Review

Uses of Antibody Panels in the Analysis of Metastatic Carcinomas of Unknown Primary

Allen M. Gown

PhenoPath Laboratories, Seattle, WA, University of British Columbia, Vancouver, B.C. and Jefferson Medical College, Philadelphia, PA

Received for publication March 19, 1999

Immunohistochemical analysis can help determine the primary site of origin of carcinomas presenting at metastatic sites. Different types of epithelium, and their corresponding carcinomas, express different subsets of cytokeratins. Thus, antibodies to unique cytokeratins, such as "high molecular weight" cytokeratins, cytokeratin 7, cytokeratin 8, and cytokeratin 20, can help distinguish among different types of carcinomas that may have similar histologic appearances. Also helpful in this analysis are antibodies to the CEA family of proteins, as carcinomas of different sites vary widely in the quantitative and qualitative expression of these proteins. Another approach is the use of antibodies to vimentin, the intermediate filament protein that is co-expressed along with cytokeratin in a limited subset of carcinomas. Additionally, determination of primary site of origin of carcinomas can be assisted by the use of organ-associated markers such as prostatic specific antigen (prostate), gross cystic disease fluid protein-15 (breast), mesothelin (ovary, mesothelium), and thyroid transcription factor-1 (lung, thyroid). Selection of the appropriate subset of these antibodies can be most helpful in clinical situations where there is a limited set of possible primary sites (e.g., breast vs. lung or bladder vs. prostate).

Key words: Carcinoma, Cytokeratins, Immunohistochemistry, CEA, Estrogen receptor

I. Introduction

Immunohistochemical analysis has become an increasingly important tool for analysis of tumors, and has traditionally been employed to help pathologists determine the nature of tumors in which analyses of hematoxylin and eosin stained slides prove insufficient to render a definitive diagnosis. The recent availability of monoclonal antibodies with defined specificities to cytokeratin subsets and organ-related markers has now made it possible to provide critical information regarding the probable site of origin of carcinomas presenting at metastatic sites, usually in the context of lymph node, liver, or brain metastases.

It is impossible to recommend a single 'screening panel' of antibodies to determine the primary site of such carcinomas, given the fact that this panel will be highly dependent upon the clinical setting, and therefore different.

Correspondence to: Allen M. Gown, M.D., PhenoPath Laboratories, 3000 1st Avenue, Seattle, Washington 98121, USA.
mediate filament family, which includes cytokeratins as well as vimentin, desmin, glial fibrillary acidic protein, and neurofilaments [13].

There are many ways of classifying and categorizing the various cytokeratin proteins. Each of the 20 cytokeratin proteins have been given a "catalog number" by Moll, et al., from 1 to 20, based upon its molecular weight and its isoelectric point, as determined by 2-dimensional gel electrophoresis [21]. From the extensive work of Franke and colleagues, it has been determined that different epithelia and different carcinomas each have characteristic cytokeratin profiles, that is, they express a reproducible subset of these 20 cytokeratins [26].

III. High vs. Low Molecular Weight Cytokeratins

Although not technically correct from a biochemical standpoint, it is convenient to divide the cytokeratin universe into two arbitrary groups: "high molecular weight" and "low molecular weight." (An alternative nomenclature system, as explained below, might be "cytokeratins of simple epithelium" and "cytokeratins of complex epithelium." ) It has been demonstrated that the use of just two cytokeratin antibodies allows subdivision of carcinomas into histogenetic types [13-15]. Moreover, it has been noted by many investigators that the immunocytochemically determined cytokeratin profile of an individual tumor type generally remains constant, both in primary and metastatic neoplasms. Thus, selected anti-cytokeratin antibodies which react with diagnostically-useful subsets of normal and neoplastic epithelium, may be strategically employed in diagnostic surgical pathology.

Two 'prototype' anti-cytokeratin antibodies which define these classes of cytokeratins are 34βE12 and 35βH11, which define 'high' and 'low' MW cytokeratins, respectively. While the former identifies cytokeratins #1, 10, 5 and 14 in the Moll catalog, and the latter cytokeratin #8, for the purposes of their use in diagnostic pathology it is more relevant to discuss the classes of epithelium and tumors that they define. In brief, antibody 34βE12 identifies 'complex' epithelium such as ductal, squamous, and transitional epithelium (and corresponding tumors), while antibody 35βH11 identifies 'simple' epithelium, such as hepatocytes, acinar cells of the pancreas, colonic mucosa, etc., and corresponding tumors. These antibodies demonstrate a complementary spectrum of tissue activity, and every cytokeratin containing tumor or normal tissue is recognized by one or the other or both.

