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ORIGINAL ARTICLE

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Intraductal papillary-mucinous tumours represent a distinct group of pancreatic neoplasms: an investigation of tumour cell differentiation and K-*ras*, p53 and c-*erb*B-2 abnormalities in 26 patients

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Abstract Intraductal papillary growth of mucin producing hypersecreting, columnar cells characterizes a group of rare pancreatic exocrine neoplasms which we propose to call intraductal papillary-mucinous tumors (IPMT). We analysed the histopathology of 26 IPMT in relation to gastro-enteropancreatic marker expression, genetic changes and biology. Four IPMT showing only mild dysplasia were considered to be adenomas. Nine tumours displayed moderate dysplasia and were regarded as borderline. Severe dysplasia-carcinoma in situ changes were found in 13 IPMT which were therefore classified as intraductal carcinomas. Six of these carcinomas were frankly invasive and two of these had lymph node metastases. The invasive component resembled mucinous noncystic carcinoma in all but one tumour which showed a ductal invasion pattern. Immunohistochemically, an intestinal marker type was found in most carcinomas, while gastric type differentiation prevailed among adenomas or borderline tumours. K-*ras* mutations (seven at codon 12 and one at codon 13) were found in 31% of IPMT (2 adenomas, 1 borderline, 5 carcinomas). Nuclear p53 overexpression was detected in 31% of IPMT (6 carcinomas and 2 borderline IPMT) and correlated with p53 mutations (one at exon 8 and the other at exon 5) in two carcinomas. p53 abnormalities were unrelated to K-*ras* mutation. c-*erb*B-2 overexpression was observed in 65% of IPMT, with various grades of dysplasia. Twenty-two of 24 patients are alive and well after a mean post-operative follow-up of 41 months. Only two patients, both with invasive cancer at the time of surgery, died of tumour disease. It is concluded that pancreatic IPMT encompass neoplasms which, in general, have a favorable prognosis, but are heterogeneous in regard to grade of dysplasia and marker expression. Adenoma, borderline tumour, intraductal carcinoma and invasive carcinoma can be differentiated. p53 changes but not K-*ras* mutation or c-*erb*B-2 overexpression are related to the grade of malignancy. Most IPMT differ in histological structure, marker expression and behaviour from ductal adenocarcinoma.

Key words Pancreatic intraductal tumours · Grade of dysplasia · Gastroenteropancreatic markers · K-*ras* mutations · p53 mutations

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Introduction

Among the exocrine tumours of the pancreas there is a particular category characterized by intraductal papillary growth. This group of tumours has received a variety of names including intraductal papilloma, intraductal papillary neoplasm, diffuse intraductal papillary adenocarcinoma, villous adenoma, mucus-hypersecreting tumour, mucin-producing carcinoma or mucinous ductal ectasia (4, 14, 17, 22, 30, 31) which reflects their variable appearance as well as uncertain biological behaviour. Grossly, two main types can be distinguished: the intraductal papillary neoplasm (6, 7, 51) and the intraductal mucin hypersecreting neoplasm (4, 11, 37, 52, 53). As transitions between these two prototypes occur and generally the same changes are found at the histological level, it seems that we are dealing with one tumour entity (18). We will therefore summarize the neoplasms investigated in this study under the term intraductal papillary-mucinous tumour (IPMT). Included among the IPMT will be intraductal tumours with grossly visible papillary proliferation and no mucin production.

The differentiation, malignant potential and prognosis of IPMT all continue to be a matter of debate and their relationship with ductal adenocarcinoma is unclear. To investigate these issues in more detail, we collected a series of 26 tumours, including six cases which had been published previously (20, 24, 37). The questions which were addressed were: (1) is there heterogeneity among the IPMT in regard to epithelial differentiation and gastroenteropancreatic marker expression; (2) are K-*ras*, p53 and c-erbB-2 gene abnormalities correlated to malignant differentiation and behaviour; and (3) can IPMT be regarded as a precursor of ductal adenocarcinoma? We will show that IPMT includes a group of heterogeneous tumours, which may be subdivided into intraductal papillary-mucinous adenoma, borderline lesion and carcinoma. Most of these neoplasms show no relationship to the usual ductal adenocarcinoma.

Materials and methods

Study material

We studied the pancreatic tumours of 26 patients (16 men and 10 women; mean age 58 years, age range 33–79) from the files and consultation archives of the Departments of Pathology of the Universities of Pavia, Varese and Verona (Italy) and the Academic Hospitals Erasmus and Jette of the Free University of Brussels (Belgium). The most important clinicopathological data are summarised in Table 1. Cases 13 and 14 were also reported by Morohoshi et al. (24) as cases 4 and 5; cases 16, 17 and 19 by Rickaert et al. (37) as cases 2, 3 and 7; and case 18 by Lemoine et al. (20) as case 22.

Multiple samples were obtained from the tumours and remaining non-tumour tissue. They were fixed in 10% formaldehyde solution and then paraffin embedded. Deparaffinized sections were stained with haematoxylin and eosin, alcian blue (1%, pH 2.5)-periodic acid-Schiff (AB-PAS) technique, high iron diamine (HID) or the diastase (1%, in phosphate buffer pH 4.5, 37° C, 1 h)-periodic acid-biotinylated concanavalin A (PACONA) technique (8).

The IMPT were classified as adenomas, borderline tumours or intraductal carcinomas according to the most severe grade of epithelial dysplasia found in the neoplasms. Adenomas showed mild dysplasia; borderline tumours moderate dysplasia; and intraductal carcinomas severe dysplasia-carcinoma in situ changes. In mild dysplasia, all papillae were well formed and had a fibrovascular stalk. In the stroma underlying some polypoid growth there were also small glands. Papillae and glands were lined by tall columnar cells containing abundant mucin in their apical portion and a round to oval nucleus of moderately increased size in the basal portion. The nuclei were usually not hyperchromatic and showed one or two small nucleoli. Because of the presence of abundant mucin containing cytoplasm the nucleocytoplasmic ratio was often lower than in normal ductal cells. Mitoses were generally absent. In moderate dysplasia, the tumour cells formed more irregular and elongated papillae with small fibrovascular stalks. The cells were still columnar and showed nuclear polarization. Their mucin content, however, was variable and the nuclei were hyperchromatic, elongated, crowded and appeared stratified. The nucleocytoplasmic ratio was increased compared to mild dysplasia, but most nucleoli were small and inconspicuous. Mitoses were present. In severe dysplasia – carcinoma in situ, the papillae were crowded and showed irregular branching and budding. In addition, some of them showed intraluminal bridging (cribriform pattern), without support by fibrovascular tissue stalks. The cells contained no or scarce mucin and distinctly enlarged and irregularly shaped nuclei, with a conspicuous nucleolus. Nuclear stratification was striking and many nuclei approached the cell surface. Mitoses were frequent.

