; Matsunou and Konishi, 1990; Zinner et al., 1990; Sclafani et al., 1991; Stömmer et al., 1991; Nishihara et al., 1993). There have been unsuccessful attempts to find any morphologic features suggestive of aggressive behaviour. However, there is some suggestion that PCTP occur-

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ring in older age (Matsunou and Konishi, 1990), having a solid, infiltrative pattern without a capsule (Matsunou et al., 1990) and nuclear pleomorphism (Cappellari et al., 1990, Matsunou and Konishi, 1990) is more likely to behave aggressively. We think that PCTP is quite similar to thyroid follicular neoplasm in regard of histologic features and the criteria for malignancy. The diagnosis of malignancy depends primarily upon the demonstration of unequivocal capsular and/or vascular invasion. Additionally a thick fibrous capsule as well as an undulating interface between the capsule and parenchyma are often found in the early-stage of thyroid follicular carcinoma (Yamashina, 1992). We attempted to compare three aggressive cases of PCTP, one of which metastasized to cul de sac with 12 non-aggressive cases and also investigated the prognostic index by the expression of nucleolar organizer region (NOR), proliferating cell nuclear antigen (PCNA) and flow cytometric analysis as well as clinicopathologic examinations of 15 cases of PCTP in order to define the biologic behaviour better and determine the predictable value of malignant potential.

MATERIALS AND METHODS

A total of 15 cases of PCTP were retrieved with medical records, diagnostic imaging studies, and surgical pathology reports from Yonsei Medical Center from 1985 to 1993. Follow-up information was obtained from referring physicians. We intended to divide these cases into 2 groups : aggressive vs non-aggressive group. These criteria mainly depended on whether the tumor limited to the pancreas or not. Thus aggressive group included the cases of which the tumor extended directly into the duodenum as well as the tumor with metastasis to the cul de sac. All resected tumors were fixed in 10 % formaldehyde and processed in paraffin blocks. Sections stained with hematoxylin and eosin were available in all cases. The average capsular thickness was determined by choosing the thickest part in each section. Formalin fixed, paraffin embedded sections of 15 cases were evaluated immunohistochemically using the avidin-biotin peroxidase complex. The sections were treated with monoclonal mouse anti-human PCNA (PC 10, M879, Dako Corp. Santa Barbara, CA) at a dilution of 1 : 100. Five-μm sections were cut and mounted on poly-L-lysine coated slides. After blocking of endogenous peroxidase with 3 % H_2O_2 for 5 minutes, a heating process in the microwave for 5 minutes with soaking in sodium citrate buffer (pH 6.0) followed. Primary antibodies were incubated for 45 min. The absolute counts of PCNA immunoreactivity were made by each scoring 400 cells by two surgical pathologists. The PCNA index represented the percentages of cells with positive nuclear staining in the total number of tumor cells counted regardless of staining intensity.

The sections were submitted for AgNOR procedure at room temperature for 30 minutes. The reaction mixture was comprised of 2 gm of gelatin in 100 ml of 1 % aqueous formic acid and 50 gm of silver nitrate in 100 ml of distilled water under dark room conditions. These solutions were mixed at 1 : 2 volumes just prior to use. After staining, the mixture was poured from the slides. Counterstaining was not performed, and the sections were dehydrated with xylene and mounted in synthetic medium. Sections were examined under an oil immersion lens at a magnification of 1000, and 200 nuclei were studied. The AgNOR "dots" in these were counted by a simple eyepiece graticule to prevent recounting.

The flow cytometric determination of the DNA content of tumor cells was done with the paraffin-embedded tissue using an earlier method (Hedley et al., 1983). Three consecutive 50-μm sections were obtained from pure solid components without necrosis or cystic change of one paraffin-embedded block. After deparaffinization and rehydration, sections were incubated for 30 minutes at 37℃ in 0.5 % pepsin(pH 1.5). The suspension was washed and passed through a 50-μm filter. After vortexing and filtration over a nylon mesh, approximately $1\text{-}3\times10^6$ cells were stained with propidium iodine according to a published method. Cellular DNA content was measured on a FACScan flow cytometer(Becton, Dickinson Corp, Mountain View, CA, U.S.A) with a 488 nm argon ion laser. Only histograms with distinct G0/1 and G2/M peaks and coefficient variation (CV) less than 7 % were accepted. Histograms of 2×10^4 cells were recorded and analyzed as follows. The first G1/0 peak was assumed to be a diploid population and was given a DNA index of 1.0. DNA aneuploidy was defined by the presence of a well-defined peak with DNA index greater than 1.1. Clinical data were available for all 15 patients, and the follow-up period ranged from 1 to 10 years.

