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Primary retroperitoneal mucinous cystoadenocarcinomas: an immunohistochemical and molecular study

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Abstract Special immunohistochemical stains for the identification of gastroenteropancreatic antigens in two cases of primary retroperitoneal mucinous cystoadenocarcinomas (PRMC) show that these tumours have patterns similar to ovarian mucinous tumours. Markers of pyloric type gastric mucosa differentiation (M1, cathepsin E, concavavalin A, pepsinogen II) are mostly positive in benign and borderline areas with endocervical type differentiation, while immunoreactivity for intestinal cell markers (M3SI and CAR–5) and for DU–PAN–2 is present mainly in frankly malignant areas, regardless of differentiation type. DNA analysis shows a point mutation of K-*ras* oncogene at codon 12 (GGT to CGT) in one case. The immunohistochemical and genotypic similarity of PRMC and ovarian mucinous tumours may indicate similar mechanisms in their histogenesis.

Key words Primary retroperitoneal mucinous cystoadenocarcinoma · Immunohistochemistry K-*ras* mutation

Introduction

Mucinous epithelial tumours (benign, borderline or malignant) similar to those occurring in the ovary have also been reported as primary retroperitoneal neoplasms. In this paper we analyse two cases of primary retroperitoneal mucinous cystoadenocarcinoma (PRMC) for possible immunohistochemical and genotypic similarities with their ovarian counterparts.

Mucinous epithelial tumours of the ovary have been the subject of several papers in the literature concerning their mucin secretion profile or the expression of gastroenteropancreatic antigens (Tenti et al. 1992). Molecular studies have shown the frequent occurrence of oncogene abnormalities, particularly of the K-*ras* gene, a feature that ovarian mucinous adenocarcinomas share with morphologically similar pancreatobiliary and colorectal tumours (Enomoto et al. 1991).

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Materials and methods

Case 1 was a 46-year-old woman. The lesion was a well-defined, multilocular cystic mass, 20 × 12 cm, located under the peritoneal serosa of the right iliac fossa, between the adnexae and the lower pole of the kidney.

Case 2 was a 45-year-old woman. The lesion was a unilocular cystic mass, 20 cm in maximum diameter, located under the retroperitoneal serosa under the lower pole of the left kidney.

Histologically, according to Hart and Norris's 1973 criteria for ovarian mucinous neoplasms, the tumours were classified as moderately and well-differentiated mucinous adenocarcinomas of mixed endocervical/intestinal type, respectively. Areas of benign and borderline mucinous epithelium were also present. In both tumours limited infiltration of the wall was detected focally, but whole thickness penetration was lacking. Exhaustive sampling did not reveal the presence of ovarian tissue.

Extensive work-up of both patients, after removal of the tumour, failed to show any residual abdominal disease. The ovaries, fallopian tubes and uterus were free from tumour. The patients are disease free 27 and 13 months after surgery respectively.

Based on clinical and pathological findings we consider both cases to be examples of primary extraovarian, retroperitoneal mucinous cystoadenocarcinoma. Our investigation was focused on the detection of expression of gastroenteropancreatic antigens, paralleling our previous study on mucinous ovarian tumours (Tenti et al. 1992). Immunohistochemistry was carried out on formalin-fixed, paraffin-embedded tissue, using the avidin-biotin peroxidase technique and monoclonal or polyclonal antibodies. The following antigens were investigated: M1 antigen, which is associated with the mucin peptic core of gastric superficial/foveolar cells (Bara et al. 1986); cathepsin E (CaE), an aspartic proteinase expressed by gastric superficial/foveolar cells (Samloff et al. 1987); pepsinogen I (PGI) and pepsinogen II (PGII), secretory products of gastric peptic and mucopeptic cells (Samloff 1982); M3SI, a mucin antigen expressed mainly in small intestinal goblet cells (Nardelli et al. 1983); CAR–5, a mucin-like antigen expressed mainly in colorectal epithelium and immature crypt cells of distal

