Use of immunohistochemical staining panel for characterisation of ovarian neoplasms

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SUMMARY Eighty five ovarian epithelial and non-epithelial tumours were studied by peroxidase histochemical staining for their reactivity with six monoclonal human milk fat globule (HMFG) antibodies, peanut agglutinin (PNA) lectin, and a monoclonal cytokeratin antibody. HMFG IIIC12 and cytokeratin antibodies distinguished epithelial from non-epithelial tumours. The staining patterns of mucinous and serous tumours were essentially different from each other; poorly differentiated anaplastic carcinomas showed similar antigenic content to that of the serous cystadenocarcinomas. Furthermore, staining with PNA lectin and HMFG antibodies was useful in distinguishing clear cell carcinomas from other malignant epithelial tumours of the ovary.

The treatment and prognosis for ovarian tumours depend on the spread, differentiation, and exact histological type.¹ Although the histopathological differential diagnostic features between various tumour types is fairly easy in most cases, about 10% of poorly differentiated ovarian tumours cannot be positively identified with morphological criteria.² To overcome this, special stainings can be used to show the presence of a variety of cellular components such as enzymes, intermediate filaments, or oncofetal proteins.

During the past decade immunohistochemical staining with a panel of monoclonal antibodies has been used to verify the presence of a large number of glycoproteins associated with tumours in ovarian neoplasms.³ Of these, antibodies against human milk fat globule (HMFG) membrane antigens have incited special interest because of their potential use in antibody guided radiotherapy in ovarian tumours.⁴ Like other antibodies, HMFG antibodies do not react exclusively with one specific ovarian tumour type.⁵⁻¹² Immunohistochemical staining of tissue sections with one antibody can only confirm or exclude¹³ a previously assumed histopathological diagnosis. We therefore stained tissue sections from 85 ovarian tumours with peroxidase using six monoclonal HMFG antibodies with differing specificities, a monoclonal anticytokeratin antibody, and peanut agglutinin (PNA) lectin to evaluate the potential use of such a staining panel for a more accurate differential diagnosis of poorly differentiated malignant ovarian tumours.

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Material and methods

ANTIBODIES AND LECTINS

The production and characterisation of murine monoclonal cell lines secreting HMFG antibodies has been described previously.^{14,15} Immunoglobulins were isolated from murine ascitic fluid by ammonium sulphate precipitation and purified with column chromatography (Sephacryl S-200, Pharmacia Fine Chemicals, Uppsala, Sweden). The biotinylated PNA lectin was purchased from Vector Laboratories and cytokeratin PKK-1 antibody from Labsystems (Helsinki, Finland).

SDS-PAGE AND IMMUNOBLOTTING

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting modifications from the methods described by Laemmli¹⁶ and Towbin¹⁷ were used. Briefly, samples were boiled in sample buffer, run in 10% SDS-PAGE, transferred electrically on to nitrocellulose paper, and stained with monoclonal HMFG antibodies as described previously.¹⁸

The tissue samples consisted of 85 ovarian tumours classified according to the World Health Organisation nomenclature.¹⁹ Tissue specimens were routinely fixed in 10% neutral formalin, embedded in paraffin, and the sections were stained with haematoxylin and eosin and with Weigert-van Gieson methods. Serial sections were stained with monoclonal anti-HMFG antibodies, PNA lectin, or monoclonal anticytokeratin antibody using the avidin-biotin-peroxidase method²⁰ as described previously.¹¹ The working con-

centration for antibodies and PNA lectin was 20 µg/ml in phosphate buffered saline (pH 7.3).

Results

To characterise the epitope specificity of monoclonal HMFG antibodies immunoblotting analyses of the protein preparations used for immunisation were performed. All six HMFG antibodies showed different staining patterns in immunoblotting of soluble skimmed milk proteins (fig 1).

SEROUS AND POORLY DIFFERENTIATED ANAPLASTIC TUMOURS

All serous benign and malignant tumours were positively stained with all six HMFG antibodies and PNA lectin, with the staining reaction being confined to the luminal membrane of the epithelial tumour cells (fig 2). The cytokeratin antibody reacted with all serous tumours. The staining pattern of the poorly differentiated anaplastic tumours was similar to that of the serous cystadenocarcinomas. Results from immunohistochemical stainings are summarised in the table.

MUCINOUS TUMOURS

Benign and malignant mucinous tumours were negative for all other HMFG antibodies (fig 2), except for HMFG IIIC12 which showed a strong luminal membrane staining. As expected, the cytokeratin antibody also stained positively all the mucinous tumours. Benign mucinous lesions showed no reactivity with PNA lectin but PNA reacted with cytoplas-

Fig 1 Immunoblotting analysis of skimmed milk antigens. Positions of molecular marker proteins are shown on the right.