RESULTS

Clinical findings

The clinical data of the patients are summarized in Table 1. All but one were female, and ranged in age from 14 to 40 years(mean, 23.5 years). In 11 cases, ultrasound and computed tomographic examination showed PCTP to be well circumscribed, and cystic with fluid-filled multiplocules alternating with solid areas partly replacing pancreas. The postoperative course was uncomplicated in all and they did not receive any other treatment. On operation, 2 cases were found to have invaded the duodenal wall down to the mucosa. In one case, there was obvious metastasis to the cul de sac. These three cases with local invasion and distant metastasis were classified as the aggressive group.

Pathologic Findings

1) Gross Findings

PTCP occurs as discrete and well-demarcated within the pancreas by a peripheral capsule in 13 cases. The mean diameter of the tumors was 6.7cm (range, 2-15 cm). Two cases (Case No. 6 & 15) showed cystic changes with extensive necrosis and hemorrhage containing dark brown soft contents and mural trabecula(Fig. 1). All but two cases were well encapsulated. Of the 15 tumors, 9 were located in the head of the pancreas, two in the body, and three in

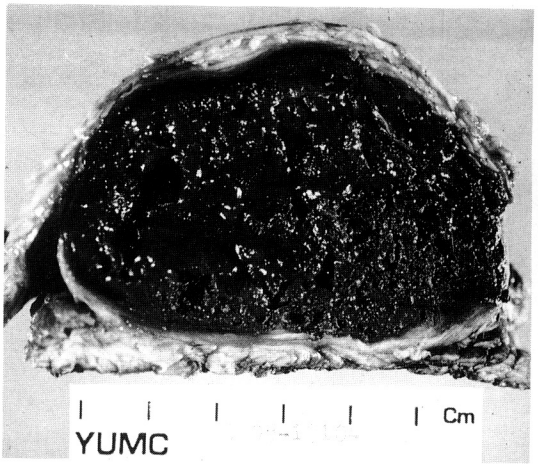

Fig. 1. Macroscopic appearance of PCTP in the case of duodenal invasion. Notice the huge, well encapsulated cystic tumor filled with muddy, hemorrhagic contents.

Table 1. Clinical Data for 15 Patients with Papillary Cystic Tumors of the Pancreas.

Case No.	Age (yrs)	Sex	Site	Operation	Size (cm)*	Metastasis and/or invasion	Prognosis (yrs)
Non-aggressive group							
1	14	M	H	Pan-duo.ectomy	9	None	AW(2)
2	15	F	H	Partial pan.ectomy	8	None	AW(1)
3	16	F	H	Pan-duo.ectomy	3	None	AW(3)
4	22	F	H	Excision	4	None	AW(1)
5	22	F	T	Excision	12	None	AW(6)
6	23	F	H	Pan-duo.ectomy	8	None	AW(1)
7	24	F	T	Excision	2	None	AW(6)
8	28	F	H	Pan-duo.ectomy	4.5	None	AW(1)
9	31	F	H	Pan-duo.ectomy	2	None	AW(2)
10	35	F	B	Excision	4	None	AW(2)
11	37	F	T	Excision	2	None	AW(4)
12	40	F	B	Enucleation	5	None	AW(1)
Aggressive group							
13	20	F	H	Pan-duo.ectomy	15	Duodenum	AW(1)
14	22	F	H&B	Pan-duo.ectomy	9	Cul de sac	AW(1)
15	24	F	H	Pan-duo.ectomy	13	Duodenum	AW(5)

M : male, F : female, H : head, B : body, T : tail

Pan-duo.ectomy : Pancreatico-duodenectomy ; pan.ectomy : pancreatectomy ; AW : alive and well

* Maximum diameter of tumor recorded in postoperative gross pathologic examination.