Table 1 Type, dilution and source of the antibodies (*MAb* monoclonal antibody, *PAb* polyclonal antibody)

Antibodies	Dilution	Source
MAbs 1–13M1, 2–11M1, 2–12M1, 9–13M1	1:32000	J. Bara, Villejuif, France
PAb anti-cathepsin E	1:4000	I.M. Samloff, Sepulveda, Calif., USA
PAb anti-pepsinogen I	1:1000	Sorin Biomedica, Saluggia, Italy
PAb R–248 anti-pepsinogen II	1:2000	I.M. Samloff
PAb DU–PAN–2	1:2	Biogenex, Calif., USA
MAb 168 M3SI	1:16000	J. Bara
MAb BD–5 anti–CAR–5	1:200	M. Prat, Turin, Italy
Carcioembryonic antigen	1:2	DAKO, Calif., USA

small intestine and gastric intestinal metaplasia (Fiocca et al. 1988); DU–PAN–2 antigen, which is normally found in biliary tract epithelium and pancreatic ducts (Borowitz et al. 1984); carcinoembryonic antigen (CEA), a non-specific tumour-associated antigen most strongly expressed by gastrointestinal tumours (Borders et al. 1973).

The selected panel of immunohistochemical staining allows the identification of gastric pyloric-type differentiation (M1, CaE, PGI and PGII), intestinal differentiation (M3SI, CAR–5) and pancreatobiliary differentiation (DU–PAN–2). Technical details on the above-mentioned methods are to be found in our previous paper (Tenti et al. 1992). The type, dilution and source of the antibodies used are indicated in Table 1.

Acidic mucins were studied with alcian blue (1%, pH 2.5) while neutral mucins were analysed by periodic acid-Schiff (PAS) and by concanavalin A (PACONA; Fiocca et al. 1987) for the neutral mucin peculiar to gastric pyloric glands and mucus neck cells. The presence of endocrine cells was investigated by Grimelius silver staining.

DNA extraction for molecular analysis was from histological sections of formalin-fixed, paraffin-embedded tissue (Higuchi 1989). Samples from benign, borderline and malignant areas from both tumours were examined. The first exon of the K-*ras* oncogene (containing both codons 12 and 13) was amplified by polymerase chain reaction with specific primers and then analysed by denaturing gradient gel electrophoresis (DGGE; Pellegata et al. 1992). After electrophoresis, the gel was stained with ethidium bromide (0.5 μg/ml) for 30 min and photographed under ultraviolet light.

Results

In both cases there were a large number of alcian-blue-positive and PAS-positive, diastase-resistant mucus cells. Reactivity to PACONA, however, was observed only in scattered, focal areas (Fig. 1a). None of the tumours expressed PGI, while there was multifocal immunoreactivity to other markers of pyloric type gastric mucosa such as M1, CaE and PGII. M1 was localized to the Golgi complex and mucin granules in the supranuclear cytoplasm (Fig. 1b) and CaE was seen in the infranuclear and supranuclear cytoplasm (Fig. 1c). The study of serial sections suggests possible co-expression of M1 and CaE from the same cell, with a pattern similar to gastric superficial/foveolar cells. The immunoreactivity for PGII was found in the perinuclear or supranuclear cytoplasm, mainly in benign and borderline areas with endocervical type morphology (Fig. 1d).

The intestinal epithelial cell antigens M3SI (Fig. 2a) and CAR–5 (Fig. 2b) were expressed in a varying percentage of cells in both tumours, in areas with intestinal-type morphology, where scattered Grimelius-positive cells were also found (Fig. 2c).

DU–PAN–2 was expressed by approximately 30% of tumour cells (Fig. 3), and the study of serial sections suggests the possible co-expression of DU–PAN–2, M1, and CaE by the same cell.

Strong cell membrane immunoreactivity for CEA was observed in both tumours (Fig. 4).

In case 2 DNA analysis by DGGE showed a point mutation at codon 12 of K-*ras* oncogene, with substitution of GGT to CGT, leading to a glycine to arginine substitution, both in bordeline and malignant areas (Fig. 5). In case 1 there was a normal GGT sequence, as in the benign mucinous epithelium of case 2.