In many cases this is an idealized classification; many squamous cell carcinomas co-express 'low MW cytokeratins', and some renal cell carcinomas and colonic adenocarcinomas can focally co-express 'high MW' cytokeratins. However, one must interpret studies of these lesions cautiously; endometrial carcinomas, for example, may also show focal HMW-CK immunoreactivity, but it is typically limited to regions of squamous metaplasia. Colonic adenocarcinoma may also express HMW-CK, but it is also typically regional in its tissue distribution, demonstrating a "sub-plasmalemmal" localization, simulating its appearance in normal colonic epithelium. Group 1 consists of a fairly broad spectrum of carcinomas, and the separation of lesions within the group requires the use of other antibodies. For example, it is difficult to distinguish among ductal type carcinomas of the lung, breast, pancreas, or biliary tract when they are present as metastases of unknown primary sites, as the cytokeratin profiles and reactivity with this panel of cytokeratin antibodies is generally identical.

IV. Coordinate Expression of Cytokeratins 7 and 20

Cytokeratin 7 is a 54 kd polypeptide found in a wide variety of simple epithelia, including the lung, cervix, breast, bile ducts and collecting ducts of the kidney as well as bladder transitional cell epithelium and mesothelium. However, many studies have found it to be absent in gastrointestinal epithelia, hepatocytes, proximal and distal tubules of the kidney, and squamous cell epithelia. The
major utility of antibodies to CK 7 is in identifying non-GI adenocarcinomas [17, 28, 31, 32]. Cytokeratin 20, originally discovered in human intestinal epithelium, was subsequently identified only in a restricted subset of epithelia, such as gastric foveolar cells, urothelial umbrella cells, and Merkel cells of the epidermis. This relatively limited tissue distribution of CK 20 has therefore been useful in the differential diagnosis of carcinomas of unknown primary site [2, 21]. More recent studies [19, 34] have demonstrated that examination of coordinate expression of these two cytokeratins can provide more information regarding carcinoma type than examination of these individual cytokeratins alone. In our recent study [34], a total of 384 cases of carcinomas primary to various organs, as well as 16 cases of malignant mesothelioma, were evaluated using commercially available monoclonal antibodies. The subset of tumors strongly expressing both CK 7 and CK 20 included virtually all bladder transitional cell carcinomas and the majority of pancreatic adenocarcinomas; the tumors negative for both CK 7 and CK 20 were largely restricted to hepatocellular, prostate and renal cell carcinomas in addition to squamous cell and neuroendocrine carcinomas of lung. The CK 7-/CK 20+ immunophenotype, on the other hand, was highly characteristic of adenocarcinomas of colorectal origin, as well as 16 cases of malignant mesothelioma, were evaluated using commercially available monoclonal antibodies. The subset of tumors strongly expressing both CK 7 and CK 20 included virtually all bladder transitional cell carcinomas and the majority of pancreatic adenocarcinomas; the tumors negative for both CK 7 and CK 20 were largely restricted to hepatocellular, prostate and renal cell carcinomas in addition to squamous cell and neuroendocrine carcinomas of lung. The CK 7-/CK 20+ immunophenotype, on the other hand, was highly characteristic of adenocarcinomas of colorectal origin, whereas CK 7+/CK 20− immunophenotype was typically seen in the vast majority of carcinomas arising from other sites, including ovary, endometrium, breast, and lung, as well as malignant mesothelioma. Gastric carcinomas were the most heterogeneous subgroup with respect to their CK 7/CK 20 immunophenotype. In the subset of mucinous tumors, striking immunophenotypic differences were noted among those primary to the breast (CK 7+/CK 20−), gastrointestinal tract (CK 7−/CK 20+), and ovary (CK 7+/CK 20+). In all cases investigated, this CK immunophenotype was invariant in metastatic versus primary tumors. Thus, in the appropriate clinical setting, the CK 7/CK 20 immunophenotype of carcinomas is a valuable diagnostic marker in the determination of primary site of origin. Recent studies have confirmed the use of these antibodies in the discrimination of ovarian from colonic mucinous carcinomas [5, 20, 29].

Carcinomas can be divided into the following four CK7/20 immunophenotypic groups (Fig. 2).