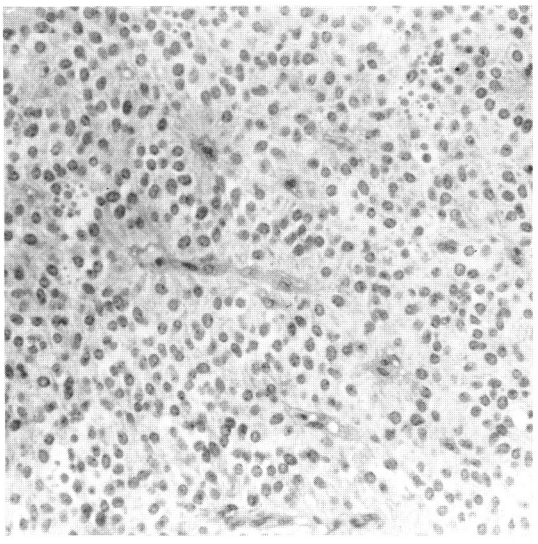

Fig. 2. The solid sheets of monotonous cells with delicate fibrovascular septa (H & E, ×100).

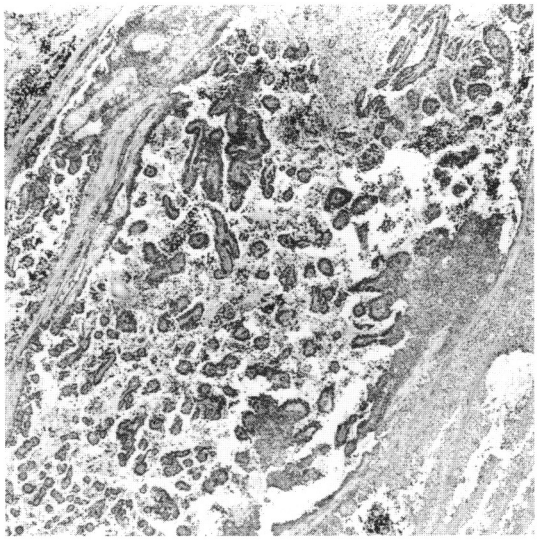

Fig. 3. Many small papillae having edematous stroma with thin vascular core (H & E, ×40).

the tail while the remaining one occurred in the head extending to the body.

2) Microscopic findings

The tumors were composed of three different-solid, papillary and cystic areas with variable extents. The solid areas were composed of sheets and cords of uniform small cells with round dark nuclei and either eosinophilic or clear cytoplasm divided by delicate fibrovascular septa (Fig. 2). Rarefaction occurred within solid areas to form microcysts between the tumor cells remote from supplying blood vessels, a pseudopapillary, pseudorosette pattern (Fig. 3). Deposition of hyalinized collagen along the blood vessels produced a trabecular pattern. Marked degenerative changes such as hemorrhage, aggregation of foam cells and cholesterol granulomas were frequently observed at the periphery of the solid areas (Fig. 4). The nuclear grades of PCTP were divided into three groups based on the nuclear size, chromatin condensation, nucleoli, nuclear pleomorphism. When the mean nuclear size of the tumor cells was 7.0 μm or less, 0 points; more than 7.0 μm, 1 point; nuclear chromatin was fine, 0; mildly vesicular chromatin, 1; nucleoli were small, 0; and moderate sized nucleoli, 1. Nuclear atypia were divided into minimal (0) to marked (2) and nuclear pleomorphism, into minimal (0) to marked (2). Then each score was added, and either 0 or 1 point was designated as

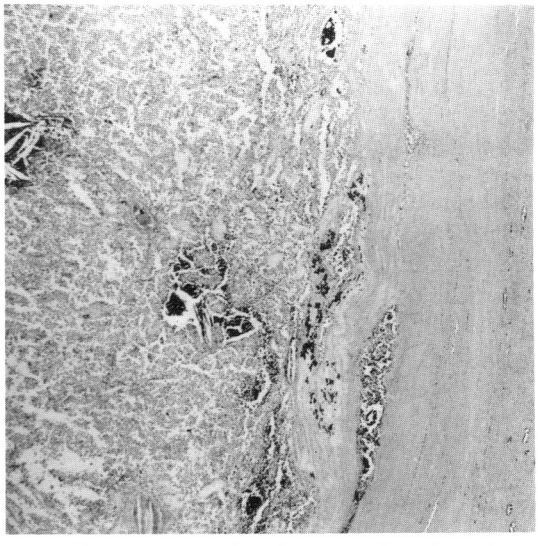

Fig. 4. Marked degenerative changes such as hemorrhage with cholesterol granuloma predominantly at the periphery of the solid tumor area. Note the very thick, dense, lamellated fibrous capsule with smooth tumor-capsule interface (H & E, ×40).

nuclear Grade 1, 2 or 3 points as nuclear Grade 2, and 4 or more points as nuclear Grade 3 (Nishihara et al., 1993). Mitotic figures were rarely perceptible. Nuclear atypism was found in only one case.

