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Intestinal differentiation in ovarian mucinous tumours*

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Summary. Fifty-five ovarian mucinous tumours, 22 benign, 16 borderline and 17 malignant, were examined for intestinal differentiation (ID). This was defined by the presence of one or more of endocrine, absorptive, goblet or Paneth cells, and identified by routine haematoxylin and eosin as well as histochemical and immunoperoxidase techniques. Twenty benign (91%), 14 borderline (88%) and all malignant tumours contained foci of ID. The frequency of ID was not significantly different between the mucinous tumour types (chi-squared test for independence). Follow-up was available on all patients with borderline tumours: 14 were stage Ia, including both cases without ID, and 2 were stage Ic at presentation. All are alive and free of disease at 9–39 months (median 15.5 months). We conclude that the presence of ID in borderline mucinous tumours is unlikely to be of prognostic significance, and that a subdivision of these tumours into müllerian and intestinal types is unnecessary.

Key words: Ovarian neoplasms – Intestinal differentiation – Mucinous tumours

Introduction

Intestinal differentiation (ID) is common in ovarian mucinous tumours (Rutgers and Scully 1988a) and is defined by the presence of goblet cells by some (Fulcheri et al. 1987; Rutgers and Scully 1988a) and endocrine cells by others (Szymarska et al. 1983). However, the role of ID in predicting the subsequent behaviour of borderline mucinous tumours is controversial. Some investigators have suggested that borderline mucinous tumours with ID are biologically more aggressive than those with purely müllerian-type epithelium (Louwerens et al. 1983; Michael et al. 1987; Rutgers and Scully 1988b). On this basis, Rutgers and Scully (1988a) subdivided their borderline ovarian mucinous tumours into those with purely müllerian mucinous epithelium and those with goblet cells (ID).

Other studies suggest that the biological behaviour of mucinous tumours is unaltered by the presence of ID (Fox et al. 1964; Spoorong et al. 1981). Klemi and Nevalainen (1978) found ID in nearly all benign, borderline and malignant tumours studied and others have identified it most frequently in benign tumours (Ueda et al. 1989).

The aim of this study was to determine if the frequency of ID differed between benign, borderline and malignant ovarian mucinous tumours. The aim also was to determine if, in borderline tumours, stage at presentation or clinical outcome correlated with the presence of ID. ID was defined by the presence of one or more endocrine, absorptive, goblet or Paneth cells. To identify these cells, a combination of routine and specialized histochemical and immunoperoxidase stains were utilized.

Materials and methods

From the pathology files of the Foothills Hospital and the Tom Baker Cancer Centre, 22 benign, 16 borderline and 17 malignant ovarian mucinous tumours were identified from August 1985 to October 1988. All patients with borderline or malignant ovarian tumours were staged in an identical manner from that date onward by the same gynaecological oncologists. All original slides and pathology reports were reviewed. The median number of tissue blocks available for review was 7 for benign, 12 for borderline and 13 for malignant tumours. All had been fixed in buffered formalin. Tumours were classified according to their most atypical areas. Borderline lesions were defined by the criteria of Hart and Norris (1973). From that review, 4 blocks from each case were selected showing the most atypical or most cellular areas. Clinical records were reviewed for patient age at diagnosis, and patient outcome for the borderline tumours only.

Additional 5-μm-thick sections were cut from the 4 selected blocks in each case and stained with haematoxylin and eosin (H &

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E), periodic acid-Schiff (PAS)-alcian blue (Wakefield and Wells 1985), high iron diamine (Spicer 1965), Grimelius (Grimelius 1968) and Masson-Fontana (Solcia et al. 1969). In control tissues, PAS-alcian blue stained neutral gastric mucins red, and acidic small and large bowel acetyl-sialomucins and sulphomucins blue. High-iron diamine stained large bowel suphomucins brown-black and small bowel *N*-acetyl-sialomucins blue (Spicer 1965).

The peroxidase-antiperoxidase technique (Aguirre et al. 1984b) was used to identify lysozyme (DAKO A099), chromogranin A (Incstar 20085) and carcinoembryonic antigen (CEA) (DAKO A115). Appropriate positive and negative tissue controls were obtained for each antibody. For the immunoperoxidase-stained sections, only discrete cytoplasmic staining was counted as positive.

Each intestinal cell type was scored as: 0, absent; 1, less than 5%; 2, 5–20%; and 3, greater than 20% of the total neoplastic epithelium. The data were analysed using the chi-squared test for independence (significance level, $p < 0.05$).

Results

Of the 55 ovarian mucinous tumours, 20 benign (91%), 14 borderline (88%) and all malignant tumours showed ID (Table 1). The frequency of ID was not significantly different between the mucinous tumour types. Intestinal areas, regardless of tumour type, contained endocrine, absorptive, goblet and Paneth cells in varying proportions, and sometimes histologically resembled "normal" intestinal mucosa (Fig. 1).