**Antibodies to other selected cytokeratins**

CKs 1/10 Antibody 34/B4 is a commercially-available monoclonal antibody to cytokeratins 1 and 10, which are expressed only in squamous epithelium. While this might suggest a utility in specifically identifying squamous cell carcinoma, in fact these cytokeratins tend to be expressed only in well differentiated tumors and not those poorly differentiated ones in which immunocytochemistry might be sought.

CYTOKERATIN 5 has recently been demonstrated to be an excellent positive marker for mesothelioma; it is not expressed to any significant degree by lung adenocarcinomas and is a powerful reagent for the discrimination of mesothelioma from adenocarcinoma [8, 25]. Cytokeratin 5 also has great utility as a marker of squamous and transitional cell epithelium and their corresponding carcinomas.

**V. Carcinoembryonic Antigen**

CEA, or carcinoembryonic antigen, was originally described in the human digestive system as a tumor-associated antigen. Although it is now known that CEA can be demonstrated, with sensitive probes and methodologies, even in normal gastrointestinal epithelium and in reactive situations, antibodies to CEA continue to have diagnostic use, especially when combined with other antibodies in the context of a larger antibody panel. In one sense, antibodies to CEA may be considered a "poor man's" anti-cytokeratin antibody, in that CEA expression, in a very crude sense, correlates with epithelial differentiation. Expression of CEA is almost never seen in non-epithelial tumors. Nonetheless, there is a restricted expression of CEA within the set of epithelial tumors that can be exploited when coupled with site or origin.

---

**Fig. 2.**
of metastatic tumors. Unlike the intermediate filament proteins, CEA expression may be directly proportional to the degree of cellular differentiation, i.e., more poorly-differentiated tumors of a given cell type tend to express less CEA than well-differentiated tumors with the same type. CEA is actually a family of high molecular weight glycoproteins (all of which are, in turn, part of a “superfamily” of proteins which include the immunoglobulins, certain cell adhesion molecules, etc.) that have recently been assigned the designation CD66 (with the variants CD66a, CD66b, etc.; the older nomenclature ‘CEA’ is equivalent to CD66e [16, 35]). The CEA family includes “true” CEA, along with “nonspecific cross reacting antigen (NCA), biliary glycoprotein (BGP), and others. It has been well-established that antisera prepared by conventional immunization with CEA contain several antibodies of different specificities, i.e., such antisera cross react with NCA and BGP. Most anti-CEA polyclonal antibodies

<table>
<thead>
<tr>
<th>Carcinomas Usually Positive For CEA Expression</th>
<th>Carcinomas Sometimes Positive for CEA Expression</th>
<th>Carcinomas Usually Negative for CEA Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung, non small cell</td>
<td>Breast</td>
<td>Endometrial</td>
</tr>
<tr>
<td>Colorectal</td>
<td>Lung, small cell</td>
<td>Ovarian</td>
</tr>
<tr>
<td>Liver*</td>
<td>Transitional cell</td>
<td>Renal cell</td>
</tr>
<tr>
<td></td>
<td>Cervical</td>
<td>Mesothelioma</td>
</tr>
</tbody>
</table>

* bile canalicular pattern of BGP expression

<table>
<thead>
<tr>
<th>Carcinomas usually showing vimentin co-expression</th>
<th>Carcinomas rarely showing vimentin co-expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal cell carcinoma</td>
<td>Breast carcinoma</td>
</tr>
<tr>
<td>Endometrial carcinoma</td>
<td>Ovarian carcinoma</td>
</tr>
<tr>
<td>Salivary gland carcinoma</td>
<td>Lung carcinoma (small cell, nonsmall cell)</td>
</tr>
<tr>
<td>Mesothelioma</td>
<td>Prostate carcinoma</td>
</tr>
<tr>
<td>Thyroid carcinoma</td>
<td>Colorectal adenocarcinoma</td>
</tr>
<tr>
<td>Spindle cell carcinomas of any location</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Antibodies to</th>
<th>Identifying</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Also identifies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostatic specific antigen [23]</td>
<td>Prostate carcinoma</td>
<td>Very high</td>
<td>Very high</td>
<td></td>
</tr>
<tr>
<td>Prostatic acid phosphatase [22]</td>
<td>Prostate carcinoma</td>
<td>Very high</td>
<td>High</td>
<td>Neuroendocrine carcinomas</td>
</tr>
<tr>
<td>Mesothelin [6, 7]</td>
<td>Serous papillary ovarian carcinomas</td>
<td>Very high</td>
<td>High</td>
<td>Mesothelioma squamous cell carcinomas</td>
</tr>
<tr>
<td>Thyroglobulin [1]</td>
<td>Thyroid carcinoma</td>
<td>High</td>
<td>Very high</td>
<td></td>
</tr>
<tr>
<td>Thyroid transcription factor-1 (TTF-1) [4, 12]</td>
<td>Thyroid and lung carcinomas</td>
<td>High</td>
<td>High</td>
<td>rare other carcinomas</td>
</tr>
<tr>
<td>Surfactant apoA Protein [24]</td>
<td>Lung non-small cell lung CA</td>
<td>Moderate</td>
<td>High</td>
<td>rare breast, other carcinomas</td>
</tr>
</tbody>
</table>
and some monoclonal anti-CEA antibodies react with both CEA and NCA, and hence will identify polymorphonuclear leukocytes in tissue sections. This can have positive as well as negative implications: while these serve as good "built-in" controls, necrotic tumors of any type will often manifest CEA positivity as a consequence of this NCA cross reactivity. New antibodies, such as the II-7 clone, do not show this cross reactivity and can be used in place of, or in conjunction with, the cross-reacting clones. Table 1 summarizes the expression of CEA within different types of carcinomas.