Table 2. Capsular Thickness and Nuclear Grade in the 15 patients.

Case No.	Capsule	Thickness (mm)	Nuclear grade
1	+	2.2	1
2	+	1.2	2
3	−	0	2
4	+	1.4	1
5	+	2.5	2
6	+	4.0	1
7	+	4.0	2
8	+	1.5	1
9	−	0	2
10	+	0.2	3
11	+	0.5	2
12	+	0.4	1
13	+	2.2	1
14	+	6.0	2
15	+	2.0	1

Marked nuclear atypism and pleomorphism were present in one case of the non-aggressive group (case 10) but not in the aggressive group. Vascular invasion by tumor cells was not observed.

The whole circumferential capsular thickness determined as an average of the maximum thickness of multiple sections ranged from 0.2 mm to 6 mm in all but 2 cases. The capsular thickness of the non-aggressive group ranged from 0.2 to 4 mm (mean 1.73 mm), whereas that of the aggressive group ranged from 2 to 6mm (Table 2 & Fig. 5). Three cases having a thin capsule and 2 cases without tumor capsule in the non-aggressive group showed shallow infiltrative nests of tumor cells into the adjacent pancreatic parenchyma(Fig. 6). The case with metastasis to the cul de sac was unique in that the papillary area had invaded into the capsule whereas the solid areas were still confined to the adjacent subcapsular area.

Immunohistochemical Findings

1) AgNOR

In all cases, clearly defined silver-stained dots were observed in 200 nuclei. These were separated or organized in small aggregates, and sometimes were placed in a different position than the hematoxylin-stained nucleoli or chromatin. All observed nuclei were taken into consideration in the counting procedure. The mean number of AgNORs was 1.57 per nucleus. The mean number AgNOR was 1.51 per

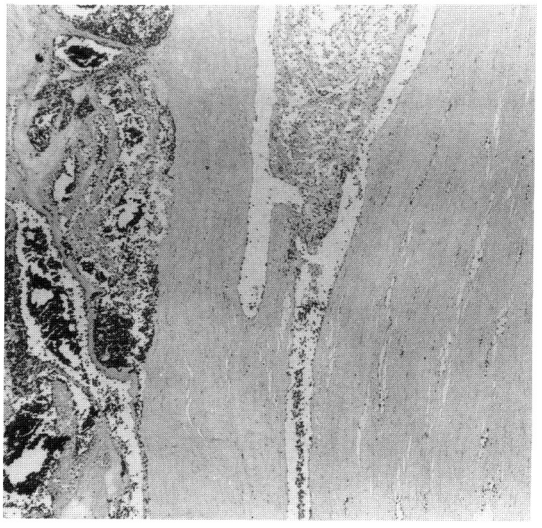

Fig. 5. A very thickened fibrous capsule with undulating margin and focal infiltration of tumor cells into the midst of the capsule in case 14 (H & E, ×40).

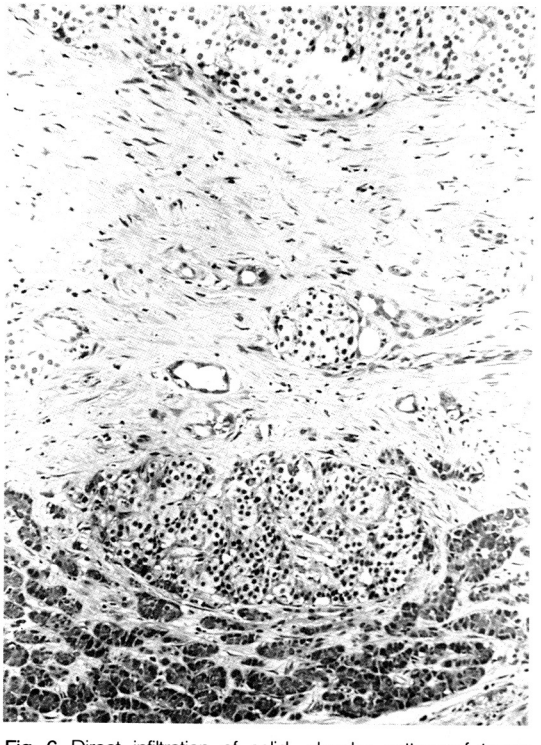

Fig. 6. Direct infiltration of solid, alveolar pattern of tumor cells into the adjacent parenchyma across the intervening thin capsule (H & E, ×100).