Endocrine cells were flask-shaped, argyrophilic, (Fig. 2A) chromogranin A positive (Fig. 2B) and mucin negative. The Masson-Fontana stain identified a much smaller population of flask-shaped endocrine cells (Table 1). Absorptive cells (Fig. 1) had basally arranged nuclei, a magenta brush border but no cytoplasmic staining with PAS-alcian blue. Goblet cells (Fig. 1) contained a large apical, alcian blue positive vacuole which stained either blue or brown with the high-iron diamine stain (Fig. 3). Paneth cells were alcian blue negative, strongly lysozyme positive (Fig. 4) and had dark, eosinophilic cytoplasmic granules which were also PAS positive. Paneth cells were also Grimelius and chromogra-

Table 1. The number (%) of mucinous tumours with intestinal differentiation

	Benign	Borderline	Malignant
n	22	16	17
ID	20 (90%)	14 (88%)	17 (100%)
APGE	8 (36%)	8 (50%)	6 (35%)
A	8 (36%)	9 (56%)	15 (88%)
P	14 (64%)	11 (69%)	11 (65%)
G	19 (86%)	13 (81%)	14 (82%)
E	13 (59%)	14 (88%)	12 (71%)
AR	2 (9%)	3 (19%)	5 (29%)
CEA	12 (56%)	10 (63%)	8 (47%)
S	16 (73%)	12 (75%)	13 (76%)
SIAL	19 (86%)	13 (81%)	14 (82%)

n, number of tumours; ID, intestinal differentiation present; APGE, each intestinal cell type present in the same tumour; A, absorptive cells; P, Paneth cells; G, goblet cells; E, endocrine (argyrophil-positive) cells; AR, argentaffin cells; CEA, carcinoembryonic-antigen-positive cells; S, sulphomucin-containing goblet cells; SIAL, sialomucin-containing goblet cells

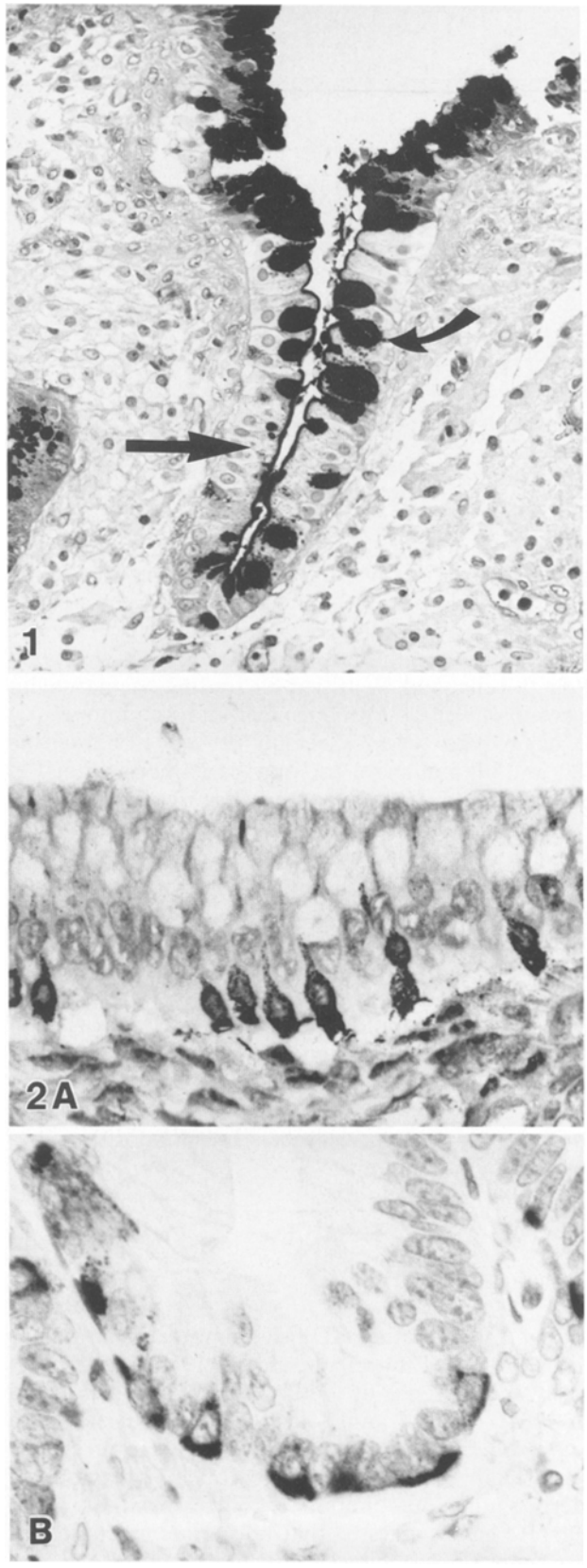
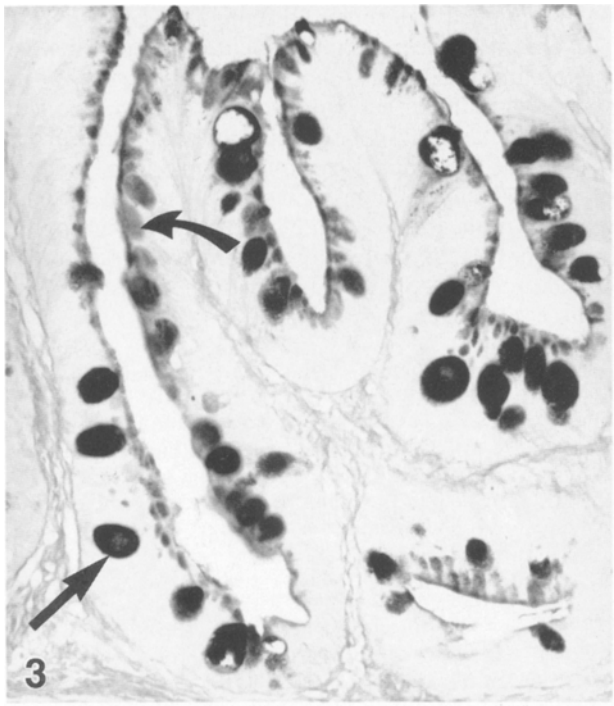