Vimentin co-expression

While co-expression of cytokeratin (the "signature" intermediate filament of carcinomas) and vimentin (the intermediate filament generally associated with mesenchymal cells) is the norm under in vitro conditions, co-expression of vimentin with cytokeratin in vivo is a characteristic of only a limited subset of carcinomas (e.g., renal cell carcinoma, endometrial carcinoma, etc.). One important overriding rule is important to remember: any spindle cell carcinoma (indeed, any spindle tumor of any kind) will co-express vimentin. This is summarized in Table 2.

Tumors in the column at the right may co-express vimentin, albeit rarely. In fact, in the case of breast carcinoma, co-expression of vimentin may be a marker of adverse outcome [11].

Antibodies to tissue-specific proteins

There are relatively few tissue-specific markers that can be exploited in the analysis of metastatic carcinomas. However, by far the most useful are the prostatic markers, PSA (prostatic specific antigen) and PAP (prostatic acid phosphatase). The former is more specific than the latter, as a subset of hindgut carcinoid tumors can also express PAP [3]. When used in combination, these two markers will identify greater than 95% of all metastatic prostatic adenocarcinomas. Other tissue-specific markers include antibodies to thyroglobulin (a thyroid-specific marker) and the gross cystic disease fluid protein-15, a breast/sweat/salivary gland marker. The latter, while characterized in the literature as a good marker of metastatic breast cancer, is, in our experience, positive in only a minority of metastatic breast cancers, especially the infiltrating ductal type, and among poorly differentiated subtypes. We have recently demonstrated that mesothelin is a highly sensitive marker of serous papillary ovarian carcinoma, and that antibodies to surfactant apoA protein identify greater than 65% of pulmonary non-small cell carcinomas. Recently published studies have also pointed to the great potential value of the nuclear protein, thyroid transcription factor-1 (TTF-1), as a powerful marker of lung and thyroid carcinomas [4].
The various tissue-specific markers are summarized in the Table 3, and graded according to their sensitivity and specificity.

**Antibodies to estrogen and progesterone receptor proteins**

Antibodies to estrogen and progesterone receptors can play an important, albeit limited role, in the identification of the primary site of carcinomas presenting at a metastatic site. It is important to note, however, that these antibodies can in no way be considered a specific marker of metastatic breast carcinoma, despite the large numbers of studies performed at many institutions specifically (and exclusively) for this marker. First, it is important to appreciate the wide spectrum of tumors that have the ability to express estrogen and/or progesterone receptors. The major utility of antibodies to ER and PR arises in the recognition that certain tumors that may be in the differential diagnosis never (or almost never) express ER and/or PR. These are summarized in Table 4.

**VI. The Concept of ‘Contextual Specificity’**

Some markers have greater value in restricted clinical situations than in a hypothetical situation in which all primary sites are considered. Thus, if a patient with a history of breast cancer presents with a pulmonary nodule, the presence of ER expression can point rather unequivocally to the diagnosis of metastatic breast cancer and rule out the diagnosis of a second lung nonsmall cell carcinoma [9, 27]. The use of many of the markers outlined above must be considered in this context. Tables 5–7 exemplify this selective use of markers.

**VII. References**