Table 3. Proliferative Cell Index of the 15 Patients.

Case No.	AgNORs	PCNA (%)	Flow cytometry	SPF (%)
1	1.70	7.8	An	(DI : 1.18)
2	1.82	56.4	NT	NT
3	1.85	3.0	NT	NT
4	1.81	11.2	Di	3.1
5	1.38	20.8	Di	0.9
6	1.80	6.8	Di	7.0
7	1.30	3.4	Di	3.1
8	1.35	63.2	Di	1.95
9	1.50	17.8	Di	7.0
10	1.17	3.8	Di	2.0
11	1.20	9.5	Di	4.4
12	1.24	37.6	Di	5.2
13	2.10	20.0	An	(DI : 1.30)
14	1.52	42.8	Di	0.95
15	1.88	17.4	An	(DI : 1.15)

PCNA : Proliferating cell nuclear antigen ; SPF : S-phase fraction ; An : Aneuploidy ; Di : Diploidy ; NT : Non-tested ; DI : DNA index

nucleus in the non-aggressive group and 1.91 in the aggressive group. Statistical analysis of the results, by means of the student t-test, showed that this difference between the two mean values was insignificant (p=0.183)(Table 3).

2) PCNA

Reactivity of PC10 was distinct although there was a gradation in the intensity of the staining. The mean PCNA index of 15 cases was 21.4 %. The PCNA index was 20.1 % in the non-aggressive group and 26.7 % in the aggressive group. There was no statistical significance between the groups (p=0.542) (Table 3). In the histologically normal exocrine parenchyma adjacent to the tumors, there was perpetual increase in immunohistologically detectable PCNA-containing cells in some, but not all cases.

3) Flow cytometry

A flow cytometric study of cellular DNA content was performed on tissue from 13 of the 15 tumors. Satisfactory DNA histograms were not obtained in 2 cases due to cellular paucity and very high coefficient variation (CV). The mean CV was 4.65 % (3.8-5.7 %). Three cases, including 2 cases with duodenal invasion and the only male patient were aneuploidy (Fig. 7). The remaining 10 cases were diploidy. These tumors showed a rather low proliferative activity, as indicated by S-phase fraction values of 0.9 to 7 % (Table 3 & Fig. 8).

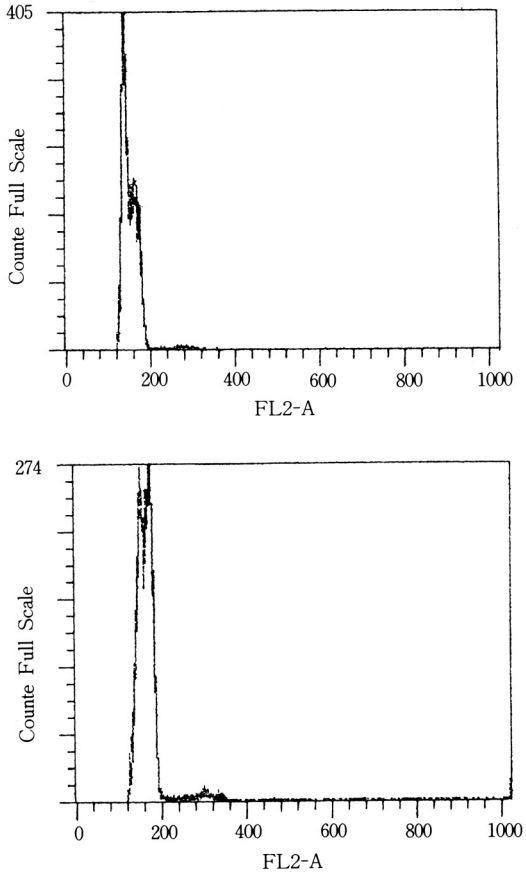

Fig. 7. DNA histogram of aneuploidy having double peaks of G_1 in case of duodenal invasion. Notice a very low S-phase fraction. DNA index is 1.15.

