Epithelial markers in prostatic, bladder, and colorectal cancer: an immunoperoxidase study of epithelial membrane antigen, carcinoembryonic antigen, and prostatic acid phosphatase

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SUMMARY Twenty prostatic adenocarcinomas, 20 transitional cell carcinomas of the bladder, and 20 colorectal adenocarcinomas were stained for epithelial membrane antigen, carcinoembryonic antigen, and prostatic acid phosphatase. Polyclonal affinity purified first and second antibodies and an indirect immunoperoxidase technique were used. All of the colorectal and bladder tumours and 16/20 prostatic tumours were positive for epithelial membrane antigen. All 20 colorectal, 7/20 bladder, and 5/20 prostatic tumours stained for carcinoembryonic antigen. All of the prostatic adenocarcinomas and none of the colorectal or bladder tumours were positive for prostatic acid phosphatase. These markers may be used to discriminate between tumours arising from these sites.

The histopathological differential diagnosis of widely invasive tumours of prostate, rectum, and bladder may, on occasion, be difficult on morphological grounds alone, especially when the tumours appear undifferentiated on a haematoxylin and eosin preparation. Even though the tumour shows evidence of adenocarcinomatous differentiation, there may be clinical doubt as to which of these organs is the primary source. Furthermore, tumours from these sites may present with nodal metastases or with a pathological fracture, with no definite signs or symptoms to indicate the likely primary site. It is important to make the correct diagnosis, since clinical management and treatment, even if only palliative, is entirely different for these three tumours.

This study was designed to investigate the distribution of epithelial membrane antigen (EMA),¹ carcinoembryonic antigen (CEA),² and prostatic acid phosphatase in tissue sections^{3–6} to see whether their expression was sufficiently different to be of value in histological diagnosis.

EMA¹ is a large glycoprotein, >440 000 daltons, which has now been partially purified.⁷ It is recognised by antisera raised against milk fat globule membranes,⁸ and has been shown in the prostate,^{1,9,10} bladder,⁹ and colon,^{1,9} as well as in a wide variety of other benign and malignant lesions.^{1,9}

CEA is an incompletely defined glycoprotein of 180 000 daltons. It was originally found in colorectal carcinomas,² and has since been shown by immunocytochemical techniques in a number of other sites including the prostate^{10–13} and bladder.^{12,14–17}

Prostatic acid phosphatase is an isoenzyme of acid phosphatase (ortho phosphoric monoester phosphohydrolase, EC 3.1.3.2) of 100 000 daltons¹⁸ and isoelectric point 4.2–5.5. Its enzymic activity is largely destroyed by formalin fixation and processing of tissue into paraffin wax, but specific antisera may be used to demonstrate prostatic acid phosphatase in sections of fixed prostatic tumours and of their metastases, as well as in benign prostatic tissue.^{4–6} Cross reactivity with pancreatic islet cells, salivary glands, renal carcinomas and breast carcinomas with some rabbit antisera has been reported,^{19–22} although no such activity has been seen in 142 various non-prostatic benign and malignant lesions stained with the affinity purified antibody used in this study (unpublished data).

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Material and methods

Formalin fixed, paraffin embedded blocks of 20 adenocarcinomas of the prostate, 20 transitional cell carcinomas of the bladder, and 20 colorectal adenocarcinomas were selected from the routine surgical files (Table 1). In this study only one block of each tissue was used, as previous experiments had indicated that where these particular markers were present all of the blocks were likely to be positive and often only one block was available from bladder biopsies. Where possible, normal tissue was included to act as an inbuilt control.

Sections were cut at 4 μ m on to slides coated with chrome gelatin to prevent the sections floating during immunostaining. An indirect immunoperoxidase technique was used as described previously,²³ and endogenous peroxidase was inhibited by a sequence of 6% hydrogen peroxide, 2.5% periodic acid, and then 0.02% potassium borohydride.²⁴ Indirect peroxidase conjugates were prepared by periodate oxidation²⁵ using affinity purified goat antirabbit immunoglobulin.

Table 1 *Differentiation in colorectal, bladder, and prostatic tumours**

	Well	Moderately	Poorly
Ca colon	1	18	1
Ca bladder	8	8	4
Ca prostate	6	11	3

*This distribution of differentiation in 20 of each type of tumour reflects that seen in tumours chosen at random from those reported during 1981-3.