Fig. 1. Absorptive (*straight arrow*) and goblet cells (*curved arrow*) in a mucinous cystadenoma focally resembling intestinal mucosa. PAS/alcian blue, ×200

Fig. 2. A Grimelius and **B** chromogranin A staining of endocrine cells in a borderline mucinous tumour, ×400



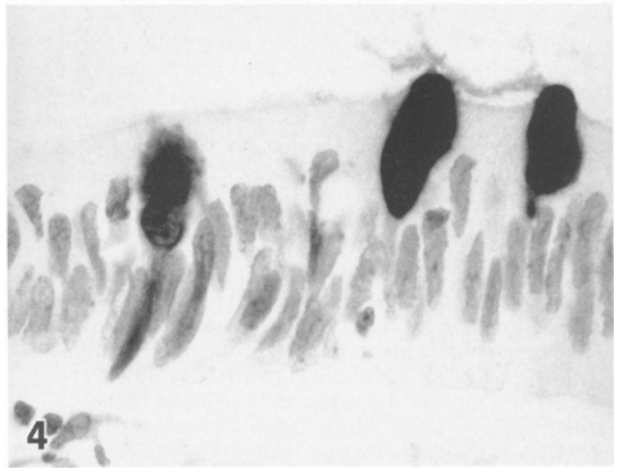


Fig. 3. Goblet cells in a borderline mucinous tumour containing sulphomucins (*straight arrow*) and sialomucins (*curved arrow*). High-iron diamine, × 200

Fig. 4. Paneth cells in a borderline mucinous tumour with strong cytoplasmic staining for lysozyme, × 400

nin A negative, preventing confusion with enterochromaffin cells.

No significant difference was found in the percentage of benign, borderline or malignant tumours which contained endocrine, goblet or Paneth cells. However, the percentage of tumours containing absorptive cells increased significantly ($p < 0.005$) between benign, borderline and malignant tumours and the percentage of neoplastic epithelium replaced by them increased correspondingly. Also, no significant difference was found between tumour types which were CEA positive or produced sulphomucins or sialomucins. However, the proportion of goblet cells which contained sulphomucins as opposed to sialomucins was significantly greater in mucinous carcinomas ($p < 0.05$).

The median age and age range at diagnosis for benign, borderline and malignant tumours was 31 years (22–71), 44.5 years (33–75) and 44 years (16–78) respectively. No patients had endocrine symptoms related to hormone production by their tumours or pseudomyxoma peritonei. Associated pathological findings noted on review included: 2 contralateral mature cystic teratomas, 1 contralateral Brenner tumour, focal serous differentiation in 6 cases (5 benign and 1 malignant) and focal endometrioid differentiation in 2 carcinomas. One malignant (6%) and 2 borderline (13%) tumours were bilateral (all with ID).

Follow-up information was available on all 16 borderline cases (14 with ID). Fourteen were FIGO stage Ia at presentation and 2 stage Ic (both with intestinal differentiation). All 16 are alive and free of disease at 9–39 months post-diagnosis (median 15.5 months).