All immunohistochemical examinations were performed with the avidin-biotin-peroxidase technique (13) using the monoclonal antibody (mAb) DU-PAN-2 specific for an antigen normally found in pancreatic and biliary ducts epithelium (19) and antibodies against several lineage specific gastrointestinal antigens (44). The latter include the aspartic proteinases cathepsin E (15) and pepsino-

Table 1 Clinicopathological data of 26 intraductal papillary-mucinous neoplasms of the pancreas (*IPMA*, intraductal papillary-mucinous adenoma; *IPMB*, intraductal papillary-mucinous borderline tumour; *IPMC*, intraductal papillary-mucinous carcinoma; *PMCM*, papillary-mucinous carcinoma with mucinous invasion pattern; *PMCD*, papillary-mucinous carcinoma with ductal invasion pattern; *LN*, regional lymph node involvement; *dead p.o.*, dead in the postoperative period; *l.f.*, later lost to follow-up)

Pt. no.	Diagnosis	Age	Sex	Site	Length of involved duct	Follow up (months)
1	IPMB	47	F	Body-tail	6 cm	alive 63
2	PMCM	75	F	Body-tail	10 cm	alive 28
3	IPMB	49	M	Head	4.5 cm	alive 60
4	PMCM	42	M	Head-body+LN	10 cm	dead 12
5	PMCM	65	M	Head-body	7 cm	alive 28
6	IPMC	50	M	Head-ampulla	6 cm	alive 120
7	PMCM	43	M	Head-ampulla	8 cm	dead 28
8	IPMC	69	F	Head	2 cm	alive 58
9	IPMA	78	M	Body-tail	1 cm	alive 38
10	IPMC	77	F	Head	5 cm	alive 18
11	IPMC	57	M	Head	4 cm	alive 18
12	IPMA	79	M	Tail	4 cm	alive 18
13	IPMB	72	F	Head-body+ampulla	7 cm	dead p.o.
14	IPMC	66	F	Head-body	8 cm	dead p.o.
15	IPMB	58	F	Head	4.5 cm	alive 37
16	IPMC	70	F	Head	8 cm	alive 120 l.f.
17	IPMB	52	M	Head	6 cm	alive 84 l.f.
18	IPMB	33	M	Head	3 cm	alive 52
19	IPMB	47	M	Head	4 cm	alive 66
20	IPMC	74	F	Head-body	4 cm	alive 23
21	IPMC	63	M	Head	1 cm	alive 19
22	IPMA	40	F	Body-tail	1.5 cm	alive 31
23	PMCM	77	M	Head+LN	5 cm	alive 13
24	IPMB	63	M	Head	2.5 cm	alive 3
25	IPMA	45	M	Head	3 cm	alive 11
26	PMCD	62	M	Head-ampulla	1.5 cm	alive 2

gens I and II, which were detected with rabbit antisera specific for each antigen (39); M1, an antigen associated with the peptide core of gastric superficial-foveolar mucin cells, using a pool of four monoclonal antibodies (2) applied to sections pretreated for 15 min with 0.004% proteinase XXIV (Sigma, St. Louis, Mo.); M3SI, a mucin antigen expressed in small intestinal goblet cells, which was detected with mAb 64 or 168 (26) and CAR5, a mucin like antigen expressed mainly in colorectal epithelium and reacting with mAb BD5 (34). For p53 protein immunohistochemistry mAb D07 directed against the 1–45 sequence of the protein (50) was used on paraffin sections pretreated with 10 mM citrate buffer pH 6.0, 2×5 min in a microwave oven. For c-*erb*B-2 oncogene product immunostaining a mAb directed against the C-terminus 1238–1255 sequence (Cambridge Res. Biochem, U.K.) was applied.

Control incubations included antisera and mAbs adsorbed with related and unrelated antigens, their substitution with nonimmune or unrelated immune sera, supernatants or ascitic fluid and their dilution in 5% pooled human serum to block non-specific immunoglobulin binding sites.

For k-*ras* and p53 gene analysis.5 μ sections of formalin-fixed, paraffin-embedded tumour tissue were obtained from all cases. Frozen tissue was available from cases 2, 3, 4 and 5. Tumour and non-tumour control tissues were selected in adjacent sections stained with haematoxylin and eosin. Only those sections where tumour cells accounted for 30 to 80% of the detected tissue were used. For DNA extraction the samples were incubated overnight at 58° C in 200 μl of extraction buffer (50 mM potassium chloride–10 mM TRIS-HCl, pH 7.5–2.5 mM magnesium chloride–0.1 mg/ml gelatin–0.45% Nonidet P-40–0.45% Tween-20–500 μg/ml proteinase K). The solution was heated at 95° C for 15 min to inactivate the proteinase K and centrifuged. Five to 10 μl of the supernatant were directly used for polymerase chain reaction (PCR) amplification.

The sequences of the primers utilised for K-*ras* and p53 gene amplification are those previously reported (32, 33, 36, 43). For denaturating gradient gel electrophoresis (DGGE) analysis one member of each primer pair contained a 5′ 40 bp GC rich sequence (GC-clamp). GC-clamped primers were used to obtain amplified sequences suitable for DGGE analysis of K-*ras* first exon and p53 exons 5 (from codon 148 to its 3′ end), 6, 7 and 8 (32, 36). PCR-amplified fragments corresponding to K-*ras* exons 1 and 2 obtained from frozen tissue were analysed by the single-strand conformation polymorphism (SSCP) method as previously described (43).