ANTISERA AND CONTROLS

The primary antisera were all raised in rabbits and affinity purified on agarose columns (AffiGel 10, BioRad Laboratories Ltd, Herts), to which the relevant antigen had been bound. Specificity was demonstrated by repassage of the affinity purified antibody down the column. Staining could be shown in the appropriate positive control tissue with the eluate from this second passage and was absent from the (unbound) effluent. This latter effluent was used as a negative control.²⁶

Milk fat globule membranes were prepared by freeze-thawing an aqueous suspension of the washed cream fraction of whole human milk⁸ and pelleted by high speed centrifugation. CEA had been prepared from a hepatic metastasis of a colorectal carcinoma and was a generous gift from Dr Westwood (ICRF, Sutton). Prostatic acid phosphatase was isolated from pooled seminal plasma obtained from the vasectomy clinic. The enzyme was precipitated with ammonium sulphate and further purified by column chromatography, first by ion

exchange on a DEAE AffiGel blue column (Biorad Laboratories Ltd, Herts) and then by gel filtration on a Sephadex G200 column (Pharmacia (GB) Ltd, Bucks).^{27, 28}

The immunogens were emulsified in a non-ulcerogenic adjuvant (Guildhay, University of Surrey) and injected subcutaneously into New Zealand rabbits.

CONTROLS

The positive control for CEA was a colonic carcinoma; for EMA a moderately differentiated infiltrating ductal carcinoma of the breast was chosen; and for prostatic acid phosphatase a prostate showing benign adenomyomatous hyperplasia was used. Each positive section of positive control or test was matched with a control section treated with the negative "absorbed" antibody.

Results (Table 2)

All of the colorectal and bladder carcinomas and 16/20 prostatic carcinomas were positive for EMA (Figs. 1 and 2). All 20 colorectal, 7/20 bladder, and 5/20 prostatic carcinomas were positive for CEA (Figs. 3 and 4). All of the prostatic tumours (Figs. 5 and 6) and none of the bladder or colorectal carcinomas were positive for prostatic acid phosphatase.

Table 2 *Results of immunoperoxidase stains for epithelial membrane antigen (EMA), carcinoembryonic antigen (CEA), and prostatic acid phosphatase (PAP)*

	EMA	CEA	PAP
Colorectal	20/20	20/20	0/20
Bladder	20/20	7/20	0/20
Prostate	16/20	5/20	20/20

Without prior knowledge, it was not possible to tell which antibody had been used because the pattern of staining was so similar for the three epithelial markers, although stains for EMA in colorectal tumours were weaker than those for CEA, and stains for CEA in prostatic carcinomas (Fig. 4) were weaker than in colorectal tumours (Fig. 3). The dilution of primary antibody had been determined on the appropriate positive control tissue—breast carcinoma for EMA, colorectal carcinoma for CEA, and benign prostatic hypertrophy for prostatic acid phosphatase. These differences were therefore relative and could be assessed only in relation to those tissues.

CARCINOEMBRYONIC ANTIGEN

There was no correlation between the presence or

Fig. 2

Fig. 1 Papillary transitional cell carcinoma of the bladder stained with antibody to epithelial membrane antigen. There is membrane staining of some of the intact superficial cells and luminal staining in foci of glandular metaplasia. $\times 125$.

Fig. 2 Immunostain for epithelial membrane antigen in a solid invasive transitional cell carcinoma of the bladder outlines a focus of squamous metaplasia. $\times 200$.

Fig. 3 Moderately differentiated adenocarcinoma of the colon stained for carcinoembryonic antigen. Necrotic debris is positively stained, as is virtually every malignant acinus. $\times 125$.

Fig. 1

Fig. 3

Fig. 5

Fig. 4 Well differentiated prostatic adenocarcinoma stained from carcinoembryonic antigen. A similar luminal pattern of staining to that seen in Fig. 3 is present, but several acini in this field are negative, and in the block as a whole only a few foci show positive staining. $\times 200$.

Fig. 4

Fig. 5 Non-neoplastic prostatic gland stained for prostatic acid phosphatase. The characteristic rather granular pattern of staining is well shown. $\times 200$.

Fig. 6

Fig. 6 Moderately well differentiated prostatic adenocarcinoma stained for prostatic acid phosphatase. There is a partly cytoplasmic, partly luminal membrane pattern of staining. $\times 200$.

Photomicrographs taken directly on to Polaroid $3\frac{1}{4}$ " $\times$ 4 $\frac{1}{4}$ " 699 film.

absence of CEA or EMA and degree of differentiation or morphology in the prostatic tumours. Where present, positivity for CEA was found mainly on the luminal membrane in well or moderately differentiated colorectal or prostatic adenocarcinomas and in the cytoplasm of poorly differentiated tumours. In sections of bladder tumours, CEA was largely confined to those areas showing squamous metaplasia and was also found on non-malignant transitional cell epithelium undergoing squamous transformation. In the prostate, CEA was mainly present on luminal membranes, but was always weaker than in colorectal carcinomas (Figs. 3 and 4).