Discussion

In this series 93% of ovarian mucinous tumours contained foci of ID which were present in 91% of benign, 88% of borderline and 100% of malignant tumours. The reported incidence of ID varies widely with previous studies identifying it in 10–100% of benign (Klemi and Nevalainen 1978; Louwerens et al. 1983), 33–100% of borderline (Spoorong et al. 1981; Takeda et al. 1982) and 17–100% of malignant tumours (Aguirre et al. 1984a; Fenoglio et al. 1976; Ueda et al. 1982). This wide variation in the reported frequency may be due to a combination of factors. Those who define ID by the presence of one cell type only will be less likely to identify it than those who define ID by the presence of one or more of several intestinal cell types. Klemi and Nevalainen (1978), who looked for several intestinal cell types, found ID in 13 of 14 (92%) mucinous tumours, which closely agrees with the results of this study.

Differences in the reported frequency of ID may also be due to the varying techniques employed to identify it. Some have confirmed ID using electron microscopy (Fenoglio et al. 1975, 1976; Klemi and Nevalainen 1978; Konishi et al. 1988; Szymarska et al. 1983), while others used various immunohistochemical reactions (Aguirre et al. 1984a; Bara et al. 1977; Charpin et al. 1982; Louwerens et al. 1983; Merchand et al. 1975; Spoorong et al. 1981; Takeda et al. 1982; Ueda et al. 1982, 1989). Other disparities in the frequency of ID could result from variations in tissue fixation and tumour sampling. A bias is introduced also since sections from a benign tumour typically contain fewer epithelial cells than those from a carcinoma, producing an artefactually low frequency of uncommon cell types in benign tumours.

In this study, Paneth, goblet and endocrine cells were approximately evenly distributed between benign, borderline and malignant tumours. Paneth cells were identified more frequently in this than any other studies, possibly because their strong cytoplasmic staining with

lysozyme (Heitz and Wegmann 1980) facilitated their recognition at low power. In the current series, occasional columnar mucus-containing cells were argyrophil positive but chromogranin A negative. These correspond to the type I argyrophil cells of Aguirre et al. (1984a), which are not true endocrine cells because the argyrophil reaction occurs within mucus granules.

However, in this series, absorptive cells showed a statistically significant increase ($p < 0.005$) in distribution between benign, borderline and malignant tumours. In addition, they were the most frequent cell type in mucinous carcinomas but the least frequent in adenomas. The significance of this finding is unclear, but a practical consequence is that any mucinous cystadenoma with prominent numbers of absorptive cells should be sampled carefully to exclude a borderline or malignant tumour.

Immunohistochemistry did not serve to delineate benign, borderline or malignant tumours. No significant difference was found between tumour types in the percentage which contained CEA-positive cells or in the percentage which expressed sulphomucins or sialomucins.

However, compared with benign tumours, a significantly greater proportion of the goblet cells in malignant tumours contained sulphomucins. This may be analogous to intestinal metaplasia in the stomach and oesophagus, where the presence of sulphomucins is associated with a significant risk of malignant transformation (Chejfec 1985). Similarly, the majority of intestinal adenocarcinomas produce sulphomucins (Allen et al. 1988; Silva and Filipe 1986). Since 73% of the benign tumours in this series contained at least some sulphomucin-positive goblet cells, their presence alone is not predictive of a poor prognosis.

Rutgers and Scully (1988a) have proposed that borderline mucinous tumours containing goblet cells are potentially more aggressive than those without goblet cells or other cellular features of intestinal differentiation (argentaffin and Paneth cells). A mechanism for their enhanced aggressiveness had been proposed previously by Michael et al. (1987), who suggested that stromal trypsin hydrolysis by extravasated mucin provided an easy route for stromal invasion. In the present series, the stage at presentation or subsequent behaviour of borderline tumours did not vary with the presence or absence of cellular markers for ID. Tumours from 14 patients contained foci of ID. All were stage Ia except 2 which were stage Ic. Only 2 tumours had no features of ID, and both these patients were stage Ia at diagnosis. Since the number of patients is small ($n = 16$) and the follow-up interval is short (median of 15.5 months with a range 9–39 months) no meaningful conclusions can be drawn, but no trend is apparent relating ID to either stage at diagnosis or survival.

From this study, we conclude that the small differences in the frequency of ID between benign, borderline and malignant tumours are not statistically significant. Enhanced tissue sampling especially of benign or borderline tumours might be expected to increase the frequency of detection of ID to the 100% found in malignant tumours. Since the frequency of ID is statistically similar between benign, borderline and malignant tumours, we suggest that it is an unreliable indicator of the potential for malignant behaviour in borderline tumours. We further suggest that the assessment of risk of subsequent malignant behaviour in borderline ovarian tumours is unlikely to be determined by conventional light microscopy alone. Instead, a more accurate assessment may require the application of some of the new technologies, such as ploidy analysis by flow cytometry (Friedlander et al. 1984) or oncogene amplification measurements (Slamon et al. 1989).

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