Genomic DNAs from samples showing variant DGGE or SSCP patterns were amplified using the appropriate primers in a total volume of 100 μl. The amplification product was purified on a 2% low-melting agarose gel. The specific band was excised from the gel, frozen in liquid nitrogen for a few minutes and incubated for 15 min at 37° C. This step was repeated two-three times to recover all DNA. The agarose fragment was centrifuged for 10 min, the surnatant was quantified and directly utilised for sequencing by the dideoxy procedure using the Circum Vent (exo) Kit (Biolabs, UK) for cycling sequencing. Following the manufacturer's instructions, 100 ng of the template DNA were mixed with 1,2 pmol of the primer, 2 units of Vent (exo) polymerase, 2 μl of ^{35}S-dATP in 16 μl of buffer: 10 mM potassium chloride, 10 mM ammonium sulphate, 20 mM TRIS-HCl (pH 8.8), 5 mM magnesium sulphate and 0,2% Triton X-100. Twenty cycles of amplification were performed in a Programmable Thermal Cycler (MJ Research, Inc.) each cycle consisting of 20 sec at 95° C, 20 sec at the annealing temperature specific for the primer utilised and 20 sec at 70° C. PCR reactions were electrophoresed on 7% polyacrylamide-7M urea gels for 2–3 h (1800 V). The gels were then washed for 15 min in 10% methanol and 10% acetic acid, dried and exposed to B-MAX films (Amersham).

Results

The majority (81%) of the tumours occurred in the head of the pancreas or in the head and body; only 5 cases were found in the tail or body and tail. Four tumours (cases 6, 7, 13 and 26) involved the ampulla. On cut surface all pancreatic specimens showed a dilated pancreatic duct with diameters ranging from 1 to 8 cm. In 11/26

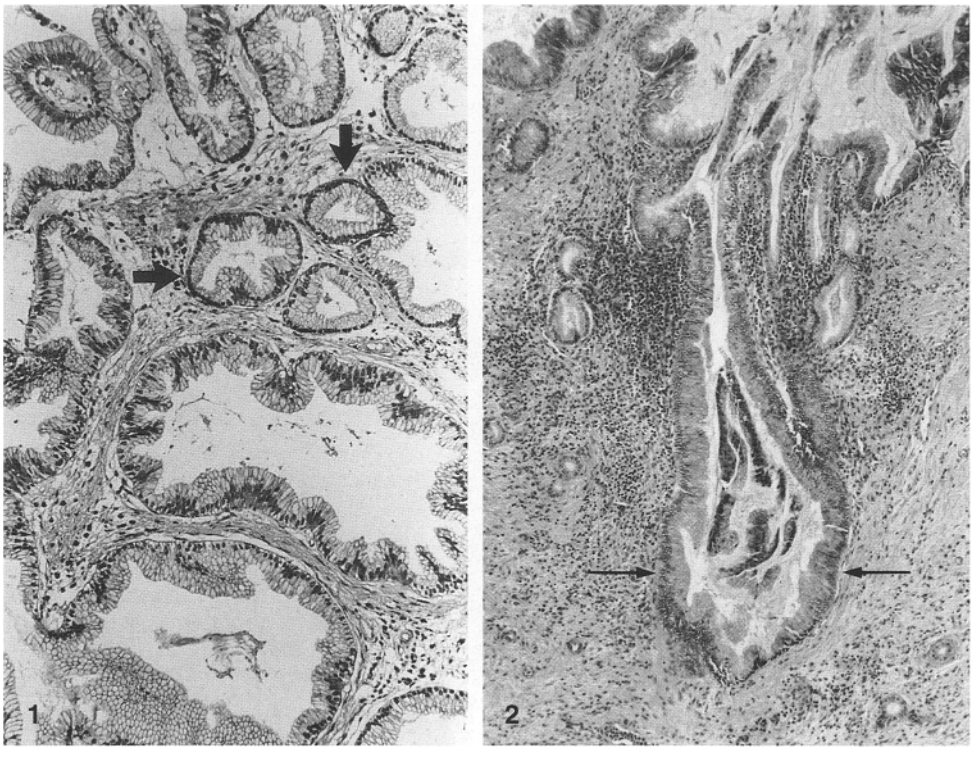

Fig. 1 Intraductal papillary mucinous adenoma. Part of a large papillary proliferation with transversly cut foldings and some stromal glands (*arrows*). The lining epithelium shows only mild dysplasia. H&E, ×60

Fig. 2 Intraductal papillary mucinous tumour with mild to moderate dysplasia (borderline tumour): Extension into secondary duct (*arrows*). H&E, ×60

cases the ectatic duct contained grossly visible or nodular tumours, the length of which ranged between 1,5 and 10 cm. In the remaining cases the main change was an extremely dilated main pancreatic duct (diameter: 1–8 cm) filled with sticky mucin. Often some secondary ducts were also involved and showed cystic dilations. In 5 cases the intraductal tumour was associated with a grossly invasive component. In case 4, the invasive component involved the whole pancreatic head, the duodenal wall, peripancreatic soft tissue and peripancreatic lymph nodes; in case 23, the pancreatic parenchyma and 9 peripancreatic lymph nodes; in case 5, the parenchyma surrounding Wirsung's duct and, through a fistula, the extrapancreatic bile duct; and in cases 2 and 26, the periductal parenchyma only (Table 1).

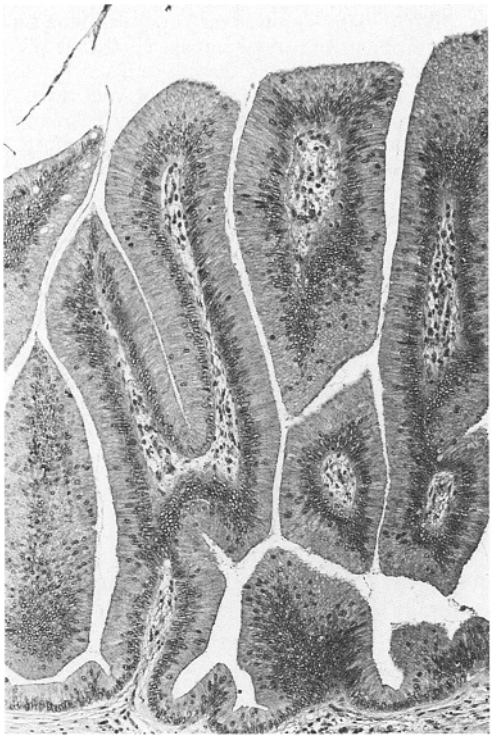

Fig. 3 Intraductal papillary mucinous tumour with moderately dysplastic epithelium (borderline tumour). H&E, ×120

Three tumours located in the body-tail and one in the head revealed the features of intraductal papillary-mucinous adenoma. In three cases, the tumour resided in the main pancreatic duct; in one case it involved also the adjacent ducts. The tumour tissue was composed of short and occasionally large papillary foldings supported by small fibrovascular stalks. In addition, the stroma of some large papillae contained glandular structures (Fig. 1). The papillae were formed by columnar epithelial cells showing only mild dysplasia in all areas examined.