Discussion

PRMC are very rare tumours: just over ten cases have been reported in the literature to date (Fujii et al. 1986; Nelson et al. 1988; Park et al. 1991; Roth and Ehrlich 1977; Storch and Raghavan 1980; Vara Thorbeck et al. 1984). Their morphology is identical to ovarian mucinous cystoadenocarcinomas, with possible co-existence of benign, borderline and invasive patterns next to each other.

According to our histochemical and immunohistochemical study, a prominent feature of PRMC is the expression of antigens characteristics of normal gastric, enteric, or pancreatobiliary epithelial cells. We detected markers of pyloric-type gastric mucosa differentiation (M1, CaE, PACONA and PGII) in benign and borderline areas with endocervical type differentiation, while immunoreactivity for the intestinal cell markers (M3SI, and CAR–5) and DU–PAN–2 was identified mainly in frankly malignant areas, regardless of differentiation.

We had similar findings in mucinous tumours of the ovary, where the expression of gastroenteropancreatic markers is a rather common feature and is apparently related to certain histological features of the tumour: PGII, a marker of pyloric-type mucosal differentiation, is more common in benign and borderline than in malignant mucinous tumours, while CAR–5 and M3SI, markers of intestinal-type mucosa, are more common in malignant tumours (Tenti et al. 1992). Moreover, intestinal markers are expressed mainly in areas with intesti-

