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Tumour marker immunoreactivity in adenocarcinoma

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SUMMARY To determine whether variations in the expression of tumour related antigen can predict the origin of tumours, the immunoreactivity of a series of adenocarcinomas from a wide range of sites was studied with a panel of monoclonal antibodies with specificity for carcinoembryonic antigen, carcinoembryonic antigen with non-specific cross reacting antigen co-specificity, epithelial membrane antigen, OC 125, and OC 19·9. A range of reactivity was seen in tumours from most sites. No distinctive results were identified for a particular site of origin. Some patterns of consistent positivity or negativity for the panel of antisera used were seen, however, which if applied to metastatic deposits have the potential to assist prediction of the site of origin.

These results also imply that immunohistological reactivity should be considered in the selection of a tumour marker for serological monitoring.

Faced with a metastatic deposit of adenocarcinoma, particularly one that is poorly differentiated, the pathologist usually has little evidence on which to base a decision about the primary site of origin. Such tumours often lack distinctive morphological characteristics that may enable the pathologist to assign it with confidence to a particular site. Histochemical staining may be useful to establish the type of carcinoma (for example, adenocarcinoma) but is generally of little help in identifying the primary site of a particular tumour.

Similar difficulty exists for the clinician. Obvious anatomical venous patterns of spread that give sufficient clues to identify the primary site occur in less than 10% of cases.[1] After ruling out prostatic carcinoma by rectal examination, breast carcinoma by palpation and mammography, and thyroid carcinoma by palpation, there is little therapeutic value in extensive clinical investigation. Most of the radiological tests produce confusing and conflicting rather than helpful information.[1]

Markers specific for the site of origin have been established for two tumours—thyroglobulin expression in thyroid carcinoma[2,3] and prostatic acid phosphatase[4,5] and prostate specific antigen (PSA)[6] in prostatic adenocarcinoma. Studies using other immunological tumour markers have been less fruitful. Investigation of carcinoembryonic antigen (CEA) immunoreactivity in tumours using polyclonal antisera has been bedevilled by problems of cross reactivity with other similar molecules—for example, non-specific cross reacting antigen (NCA).[7]

Well characterised monoclonal antisera are now available that are specific for CEA and NCA.[8] Preliminary studies have shown variable expression of these antigens in carcinoma from different sites: colonic carcinoma invariably expresses both CEA and NCA, and breast carcinoma commonly expresses NCA and rarely CEA (Robertson JFR *et al*, unpublished observations). Other markers show variability in expression—for example, epithelial membrane antigen (EMA) is present in tumours from many sites but does show variation in immunoreactivity according to the primary tumour site.[9] It is expressed strongly by virtually all adenocarcinomas of breast but is poorly expressed by colonic adenocarcinoma.

Two further well characterised antisera (CA125 and CA19·9) to tumour related antigens have been described. CA125 is a high molecular weight glycoprotein expressed by coelomic epithelium and its derivatives.[10] It is detectable in large amounts in non-mucinous carcinomas of the ovary. Preliminary studies[11] have shown positive immunoreactivity in about 95% of serous adenocarcinomas of the ovary and no immunoreactivity in metastatic adenocarcinoma deposits from non-Mullerian sites. The CA 19·9 antigen (lacto-N-fucopentanose II) is a sialated derivative of the Lewis A blood group that is expressed in adenocarcinomas of the gastrointestinal tract and pancreas.[12]

These well characterised antisera illustrate the range of antisera to tumour related antigens that are now

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Table 1 *Consistent positivity: antibody v primary site of tumour*

Antibody	*Strong or diffuse positivity*	*Weak or focal positivity*
EMA	Breast	Kidney, urothelium, lung, stomach, endometrium, mesothelium, gall bladder, and ovary (non-mucinous)
CEA	Large bowel	0
CEA/NCA	Lung, bile ducts, and large bowel	Gall bladder, cervix (adenocarcinoma), and ovary (mucinous)
CEA (CIS)	Large bowel	0
CA12·5	0	Ovary (non-mucinous)
CA19·9	Bile ducts	Gall bladder, ovary (mucinous), and endometrium Cervix (adenocarcinoma)

widely available. Use of a panel of such antibodies may allow more accurate assessment of carcinomas and could assist in identification of the primary site in the investigation of metastatic deposits. This study investigates the immunoreactivity of the antisera described above in a range of primary adenocarcinomas. A small series of malignant tumours of squamous and urothelial origin and malignant mesotheliomas were included for comparison.