The remaining 22 intraductal tumours revealed papillary proliferations with moderate dysplasia and/or severe dysplasia-carcinoma in situ-changes. The papillary proliferations were found in the main duct, but usually extended into the secondary ducts (Fig. 2). The dysplastic changes were only mild to moderate in 9 tumours (Fig. 3). In 13 there were, in addition to moderate dysplasia, foci of severe dysplasia-carcinoma in situ changes. These cases included the 5 macroscopically invasive tumours as well as tumour 7, in which multifocal microinvasion was detected. In two non-invasive IPMT (cases 14 and 21) the entire neoplastic tissue displayed severely dysplastic papillary proliferations forming cribriform structures (Fig. 4). Such a pattern was also seen as focal

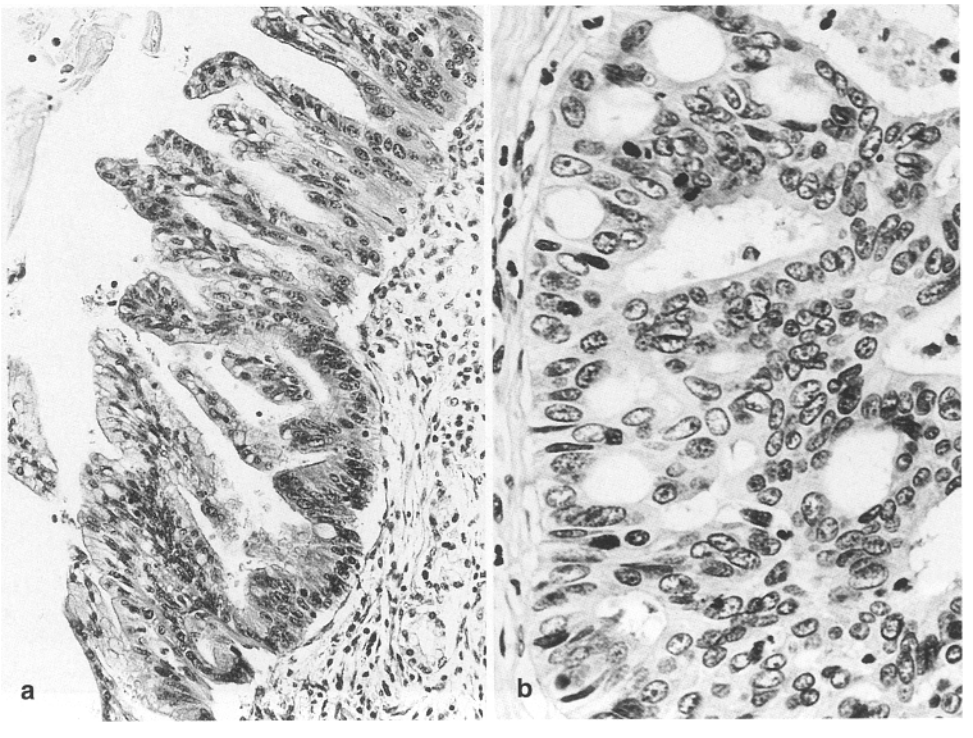

Fig. 4 Intraductal papillary mucinous carcinoma showing (**a**) severe dysplasia and (**b**) carcinoma in situ changes. H&E, ×120 and 250

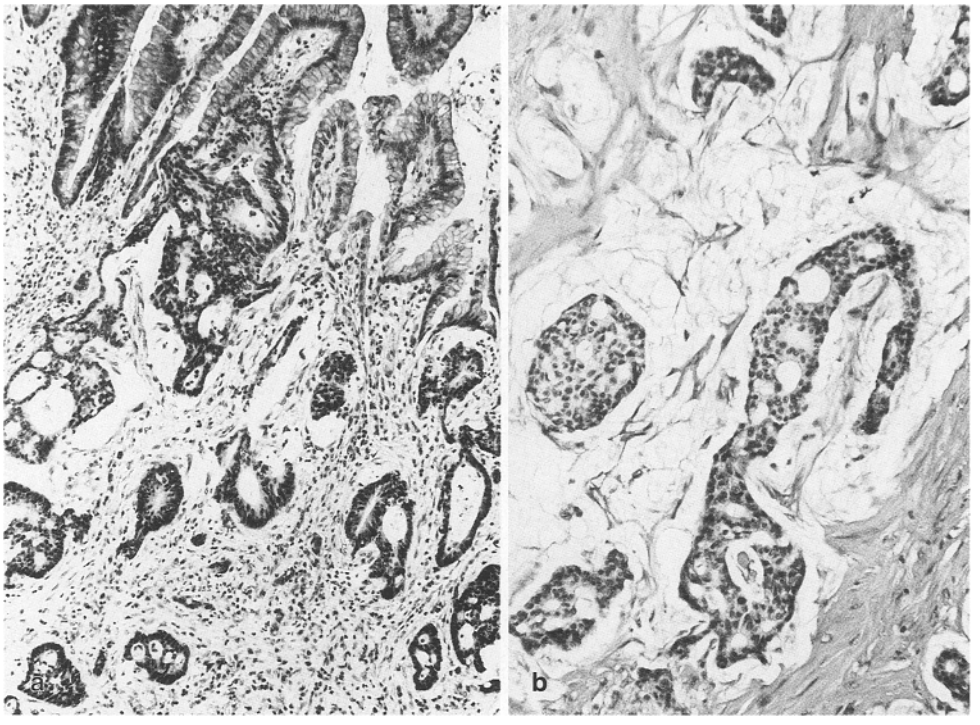

Fig. 5 Papillary mucinous carcinoma (**a**). Invasive component shows the features of a mucinous noncystic carcinoma (**b**). H&E, ×120