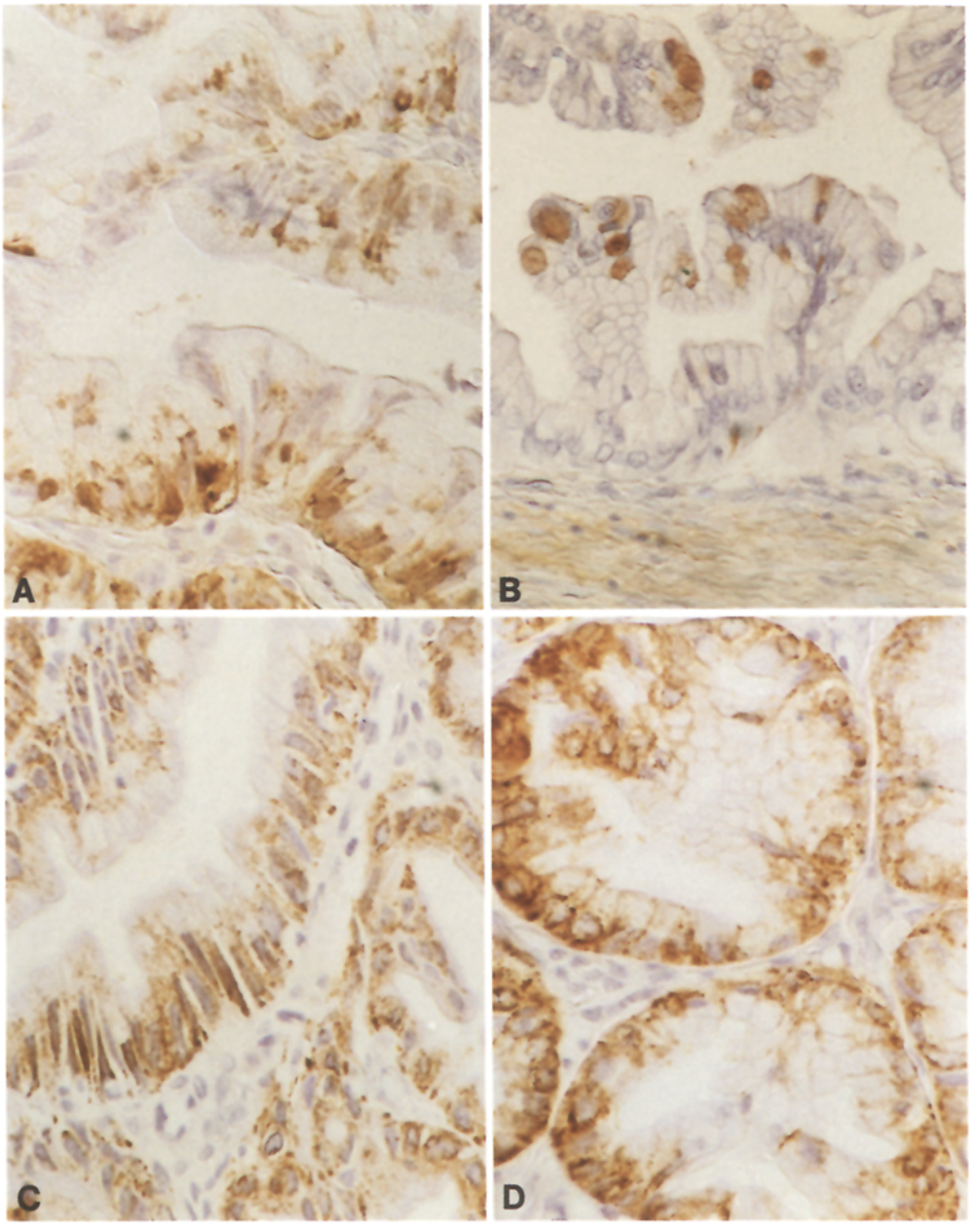


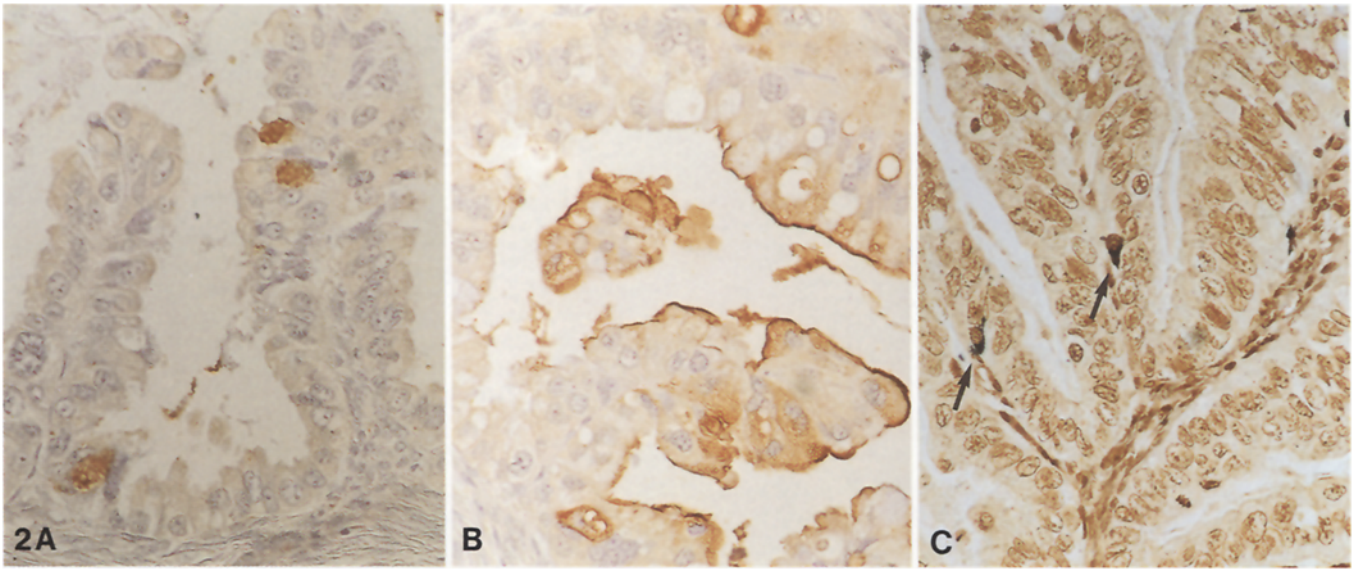
Fig. 1 Gastric epithelial cells markers: concanavalin A staining is present in focal areas, mainly in the perinuclear cytoplasm (**a**). Immunostaining for (**b**) M1, (**c**) cathepsin and (**d**) pepsinogen II is present in a high percentage of tumour cells in benign and borderline areas of endocervical type (×330)