Material and methods

One to two representative blocks were selected from 120 cases of primary carcinoma from the following sites: colon, stomach, pancreas, salivary gland, gall bladder, bile ducts, ovary, endometrium, cervix, breast, prostate, lung, kidney, bladder, liver, thyroid, and pleura.

Four micrometric sections of formalin fixed paraffin wax embedded tissue were cut. Tissue sections were stained immunocytochemically by a standard indirect immunoperoxidase technique[13] using diaminobenzidine as the substrate with imidazole and copper sulphate end product enhancement. The following primary antisera were used: OC 19·9 (CA 19·9) (Histocis, CIS Laboratories), OC 125 (CA 125) (Histocis, CIS Laboratories), CEA (CIS) (Histocis, CIS Laboratories), CEA (CRC Laboratories, Nottingham),[8] CEA/NCA (CRC Laboratories, Nottingham),[8] and NCRC11 (EMA) (CRC Laboratories, Nottingham).[14] Each section was examined microscopically to ascertain positive immunoreactivity and whether the staining was diffuse or focal.

These results were tabulated in association with the site of the tumour to determine consistency of positive or negative immunoreactivity for each antibody. Consistent positive immunoreactivity was defined as all cases of a particular tumour showing positive staining for a particular antibody. Note was made as to whether consistent positivity was of a diffuse or focal (heterogenous) nature.

Table 2 *Combinations of consistent positivity: site of primary tumour v antibody*

Site of primary tumour	*Strong or diffuse positivity*	*Weak or focal positivity*
Breast	EMA	0
Kidney	0	EMA
Bladder	0	EMA
Endometrium	0	EMA, CA19·9
Bile ducts	CEA/NCA, CA19·9	0
Gall bladder	0	CA19·9, EMA
Stomach	0	CEA/NCA, EMA
Large bowel	CEA, CEA (CIS), CEA/NCA	0
Lung	CEA/NCA	EMA
Mesothelium	0	EMA
Ovary (non-mucinous)	0	CA12·5, EMA
Ovary (mucinous)	0	CA19·9, CEA/NCA
Cervix (adenocarcinoma)	0	CA19·9, CEA/NCA

Results

The staining patterns seen for each tumour primary site are shown in tables 1–5. Tables 1 and 2 show comparisons of the sites of the primary tumours with each antibody with respect to consistent strong and focal positive immunoreactivity. Tables 3 and 4 show comparisons of the sites of the primary tumours with each antibody with regard to consistent negative immunoreactivity. Table 5 summarises the results shown in tables 1–4.

Discussion

We have shown that there are many combinations of positive immunoreactivity for all the tumour types that we examined. Unfortunately we did not see strikingly characteristic staining patterns for the panel

Table 3 *Consistent negativity: antibody v primary site of tumour*

Antibody	*Negativity*
EMA	Thyroid (follicular)
CEA	Thyroid (follicular), thyroid (all), kidney, liver, and prostate
CEA/NCA	Kidney, thyroid (follicular), and mesothelium
CA19·9	Salivary gland, and thyroid (follicular)
CA125	Salivary gland, stomach, small intestine, gall bladder, kidney, liver, bladder, prostate, thyroid (follicular), and ovary (mucinous)
CEA (CIS)	Thyroid (follicular), kidney, liver, prostate, and salivary gland

Table 4 *Consistent negativity: site of primary tumour v antibody*

Primary site	*Negativity*
Thyroid (follicular)	All negative
Thyroid (all)	CEA
Kidney	CEA, CEA (CIS), CEA/NCA, CA125
Mesothelium	CEA/NCA
Liver	CEA, CEA (CIS), CA125
Bladder	CA125
Prostate	CEA, CEA (CIS), CA125
Stomach, small intestine, and gall bladder	CA125
Salivary gland	CA125, CA19·9, CEA (CIS)
Ovary (mucinous)	CA12·5

of tumour markers that we used. We accept that to use negative immunoreactivity as a diagnostic criterion is still controversial; however, with the increasing purity of monoclonal antibodies, the use of appropriate control material, and the reliability of the staining techniques, we believe that a negative result may be helpful. The reliability of this type of result is strengthened when a panel of antibodies is used. Certain patterns of immunoreactivity do emerge when consistency of positive and negative results are considered together (tables 1–4).

In this small series only breast tumours showed consistent strong positivity for EMA. More extensive studies of breast tumours for this antigen have shown more variability[15] but in general breast tumours seem to express EMA and this expression is usually strong. Colorectal carcinomas show consistent strong positivity for CEA both with and without NCA, whereas lung tumours only show strong positivity for CEA/NCA. Bile duct tumours show strong positivity for both CEA/NCA and CA 19·9.