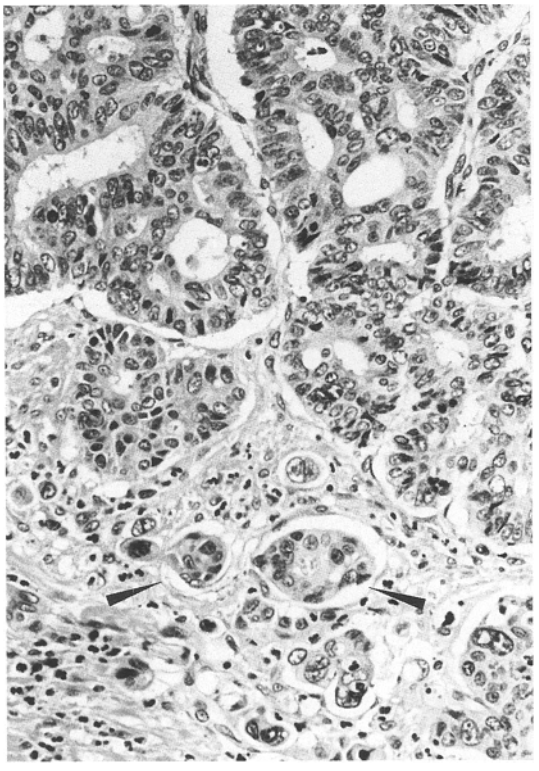

Fig. 6 Papillary mucinous carcinoma with features of ductal adenocarcinoma and lymphatic invasion (*arrow*). H&E, ×120

change in the intraductal component in one of the invasive tumours (case 26).

Among the six invasive carcinomas two distinct patterns of invasive growth were identified. In five, the invasive component showed the features of mucinous noncystic adenocarcinoma; that is to say, mucin lakes containing freely floating neoplastic glands formed by moderately atypical adenocarcinoma cells (mucinous invasion pattern or "muconodular" pattern according to Yamada et al. (52) (Fig. 5). In one tumour, the invasive component formed tubular and glandular structures, thus mimicking the features of a usual ductal adenocarcinoma (ductal invasion pattern or "tubular" pattern according to Yamada et al. (52). The tumour cells of these invasive structures exhibited considerable atypia. In addition, there was prominent lymphatic invasion (Fig. 6).

The 4 adenomas showed prominent PAS and PACONA positivity with variable alcian blue staining marked in case 12, less marked in cases 9, 22 and 25, and diffuse immunoreactivity for gastric-type markers like M1, cathepsin E and, in particular, PGII (Fig. 7). There was no reaction for PGI or intestinal and pancreatic markers (Table 2).

All the 22 papillary tumours with moderate and/or severe dysplasia-carcinoma in situ changes showed extracellular and/or intracellular mucin. Abundant extracellular mucin was found in 17 tumours. The mucins were either sulphomucins stained with both HID and alcian blue, like most normal pancreatic duct and colorectal mucins, or sialomucin reactive with alcian blue only, like small intestine mucin (8, 9, 44). Six of the 9 borderline tumours showed a mixed pattern of markers, usually with prevalence of superficial-foveolar over pyloric gland markers; two had an essentially "gastric" pattern and one remained poorly reactive (Table 2).

Nine of the 13 tumours with severe dysplasia, including both the intraductal and extraductal components of the 5 invasive tumours with a mucinous invasion pattern, showed strong expression of intestinal markers with poor

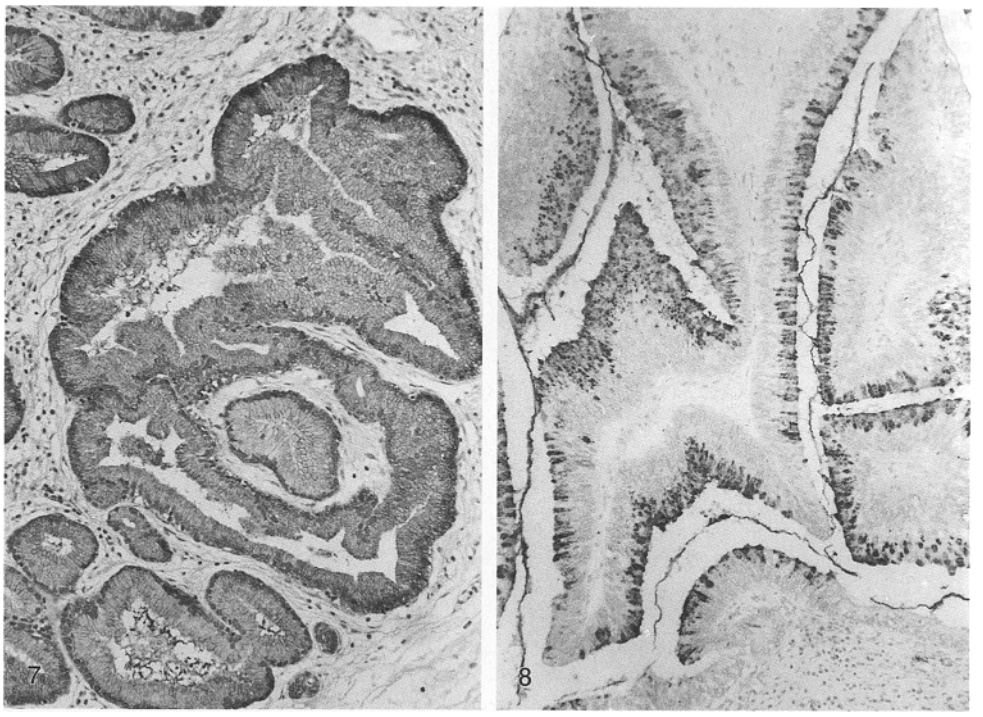

Fig. 7 Intraductal papillary mucinous adenoma with intense immunostaining for PGII. ×60

Fig. 8 Intraductal papillary mucinous tumour with intense immunostaining for the M3 antigen. ×60

Table 2 Histochemical and immunohistochemical findings in IPMT (% of cells immunoreactive for the following antigens: *DUPAN-2*, a glycoprotein expressed by pancreatobiliary ducts; *M1*, the protein core of the gastric superficial-foveolar cell mucin; *CaE*, cathepsin E, an enzyme of gastric superficial-foveolar cells and enterocytes of proximal villi; *PGII*, pepsinogen II, an enzyme of gastric oxyntic, pyloric and cardial glands as well as Brunner glands; *CAR5*, the protein core of a glycoprotein expressed by all colorectal cells and by a few immature enterocytes of deep crypts in the ileum; *M3SI*, mucin of small intestine goblet cells; – negative; 1<5%; 2≥5%; 3≥20%; 4≥50%; 5≥75% of reactive cells)