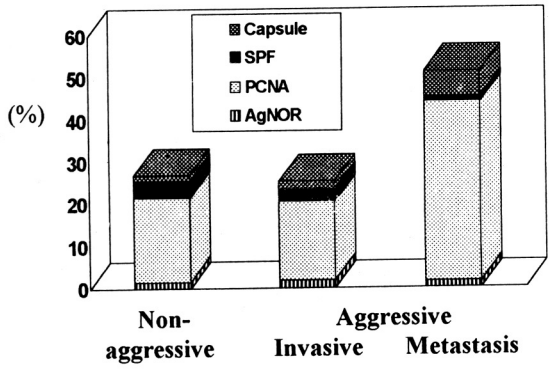

Fig. 8. Mutual correlation between capsular thickness and some kinds of proliferating indices. Degree of capsular thickness only shows significant differences between each group.

DISCUSSION

The biologic behaviour of PCTP has not been clarified although approximately 130 cases of PCTP have been reported since it was first described by Frantz in 1959 as "Papillary tumor of the pancreas-benign or malignant?". The tumor usually occurs in young women. Only eight cases of PCTP in men have been reported (Frantz, 1959 ; Friedman et al., 1985 ; Choi et al., 1988 ; Jaffe amd Newman, 1990 ; Wilson et al., 1990 ; Sclafani et al., 1991). This tumor may occur anywhere in the pancreas, but has a strong predilection for the body and tail (Yamagichi et al., 1989 ; Pettinato et al., 1992). Our cases occurred predominantly in the head of the pancreas. Most tumors behave in a benign fashion in which they merely displace surrounding structures instead of invading them and complete removal of this tumor is usually possible. Aggressive behaviour such as extension of the tumor into contiguous organs or vessels, local recurrence or distant metastasis, occur less frequently and have been documented only in 21 patients (Compagno et al., 1979 ; Benhamin and Wright, 1980 ; Warren, 1985 ; Kaufman et al., 1986 ; Rustin et al., 1986 ; Todani et al., 1988 ; Hernadez-Maldonada et al., 1989 ; Yamaguchi et al., 1989 ; Cappellari et al., 1990 ; Matsunou et al., 1990 ; Matsunou and Konishi, 1990 ; Zinner et al., 1990 ; Sclafani et al., 1991 ; Stömmer et al., 1991 ; Nishihara et al., 1993). Only 5 cases were documented to have metastasized to the liver (Cappellari et al., 1990 ; Matsunou and Konishi, 1990 ; Sclafani et al., 1991). The three cases found in this study revealed aggressive behaviour, two of which invaded the duodenal wall. The other one metastasized to the cul de sac. It has not yet been established how to define parameters indicating aggressive behaviour by morphological evaluation alone. There are several factors considered to predict aggressive behaviour, that is, incomplete resection (Cappellari et al., 1990), older age (Matsunou and Konishi, 1990) with cellular atypism (Cappellari et al., 1990 ; Matsunou and Konishi, 1990) and solid infiltrating tumor margin without capsule (Matsunou et al., 1990). However, we could hardly find any appreciable value in ordinary features. In a previous report of 9 cases of PCN (Matsunou and Konishi, 1990), the fact that a 47 years-old patient died 6 years later while a 60 years-old patient died 29 years later was used as evidence that age is related to the prognosis of this tumor. However, the age of the aggressive group was slightly younger in the present 15 cases reviewed, the correlation is rather ambiguous. Cellular atypism, once considered a useful prognostic parameter (Cappellari et al., 1990 ; Matsunou and Konishi, 1990), was not present in the aggressive group but prominent in the non-aggressive group. Thus, cellular atypism does not appear to be related after all. The case with solid, infiltrative variety but no definite capsule did not show aggressive behaviour, whereas interestingly prominent capsular thickness was found to be correlated well with aggressive behaviour in our study. A thick fibrous capsule more than 2 mm in thickness was almost, if not always, seen in the aggressive group. There were 4 cases having thick fibrous capsule more than 2 mm in thickness also in the non-aggressive group. The metastatic case had the thickest capsule of 6 mm in thickness among all the cases reviewed.PTCPs have some characteristics similar to follicular neoplasm of the thyroid gland in that both are an endocrine organ with well-developed capsule and histologic pattern as well. Thick fibrous capsule as well as an undulating interface between the capsule and parenchyma and capsular/vascular invasion were often found to be characteristic of early-stage carcinoma of follicular neoplasm (Evans, 1984 ; Rosai et al., 1990 ; Yamashina, 1992). Even when follicular neoplasm of the thyroid gland penetrates through the capsule, it usually does not permeate the surrounding parenchyma but forms a new fibrous capsule along the advanced margin (Evans, 1984). The fibrous capsule tends to be thicker and more irregular in carcinomas than in adenomas (Evans, 1984 ; Rosai et al., 1990 ; Yamashina, 1992). The tumors, in general, would be benign if they expand being incorporated by condensation of pre-existent stromal fibers. It can view as a sign of malignancy if explained as a component of newly laid down collagen fibers as an expression of host response to the malignancy especially in the thyroid gland (Evans, 1984 ; Rosai et al., 1990 ; Yamashina, 1992).