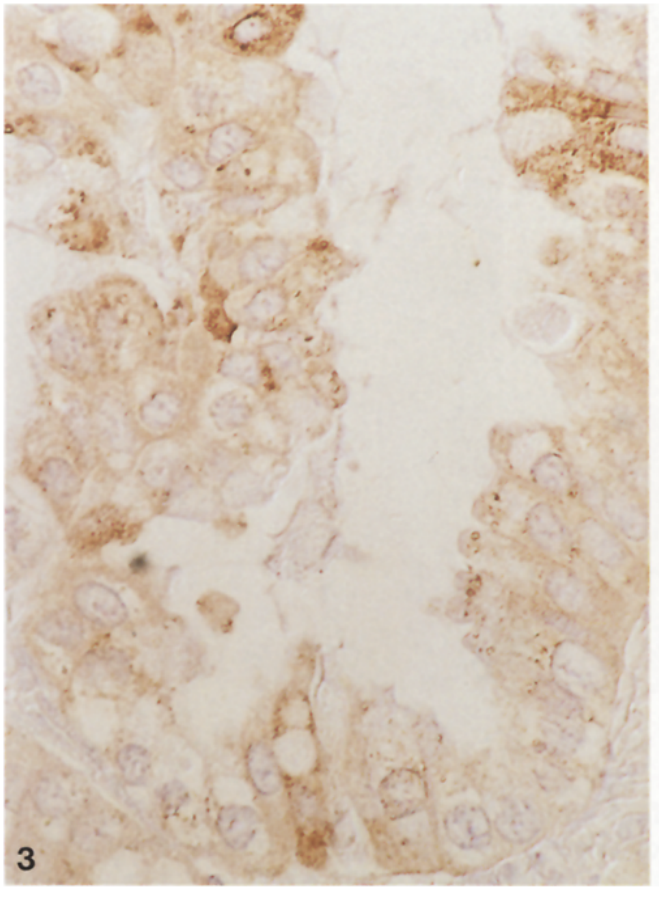
nal morphology, while gastric markers are detected mainly in areas of endocervical type differentiation. In addition to markers of gastrointestinal epithelial cells, ovarian mucinous tumours also express DU–PAN–2, which is a marker of normal and neoplastic pancreatic and biliary ducts.

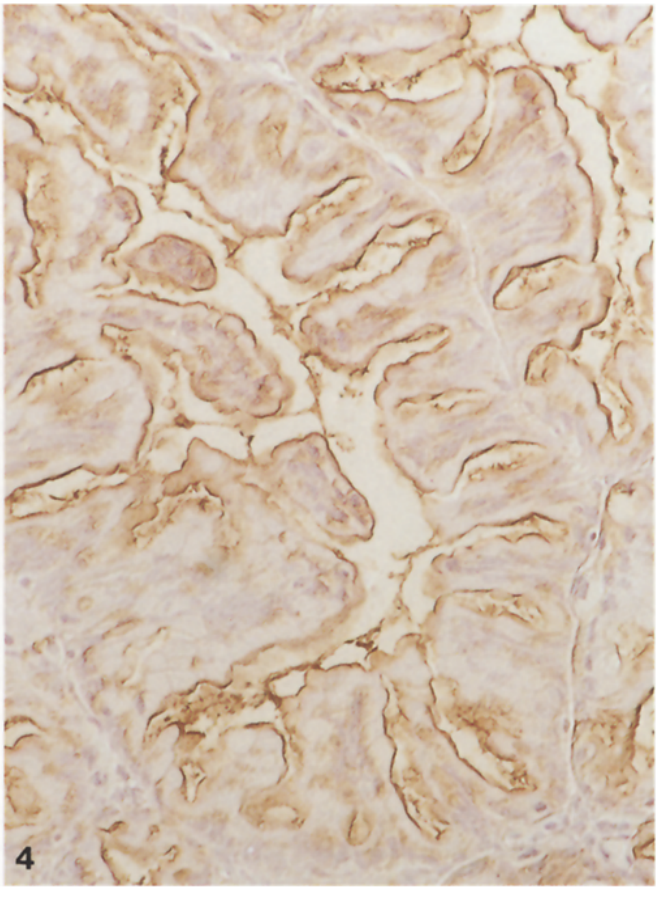
The correlation found between Grimelius staining and the intestinal markers in PRMC suggests that, as in ovarian mucinous tumours, the differentiation of an endocrine component tends to parallel the differentiation of the exocrine cell component.