Pt no.	Diagnosis	Markers						Mucin tests			
		Pancreatic	Gastric			Intestinal		HID	Alcian Blue	PAS	PACONA
		DUPAN-2	M1	CaE	PGII	CAR5	M3SI				
1	IPMB	5	10	80	–	5	–	3	3	2	2
2	PMCM	5	60	20	20	–	30	1	5	2	–
3	IPMB	–	50	–	–	70	–	3	4	2	1
4	PMCM	–	–	5	–	–	30	1	5	–	–
5	PMCM	–	–	–	–	5	40	–	2	1	1
6	IPMC	–	5	–	–	–	80	3	4	2	–
7	PMCM	–	5	10	–	10	80	3	4	2	1
8	IPMC	–	20	10	–	40	40	3	4	1	–
9	IPMA	–	80	30	90	–	–	2	2	5	4
10	IPMC	–	5	–	–	–	5	2	2	2	–
11	IPMC	–	10	–	–	–	10	1	2	–	–
12	IPMA	–	40	20	60	–	–	2	4	3	3
13	IPMB	10	10	10	50	5	–	2	2	2	–
14	IPMC	–	–	10	40	–	–	1	2	2	2
15	IPMB	–	80	20	–	30	40	4	3	1	–
16	IPMC	–	–	20	–	20	70	4	3	2	2
17	IPMB	–	30	50	20	–	70	4	3	2	1
18	IPMB	–	70	70	–	25	20	3	3	2	–
19	IPMB	5	80	20	–	15	80	3	2	2	1
20	IPMC	–	60	70	–	10	40	2	3	–	–
21	IPMC	10		10	–	–	–	1	1	–	–
22	IPMA	–	50	70	60	5	–	1	2	4	3
23	PMCM	5	5	5	–	20	80	4	5	1	1
24	IPMB	–	70	40	–	10	80	4	5	1	1
25	IPMA	–	60	80	60	–	–	–	2	3	3
26	PMCD	80	20	90	80	10	–	3	4	2	2

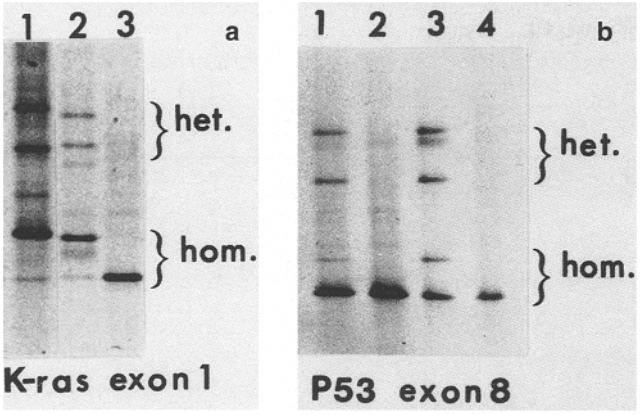

Fig. 9 Negative image of ethidium bromide-stained denaturating gradient gels. (**a**) Tumour DNAs were amplified with 5′ and 3′-GC ampliprimers specific for the first exon of the K-*ras* gene, and the PCR products were loaded onto a 45–75% gradient of denaturants. Sample 1: case 14, GGC→GAC mutation at codon 13 (Gly→Asp). Sample 2: case 16, GCT→GAT mutation at codon 12 (Gly→Asp). Sample 3: normal sequence. (**b**) Tumour DNAs were amplified with ampliprimers specific for exon 8 of the p53 gene, and the PCR products were loaded onto a 50–75% gradient of denaturants. Samples 1 and 3: case 20, CGT→CAT mutation at codon 273 (Arg→His). Samples 2 and 4: normal sequence. het., heteroduplexes, hom., homoduplexes.

or no expression of the pancreatic duct marker DU-PAN-2 and with either poor (6 "intestinal" cases) or abundant (3 "mixed" gastrointestinal cases) expression of the gastric type markers M1, cathepsin E and pepsinogen II. The tumour with ductal type invasion was characterized by an extensive immunoreactivity for both the pancreatic duct marker DU-PAN-2 and gastric type markers, usually by the same "hybrid" tumour cells, a common finding in ductal adenocarcinoma (44).

Excluding the adenomas and the carcinoma with ductal invasion type, which displayed dinstinctive morphological and histochemical patterns, the remaining 21 IPMT showed M1 antigen in 76% of cases, cathepsin E in 76%, pepsinogen II in 19%, CAR-5 antigen in 62%, M3 antigen in 76% (Fig. 8) and DUPAN-2 in 29% (in 5 to 10% of tumour cells only) of cases. Gastric type markers were commonly expressed in adenomas and borderline tumours, intestinal type or hybrid pancreatic-gastric type markers in carcinomas (Table 2).

K-*ras* mutations were found in 31% of IPMT (Fig. 9 and Table 3); seven were detected by the DGGE procedure applied to paraffin sections and in one tumour (case 3) by the SSCP technique on fresh tissue. Mutations occurred in 50% of the adenomas, 11% of the borderline tumours and 38% of the carcinomas.

The p53 tumour suppressor gene was found to be mutated in exon 8 (codon 273:CGT→CAT) in case 20 and in exon 5 (codon 175:CGC→CAC) in case 21 (Fig. 9 and Table 3). Both tumours showed severe dysplasia-carcinoma in situ changes. Nuclear p53 protein immunoreactivity was detected in 31% of IPMT, including the two neoplasms with p53 mutations. In 3 tumours without mutations only a few (2 to 5%) tumour cells were p53 positive, while in the remaining tumours immunoreactivity was found in up to 15% of cells. p53 positive tumours included 3/6 invasive carcinomas, 3/7 intraductal carcinomas and 2/9 borderline tumours. Positive cells usually showed the highest grade of atypia within a given tumour.

Sixty-five percent of IPMT were positive for c-*erb*B-2, usually in the cytoplasm of the cells (Fig. 10 and Table 3). There was no clear correlation with differentiation and marker expression. Five of 6 tumours (including both p53 mutated carcinomas) with p53 positivity of more than 2% of cells failed to show c-*erb*B-2 staining.

Clinical history and follow-up

Most patients had a long preoperative history of symptoms (1 to 18 years; mean 6 years) usually mimicking chronic pancreatitis. All patients underwent surgical treatment, which was Whipple resection for tumours involving the pancreas head or head-body and distal pancreatectomy for tumours in the body tail. Twenty-one of the 24 patients followed after surgery for a mean of 40 months were alive and apparently free of tumour, including one patient with an intraductal carcinoma (case 21) and three patients with invasive carcinomas (cases 2, 23 and 26). The fourth patient with invasive carcinoma (case 5), had recurrence one year after surgery showing intraperitoneal carcinomatosis, which was successfully treated by intraperitoneal cisplatin administration. Only two patients, both with invasive carcinoma (cases 4 and 7), died of disseminated intra-abdominal tumour disease 12 and 28 months, after operation respectively (Table 1).