It may be argued that it is not valid to introduce a semiquantitative factor into the assessment of positivity. Our results suggest that to distinguish between the two types of positivity may be helpful in that focal positivity for a given antibody may mitigate against a particular primary site, where diffuse strong positivity for that particular antibody is the rule.

Consistent negativity may be of assistance where a particular metastatic tumour shows negative immunoreactivity for all antibodies. Such a tumour is more likely to be in the prostate than in the pancreas (table 5). Similarly, if focal positivity is only seen for EMA the primary site is more likely to be kidney than stomach, as consistent negativity is seen for four of the six antibodies in renal tumours as opposed to only one in gastric tumours. Only by examining cases prospectively can the validity of this hypothesis be tested.

We believe that the value of this particular study is to illustrate the potential of tumour marker immunoreactivity in the assessment of metastatic tumours. Up to 15% of patients with histologically confirmed metastatic tumours have no evidence of a primary tumour.[16,17] Nearly 40% of these are adenocarcinomas.[18] The prognosis of these patients is poor, with a reported median survival of less than six months and under 25% surviving more than one year.[16–24] More accurate and earlier recognition of the source of a particular tumour may assist palliation and prolong survival in these patients.

Table 5 *Summary of findings*

Primary site	*EMA*	*CEA*	*CEA/NCA*	*CEA (CIS)*	*CA 19·9*	*CA 125*
Salivary gland	O	O	O	N	N	N
Stomach	P	O	O	O	O	N
Small bowel	O	O	O	O	O	N
Large bowel	O	PP	PP	PP	O	O
Liver	O	N	O	N	O	N
Gall bladder	P	O	P	O	P	N
Bile ducts	O	O	PP	O	PP	O
Pancreas	O	O	O	O	O	O
Thyroid (follicular)	N	N	N	N	N	N
Thyroid (all)	O	N	O	O	O	O
Lung	P	O	PP	O	O	O
Mesothelium	P	O	N	O	O	O
Breast	PP	O	O	O	O	O
Ovary (non-mucinous)	P	O	O	O	O	P
Ovary (mucinous)	O	O	P	O	P	N
Endometrium	P	O	O	O	P	O
Cervix (squamous cell carcinoma)	O	O	O	O	O	O
Cervix (adenocarcinoma)	O	O	P	O	P	O
Kidney	P	N	N	N	O	N
Bladder	O	O	O	O	O	N
Prostate	O	N	O	N	O	N

PP = consistent extensive positivity; P = consistent focal positivity; N = consistently negative; and O = no consistent immunoreactivity.

Unfortunately diagnostic investigations carried out at presentation (including serology, endoscopy, radiology, imaging techniques and haematology) have an extremely poor yield.[19,22] Many of these techniques are extremely costly and time consuming and are not without risk. Identification of the tumour marker phenotype of a given metastatic tumour by immunohistological staining is by comparison cheap and simple to perform. Use of an appropriate panel of antibodies has the potential to allow accurate identification of the precise site of origin of a given tumour. With the range of antibodies available at present this appears unlikely in most cases; in many, however, the range of potential sites could be reduced and other diagnostic investigations could concentrate on more likely primary sites.

The lung and the pancreas,[24] and the lung, colon, and ovary[19] have been identified as the most common

sites of primary tumours in patients who had metastatic malignant disease but in whom the primary site was not identified until necropsy. Table 5 shows that a given staining pattern may exclude certain of these primary sites and may give a rough indication of the likelihood of one of a number of primary sites being the correct one.

There were no distinctive immunoreactivity patterns that were characteristic for more than one primary site, although one particular combination may fit more than one tumour.

We believe that this approach is possibly of more use in excluding certain primary sites, especially those where treatment of advanced disease is potentially beneficial (for example, breast, ovary, endometrium, and prostate), and that it both assists investigation and avoids dangerous, unpleasant treatment where it would be of little or no value. A limited panel of antibodies could be used where only a few primary sites are considered—for example, EMA and CEA/NCA where only lung adenocarcinoma and mesothelioma are being considered.

Finally, the pronounced variability of immunoreactivity for this panel of tumour markers seen in tumours from the sites that we investigated indicates that selection of a tumour marker for sequential serological monitoring of patients during and after treatment should not be based solely on the site of origin. Screening for immunohistological reactivity with a panel of antisera to appropriate tumour markers would give an accurate assessment of expression of tumour markers and indicate the most suitable marker for monitoring.

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