EPITHELIAL MEMBRANE ANTIGEN

Stains for EMA were present on the cell membranes of transitional cell carcinomas and outlined foci of glandular (Fig. 1) and squamous differentiation (Fig. 2). It was interesting that many more transitional cell carcinomas contained foci of glandular metaplasia shown by the EMA stain than was apparent with haematoxylin and eosin or periodic acid Schiff preparations. In colorectal and prostatic adenocarcinomas both CEA and EMA were present mainly on the luminal membranes of malignant acini, with some cytoplasmic staining particularly in poorly differentiated carcinomas.

PROSTATIC ACID PHOSPHATASE

Positive staining for prostatic acid phosphatase was found in non-neoplastic prostatic glands (Fig. 5) and in 20/20 prostatic tumours (Fig. 6), although not all benign or malignant glands in any one section were positive. It was found mainly in the cytoplasm, with some luminal membrane staining. No positivity was found in non-prostatic tumours.

No staining was seen with the negative "absorbed" controls for any of the three markers.

Discussion

These results confirm previous reports that the demonstration of prostatic acid phosphatase in a tumour is virtually diagnostic of prostatic origin. A further 142 primary and metastatic tumours from a wide variety of sites outside the prostate have been stained with the antibody used here, and none has been positive (unpublished data). Although there were no false positives, the possibility of false negatives cannot be excluded, although in most studies between 93% and 100% of prostatic carcinomas have been found to be positive.^{5 6 10 21 29-32} Shevchuk *et al*³³ found lower rates of positivity using polyclonal goat (83% positive), rabbit (70%), or monoclonal antibodies (59%).

Since this study was carried out, and the high

degree of specificity of the antibody to prostatic acid phosphatase was demonstrated, the antibody has been shown to be particularly valuable in distinguishing between solid transitional cell carcinomas of the bladder and poorly differentiated prostatic carcinoma in cases verified at operation or necropsy. Prostatic ducts lined by transitional type epithelium show some positivity, however, and transitional cell carcinomas of prostatic ducts are currently being studied. Determination of the physical site of origin of tumours at the bladder neck may be difficult when prostatic chippings, rather than a prostatectomy specimen, are available.

Antibodies to a smaller protein, prostate specific antigen of 34 000 daltons and isoelectric point 6.9,²⁷ do not apparently show the cross reactivity with non-prostatic tissues reported with some antisera to prostatic acid phosphatase and show similar positivity rates in prostatic carcinoma. Antibodies to prostatic acid phosphatase have an advantage over anti-prostate specific antigen, however, in that the effects of various fixatives and processing schedules in terms of both antigenic and enzymic reactivity can be studied and the affinity purified antibody used in this study has not so far been found to stain non-prostatic tissues.

Eighty colorectal carcinomas have now been stained by us for CEA¹¹ (unpublished data). As found by others,^{12 34} all have been CEA positive, so that the absence of CEA makes the diagnosis of a colorectal primary tumour unlikely. Wiley *et al*,³⁵ however, found only 21/41 (51%) colonic tumours positive for CEA using an unpurified and unabsorbed rabbit antibody (source not stated).

Our finding of CEA in 7/20 (35%) bladder carcinomas is comparable with that of Wahren,¹⁶ who examined cytological preparations with a positivity of 18/40 (48%). Wiley *et al*¹⁷ found CEA in 26/48 (58%), but since they used a commercial rabbit antibody without mention of spleen absorption they may well have been showing the shared normal cross reacting antigen (NCA) determinant.^{36 37} The number of CEA positive tissues reported by Goldenberg *et al* in 1976¹⁴ is generally lower than is presently found with more recently developed reagents: 23/37 (62%) of their colorectal carcinomas, 1/10 (10%) of bladder, and none of their 10 prostatic tumours were positive for CEA. In their 1978 paper¹⁵ 60/71 (85%) colorectal and 4/38 (11%) bladder tumours were positive.

In the study reported here 5/20 (25%) of the prostatic carcinomas were positive for CEA, a similar figure to the 7/30 (23%) reported by Heidl.¹² This is different from the figures of 16/38 (42%) before absorption with spleen and 1/38 (2.6%) after absorption reported by Purnell *et al*.¹³

The main value of the EMA immunostain in these tumours lies in the distinction of carcinoma from lymphoma or sarcoma.^{19,38} Fifty six of the 60 carcinomas in this study were positive. Sloane and Ormerod⁹ found 2/2 prostatic carcinomas, 4/4 bladder tumours, and 6/6 colorectal carcinomas were positive, and using a similar rabbit unpurified antibody Ellis *et al*¹⁰ found EMA in 47/60 (78%).

Variations in the percentage of positive tumours found in published reports are probably largely due to the use of antisera from different sources, although the immunoperoxidase technique employed and differences in fixation and processing schedules are important. It is advisable for departments which intend to use immunocytochemical stains as an adjunct to diagnostic histopathology to carry out immunostains on a number of tissues fixed and processed at that institution. The incidence of positive tumours stained under local conditions may be ascertained and the potential value of the antibodies established.

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