Discussion

Intraductal papillary-mucinous tumours (IPMT) are slowly growing pancreatic neoplasms which usually have a favorable prognosis after adequate resection (10, 17, 22, 24, 27, 29, 37, 38, 47, 52). Our study of 26 IPMT confirms the relatively indolent nature of this neoplastic disease. From 21 patients who were available to follow-up over 12 to 120 months only two died of the tumour and one had tumour recurrence while the remaining patients were tumour free during a mean of 47 months. Despite this apparent biological uniformity, the 26 IPMT of our study revealed considerable heterogeneity regarding the degree of epithelial dysplasia present, the type of gastro-enteropancreatic marker produced and the genetic changes found.

Using well defined criteria for dysplasia we classified the 26 IPMT into four adenomas (15%), nine borderline tumours (35%) and thirteen intraductal carcinomas (50%), including six with invasive carcinoma. The adenomas showed only mild dysplasia and intense reactivity

Table 3 Relationship between grade of dysplasia and K-*ras*, p53 and c-*erb*B-2 abnormalities in IPMT (Grade of dysplasia in IPMT: *mild*; *mod*, moderate; *sev*, severe; % of reactive cells)

Pt. no.	Diagnosis	Dysplasia	K-*ras* mutated codon	aminoacid substitution	p53 mutated codon	aminoacid substitution	p53 staining	c-*erb*B-2
1	IPMB	mod	–		–		2	40
2	PMCM	mod/sev	–		–		2	30
3	IPMB	mild/mod	12	gly→asp	–[a]		–	–
4	PMCM	mod/sev	–		–		–	40
5	PMCM	mod/sev	–		–		–	20
6	IPMC	mod/sev	–		–[a]		–	20
7	PMCM	mod/sev	12	gly>asp	–[a]		–	60
8	IPMC	mod/sev	–		–		–	20
9	IPMA	mild	12	gly>val	–		–	50
10	IPMC	mod/sev	12	gly>val	–		–	–
11	IPMC	mod	–		–		–	–
12	IPMA	mild	–		–[a]		–	–
13	IPMB	mild/mod	–		–		–	50
14	IPMC	sev	13	gly>asp	–		10	60
15	IPMB	mild/mod	–		–		–	5
16	IPMC	mod/sev	12	gly>asp	–		–	80
17	IPMB	mild/mod	–		–		–	75
18	IPMB	mild/mod	–		–		–	15
19	IPMB	mild/mod	–		–		–	70
20	IPMC	mod/sev	–		273	arg>his	15	–
21	IPMC	sev	–		175	arg>his	10	–
22	IPMA	mild	12	gly>asp	–		–	10
23	PMCM	mod/sev	–		–		10	–
24	IPMB	mild/mod	–		–		5	–
25	IPMA	mild	–		–		–	40
26	PMCD	mod/sev	12	gly>val	–		10	–

[a] Exon 6 not done

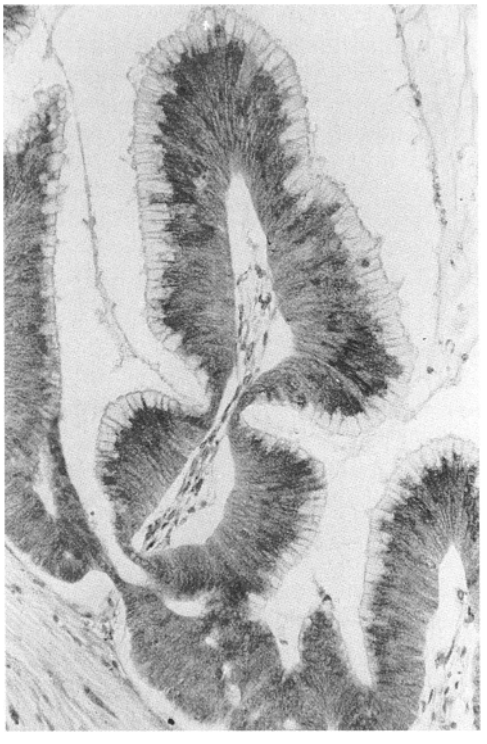

Fig. 10 Intraductal papillary-mucinous tumour of borderline malignant potential showing intense c-*erb*B-2 immunostaining

for neutral mucins as well as specific markers of gastric foveolar (M1 antigen, cathepsin E) or pyloric gland epithelium (pepsinogen II and PACONA) in the absence of any reactivity for the pancreatic duct marker DU-PAN-2, the intestinal markers CAR-5 and M3SI, or the gastric marker pepsinogen I. These pyloric type features resembled those of pyloric type metaplastic and hyperplastic lesions frequently occurring in main and interlobular pancreatic ducts (44). Interestingly, these tumours also seem to differ from most of the remaining 22 cases in being smaller (mean diameter: 2.4 vs 5.4 cm), and occurring preferentially in the tail of the pancreas. All the 4 patients are alive and well with a mean follow-up of 25 months after surgery. Judged by the illustrations and descriptions Obara et al. (28) reported similar tumours.

Mild to moderate dysplasia in the absence of severe dysplasia-carcinoma in situ changes characterized the nine borderline tumours. Their marker pattern resembled that of the adenomas, exhibiting a prominent gastric type differentiation. We considered these neoplasms to be borderline tumours because their epithelial dysplasia suggests a potential evolution into overt malignancies. Biologically, the borderline tumours revealed benign behaviour, as all 8 patients who could be followed after surgery were alive and well after a mean follow-up of 4 years.

Severe dysplasia-carcinoma in situ changes characterized the seven intraductal papillary-mucinous carcinomas, regardless whether these changes were only focally

present (six tumours) or diffusely developed (two tumours). Their mucin pattern was dominated by the expression of the M3SI antigen, a marker of small intestine goblet cell mucin (26), which was associated with a substantial lack of expression of DU-PAN-2, a pancreatic duct marker. Despite clear signs of cellular malignancy, all eight patients are found alive and well after a mean follow-up of 68 months. Nevertheless, the diagnosis of intraductal papillary carcinoma should be maintained in these patients, since the chance of missing small invasive tumour foci in the relatively large tumours and their presently unpredictable risk of progression into invasive disease has to be considered.