Table Immunohistochemical reactivity of 85 ovarian tumours with HMFG antibodies, cytokeratin antibody, and PNA lectin

Morphological diagnosis	No of cases	Antibody			Lectin
		HMFG IIIC12	SM IF3	Anti-cytokeratin	PNA
Serous tumours:					
Cystadenoma	10	+	+	++	+++
Borderline	2	+++	+	++	+++
Cystadenocarcinoma	10	+++	+	++	++
Poorly differentiated anaplastic carcinoma	10	+++	+	++	++
Mucinous tumours:					
Cystadenoma	10	+++	—	+	—
Borderline	2	++	—	+	—
Cystadenocarcinoma	15	+++	—	+	++
Endometrioid carcinomas	5	+++	—	++	—
Clear cell carcinomas	5	+++	++	++	—
Brenner tumours	8	+++	—	+++	—
Granulosa cell tumours	8	—	—	—	—

+++ Intensive staining, ++ moderate staining, + slight staining, — no staining. The staining reactions with monoclonal HMFG antibodies SM IC6, HMFG IIE9, SM IVC7 and SM VH9 were similar to those obtained with antibody SM IF3.

Fig 2 Peroxidase staining of malignant ovarian tumours with monoclonal antibody SM IVC7 and PNA lectin. Serous cystadenocarcinoma stained with monoclonal antibody SM IVC7 (upper left) and PNA (upper right); mucinous cystadenocarcinoma stained with SM IVC7 (middle left) or PNA lectin (middle right); clear cell carcinoma stained with SM IVC7 (bottom left), or PNA lectin (bottom right).

mic material in all borderline tumours and mucinous cystadenocarcinomas.

OTHER EPITHELIAL AND GRANULOSA CELL TUMOURS

Other epithelial tumours were all positive for cyto-keratin antibody but negative for PNA lectin. As for HMFG-antibody reactivity, the antibody IIC12 positively stained all tumours, including the Brenner tumours, in which a cytoplasmic reaction was shown. The other HMFG antibodies generally failed to detect any antigenic material in these tumours. Clear cell carcinomas were an exception: a cytoplasmic staining reaction was shown in sections treated with other HMFG antibodies except HMFG IIC12, which again showed only luminal membrane reactivity. Granulosa cell tumours gave negative staining results.

Discussion

We analysed 85 ovarian tumours for their reactivity with six monoclonal antibodies against HMFG and with PNA lectin. All epithelial tumours strongly stained with HMFG IIC12 antibody; non-epithelial tumours showed no reactivity with the antibody. Antibodies directed against cytokeratins have shown similar immunohistochemical staining characteristics.^{21,22} Like cytokeratin antibodies, HMFG IIC12 antibody may therefore be useful in distinguishing epithelial from non-epithelial tumours. Similar results with other HMFG antibodies have been obtained by Wells *et al.*,²³ and by Heyderman *et al.*, who were able to diagnose anaplastic tumours of unknown origin¹³ using an antibody against epithelial membrane antigen.

To date no monoclonal antibody has been shown to be specific for a certain type of tumour or even ovarian tissue,²⁴ although some antibodies react preferentially either with mucinous²⁵ or serous²⁶ ovarian tumours. In our material serous, mucinous, endometrioid and clear cell carcinomas all showed a distinctive staining pattern when an immunohistochemical staining panel of six HMFG antibodies and peanut agglutinin lectin were used. The well defined staining pattern of the serous and anaplastic tumours suggests that the 10 anaplastic tumours previously identified as papillary ovarian carcinomas might, in fact, have been poorly differentiated serous cystadenocarcinomas of the ovary.

The reactivity of mucinous tumours with peanut agglutinin lectin was of interest as intracytoplasmic staining with PNA lectin was shown in borderline and malignant, but not in benign lesions. These results suggest that cytoplasmic PNA lectin positivity is a marker for ovarian epithelial cell differentiation. Similar PNA lectin binding characteristics have

previously been observed in epithelial cells in breast cancer^{27,28} as well as in intestinal neoplasms.²⁹

As for other epithelial tumours, clear cell carcinoma, a relatively rare ovarian tumour belonging to the "common epithelial tumours of the ovary" in the WHO classification,¹⁹ has been the object of great controversy in the past. It is now considered to be a variant of the endometrioid tumour of the ovary.^{30,31} Interestingly, in our material clear cell carcinomas could be distinguished from other epithelial neoplasms by their cytoplasmic staining reaction with monoclonal HMFG antibodies together with PNA lectin negativity. The observed antigenic differences between endometrioid and clear cell carcinomas might be due to the differing origins of these tumours. Our material is insufficient to draw definite conclusions on the histogenesis of these tumours.

Taken together, although immunohistochemical staining with one antibody can seldom be used to classify positively poorly differentiated ovarian tumours, an immunohistochemical staining panel including several HMFG and other antibodies³² and PNA lectin may be useful in the differential diagnosis of ovarian tumours.

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