Surprisingly, proliferation indices have seldom been utilized for assessing tumor behaviour in most pancreatic tumor studies. There are several methods (Woosley, 1991) for measuring cell proliferation, including incorporation of modified nucleotides into newly synthesized DNA(tritiated thymidine or bromodeoxyuridine), proliferation-associated antigens (PCNA, Ki 67, p105, ribonucleotide reductase, and DNA-directed DNA polymerase α), silver-stained

nucleolar organizer regions(AgNORs), flow cytometric analysis and in situ hybridization of a cDNA probe to mRNA of histone H3 as well as mitotic figure counting. Of these methods, PCNA, AgNORs and flow cytometric analysis are advocated to be performed in conventionally processed, paraffin-embedded tissues. Silver-stained nucleolar organizer regions can be readily identified in the interphase nuclei of tumor cells following conventional formaldehyde solution fixation and processing (Derenzini et al., 1989 ; Derenzini et al.,1990 ; Derenzini and Trerè D, 1991 ; Shi et al., 1991). Increase of AgNOR number per nucleus in hyperplastic or malignant cells has been reported to be also related with DNA ploidy and transcriptional activity (Derenzini and Trerè, 1991 ; Shi et al., 1991). PCNA is a 36kd acidic non-histonic nuclear protein, an auxilliary protein to DNA polymerase delta. Its presence appears to be necessary for the initiation of cell proliferation. As regards the PCNA index, previous studies have shown that it correlates with the S-phase fraction of tumor cells determined by DNA flow cytometry (Garcia et al., 1989 ; Hall et al., 1990 ; Ottavio et al., 1990). However, in this study there was discordance between PCNA index with S-phase fraction in flow cytometry. There is no correlation between AgNOR with PCNA and S-phase fraction to determine or predict the behaviour of this tumor at all. The intriguing observation is that in histologically normal lobules adjacent to tumors, there was perpetual increase in immunohistologically detectable PCNA containing cells in some, but not all cases. This finding has been observed in some tumors such as breast tumor and pancreatic exocrine and endocrine tumors (Chang et al., 1990 ; Hall et al., 1990 ; Lee et al., 1993). Recent evidence suggests that platelet derived growth factor (PDGF) secreted by tumor can induce increased PCNA mRNA stability and consequently PCNA expression and thus induce PCNA protein accumulation in the surrounding normal cells (Hall et al., 1990). Until now there have been only 20 previous case reports of flow cytometric analyses in PCTP (Cappellari et al., 1990 ; Pettinato et al., 1992 ; Nishihara et al, 1993). The previous studies using DNA flow cytometry in PCTP revealed that only 2 cases showed aneuploidy. One had been still alive at 3 years after surgery, and the other later developed widespread intraabdominal metastasis. In our study 3 cases were of aneuploidy, of which 2 cases showed duodenal invasion. The other case showing aneuploidy was from a male patient among the non-aggressive group. Although these results suggest that flow cytometric analysis may provide useful guidance to predict aggressive behaviour, this experience is limited and further studies are needed. In conclusion, although there is no solid criterion of malignancy in PCTP, we would suggest that the aggressive group has a tendency to have thicker capsule and aneuploidy.

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