Invasive carcinoma on the basis of an IPMT with severe dysplasia-carcinoma in situ changes was found in six patients. In five of these neoplasms the invasive component fullfilled the criteria of a mucinous noncystic carcinoma (18). Yamada et al. (52) who reported similar cases described this pattern of invasion as "muconodular". These lesions expressed the marker M3SI, but were unreactive with DU-PAN-2. This suggests that invasive IPMT with a mucinous invasion pattern and the intraductal carcinomas displaying the same marker pattern are closely related neoplasms.

In only one of the six invasive carcinomas the invasive growth resembled that of a ductal adenocarcinoma. A distinctive feature of the tumour's intraductal as well as extraductal component was a strong co-expression of both pancreatic duct and gastric markers by the same cells. In a previous investigation this hybrid gastropancreatic phenotype was found to be a characteristic finding in ductal adenocarcinomas (44). This indicates that IPMT with ductal invasion pattern derive from another cell lineage than those showing a mucinous invasion pattern. Whether these two tumour types also differ in prognosis, is not clear. Yamada et al. (52) who observed six tumours with a muconodular invasion pattern and three tumours with a ductal invasion pattern did not report any follow-up. Milchgrub et al. (22) described three IPMT, two with a ductal invasion pattern and one with a mucinous pattern. Of the two patients with a ductal invasion pattern one was alive 6 months after surgery, while the other had died 10 months after tumour resection. The patient with a mucinous invasion pattern was reported to be dead 6 months after surgery. In our five cases with mucinous-type invasion, two died of the tumour after 12 and 28 months, respectively, while the other patients are alive with a mean follow-up of 23 months. The patient with the ductal invasion pattern has so far only a follow-up of two months and cannot be considered. Future studies have therefore to clarify whether IPMT with a mucinous invasion pattern have a prognostic advantage over those with a ductal invasion pattern. This question is of interest because IPMT with a mucinous invasion pattern appear to be more frequent than those with a ductal invasion pattern.

From the presented histological, histochemical and immunohistochemical data it is obvious that IPMT differ rather sharply from the usual ductal cancers (11, 22, 44). Lack of DU-PAN-2 antigen, reduced expression of pepsinogen II and increased expression of M3SI antigen are among the most prominent differences. Thus, with the possible exception of the single tumour showing a ductal invasion pattern, the IPMT of our series are unlikely to represent a precursor stage of ductal adenocarcinoma, as suggested by Mizumoto et al. (23). IPMT rather seem to be related to mucinous noncystic carcinomas, which have also been described under the term "colloid carcinoma" (6, 17).

In only four of the IPMT (15%) the papillary growth was not associated with mucin hypersecretion. It seems therefore that the "ductectatic mucin hypersecreting" IPMT variant which often present with mucin extrusion through the ampulla of Vater (4, 37, 52, 53) is more common than IPMT variant showing a papillary tumour without significant mucin production (24, 51).

IPMT are similar to mucinous cystic tumours of the pancreas regarding histological and histochemical patterns as well as prognosis (52). However, the preferential occurrence of mucinous cystic tumours in the distal rather than the proximal pancreas, the high preponderance of female patients as well as the distinctive gross morphology clearly separate these tumours from IPMT (5, 37, 44, 52). Considering the histological and histochemical similarities of many IPMT (especially the malignant ones) with well differentiated papillary-villous colorectal, ampullary and biliary tract tumours (31), and the reported cases with simultaneous involvement of biliary tract, ampulla and pancreas by papillary tumours (21, 24, 25), it seems possible that some relationship may exist between these groups.

The 31% incidence of K-*ras* mutations in our IPMT, which increases to 44% if we add the 19 positive tumours of the total of 36 cases reported by Lemoine et al. (20), Satoh et al. (41), Tada et al. (49) and Yanagisawa et al. (55), is distinctly lower than the 70–90% incidence seen in ductal adenocarcinomas of the pancreas (1, 32, 33, 46). Also, the 8% incidence of p 53 mutations and the 19% incidence of p 53 protein expression fails to reach the 44–50% incidence of p 53 mutations (3, 33, 42) or the approximately 60% of p 53 immunostaining of ductal adenocarcinomas (3, 16, 42). This supports the interpretation that in most IPMT the pattern of genetic changes differs from ductal cancer (20). In line with this assumption is the common overexpression of the c-*erb*B-2 oncogene product, which was found in 76% of the IPMT investigated by Satoh et al (40) and in 65% of our IPMT. These data contrast with the uncommon reactivity of most pancreatic ductal adenocarcinomas which showed immunostaining in only 7 to 22% (12, 40, 45). In a recent paper on 76 (ductal?) pancreatic cancers, 45% were found to express c-*erb*B-2 (HER2/*neu*) protein (54).

The frequency in K-*ras* mutations of our IPMT did not increase with the degree of dysplasia or the presence of invasion. K-*ras* mutations were not only found in malignant IPMT but also in two of our four adenomas. This finding confirms the observation by Tada et al. (49) that

in IPMT K-*ras* mutations may occur independently from severe dysplasia or frank tumour invasion and thus represent a very early event in tumorigenesis. Recently, K-*ras* mutations were even found in ductal papillary hyperplasia of chronic pancreatitis (56). This finding, however, remains to be established, as other studies were not able to confirm it (20, 48).

In contrast to K-*ras*, the two p53 mutated tumours as well as 6 of the 8 cases with nuclear p53 staining were found among the IPMT classified as carcinomas. In addition, usually only the most anaplastic tumour cells were p53 positive. These findings suggest that, in some IPMT a relationship may exist, as already discussed for other tumours (35, 43), between histological differentiation and p53 gene mutation and/or nuclear p53 staining.

It is concluded that IPMT, although generally sharing a favorable prognosis, are histologically and phenotypically heterogeneous lesions. We propose to classify these tumours according to the highest grade of dysplasia encountered in the neoplasms as intraductal papillary-mucinous adenomas, intraductal papillary-mucinous tumours of borderline malignant potential and intraductal papillary-mucinous carcinomas. If they present with an invasive component they may be called papillary-mucinous carcinomas. Future studies have to determine whether this separation is valid and may be of biological significance. There is however, no doubt that most IPMT are phenotypically, genetically and behaviourally distinct from usual ductal adenocarcinomas.

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