The activation of oncogenes is a genetic event which is involved in the genesis and progression of a variety of human neoplasms. It may ensue through several mechanisms, including amplification, overexpression, deletion, mutation or rearrangement. Mutations in the *ras* family of proto-oncogenes occur in a variety of tumour types, the highest incidences being found in mucus secreting carcinomas. K-*ras* activation in particular has been identified in ovarian mucinous adenocarcinomas (Enomoto et al. 1991) and in morphologically similar pancreatobiliary (Almoguera et al. 1988; Smit et al. 1988; Tada et al. 1990) and colorectal (Bos et al. 1987; Forrester et al. 1987) carcinomas.

In one of our cases of PRMC we were able to demonstrate, by the DGGE technique, a point mutation of the K-*ras* gene with base substitution at codon 12 (GGT to CGT), a finding that adds to the similarity of PRMC







and ovarian mucinous tumours. The mutation is not present in benign mucinous epithelium, but is found in frankly malignant as well as in borderline areas. This might imply its occurrence as a relatively early event along the path leading to malignant transformation.

We can conclude that PRMC may share other biological features with ovarian mucinous tumours in addition to morphology, including genotypic abnormalities and patterns of differentiation of neoplastic epithelium. This is also reflected in their biological behaviour, which

Fig. 2 Intestinal epithelial cell antigens and endocrine cells: immunostaining for (**a**) M3SI and (**b**) CAR–5 is present in intestinal-type areas. Immunoreactivity for M3SI is limited to the cytoplasm of goblet cells. Intense CAR–5 immunoreactivity is present over the luminal surface and in the subapical cytoplasm. Scattered endocrine Grimelius-positive cells are present in papillary intestinal type area (**c**) (× 330)

Fig. 3 Immunostaining for DU–PAN–2 is present in frankly atypical cells (× 330)

Fig. 4 Immunostaining for carcinoembryonic antigen shows intense and diffuse positivity over the luminal surface (× 330)

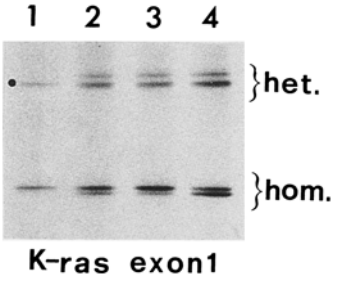


Fig. 5 Negative image of ethidium bromide stained denaturing gradient gel electrophoresis (DGGE) of K-*ras* exon I amplified fragments from different DNA samples of case 2. *Lane 1* DNA from area of benign mucinous epithelium; *lane 2* DNA from borderline area; *lanes 3 and 4* DNA from two different malignant areas. Sample 1 shows a normal DGGE pattern whereas samples 2, 3, and 4 show the same GGT to CGT base change at codon 12 leading to a glycine to arginine substitution. *Het* Heteroduplex; *hom* homoduplex. The K-*ras* pseudogene sequence can occasionally be amplified and detectable by DGGE analysis as in sample 1 (*dot*)

is akin to ovarian mucinous tumours. Whatever the histogenesis of PRMC, whether from teratomas, from ectopic ovarian tissue or from mucinous metaplasia of the coelomic epithelium, their morphological and biological similarities to ovarian mucinous tumours suggest that the process of tumour formation follows similar steps.

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