Potential Pathological Application of Immunocytochemical Methods to the Detection of Micrometastases

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Abstract

Immunocytochemical methods can be used to demonstrate tumor products and antigens at the cellular level. This approach facilitates the classification of tumors and the detection of small metastatic tumor foci in biopsy material from various sites including the bone marrow.

Introduction

During the past few years there has been a renewed interest in the use of radiolabeled antibodies to tumor cell components and/or their secreted products to localize neoplasms in vivo (1, 2, 5). Following the initial experimental animal tumor studies (1, 3), radiolabeled antibodies to CEA2 were used and were found to localize in xenografts of human colorectal carcinomas (11, 15).

Such investigations have now been extended to patients when the use of radiolabeled antibodies to CEA has been shown to result in the in vivo localization of primary and metastatic colorectal carcinomas (5, 6). While this approach has largely confirmed the presence of tumor detected by other clinical or physical diagnostic methods, there are examples where occult tumor deposits have also been found (5, 6). Although future improvements in scanning technology may offer the hope of detecting smaller lesions than are presently detected, other avenues to detect small metastatic tumor deposits seem worthy of consideration.

The ability to demonstrate a wide variety of antigens and products at the cellular and ultrastructural levels by using immunocytochemical methods (6, 15)3 has opened a new era for the pathological classification and histogenesis of tumors. To date, this has had its widest application with respect to germ cell tumors (6). However, the high-quality suitable reagents are also now available to enable this technique to be extended to the commoner tumors.

A new antigen called EMA can be detected immunocytochemically on a wide variety of normal and neoplastic human epithelial tissues including the breast (8).3 It is localized to the luminal membrane of normal breast acini; it is also expressed on the cell membrane and in the cytoplasm of all mammary carcinomas examined to date. Cytoplasmic staining is most intense in isolated carcinoma cells.

Another potential pathological application of immunocytochemical methods is to aid cell detection and identification. For example, is it possible to evolve a more sensitive histological method to detect single or several foci of tumor cells? Pilot studies have been initiated to ascertain whether the immunocytochemical demonstration of EMA will enable the reader detection of micrometastatic mammary carcinoma cells in the bone marrow.

Patients

All patients attending the Breast Clinic, Royal Marsden Hospital, Sutton, England, and who are found to have mammary carcinoma are subjected to a detailed staging protocol before operation and at 3 to 6 monthly intervals thereafter. The staging tests, apart from clinical examination, include: chest X-ray; bone scintigraphy and, where indicated, skeletal surveys; liver ultrasonography and scintigraphy; iliac crest marrow aspiration; full blood count, liver function tests, and various serum and urinary tumor markers. The value of these tests in detecting metastases is the subject of a recent report (3).

Materials and Methods

Tissues and the marrow aspirates were processed for histological examination. The aspirates were fixed in Zenker’s solution, while the tissues were fixed in neutral formalin or Bouin’s solution. All were then processed and embedded in paraffin wax. Sections (5 μm) were cut and either stained with hematoxylin and eosin or used to demonstrate EMA immunocytochemically (7). For this purpose, absorbed rabbit antisera to EMA were used together with affinity purified sheep antirabbit γ-globulin conjugates or peroxidase (8). In addition, alkaline phosphate conjugates were used for bone marrow studies. The tissue distribution of EMA and its nonidentity with other known tumor antigens has been presented elsewhere (13).

Results

Previous studies have demonstrated clearly that EMA withstands conventional histological fixatives and wax embedding (8). Not only is the antigen expressed by primary breast carcinomas but also by their metastases (Fig. 1). Three xenografts of human breast tumors growing in immune-deprived mice also continue to express EMA (Fig. 2).

These techniques have been applied in pilot studies to the marrow aspirates from 2 groups of breast cancer patients. In the first group of 20 patients who have remained clinically disease free for 5 years following mastectomy, none of the 20 marrow aspirates revealed metastatic deposits as judged by conventional histology or using the immunocytochemical methods for EMA.
In the second group of 43 patients who developed metastatic disease, not necessarily involving the skeleton, none of the aspirates were considered to contain a tumor as judged by conventional hematoxylin and eosin morphology. However, with the immunocytochemical method to demonstrate EMA, 8 of the 43 were found to contain tumor deposits mostly occurring as single cells but occasionally in the form of small clumps of cells. Such small deposits were not recognizable on conventional histology. All but one had radiological or scanning evidence of bone involvement (Table 1).

Discussion

Current approaches to the staging of malignant neoplasms involve the use of a wide variety of physical and biochemical methods. The results to date in relation to breast cancer suggest that available methods are far from ideal; most results become positive only late in the course of the disease (3). While the in vivo use of radiolabeled antibodies may represent one approach to the earlier detection of metastases (5), other methods, directed particularly at the finding of micrometastases, are also required.

The skeleton is a major location for metastases from carcinomas of diverse site and histology, including breast cancer. Such lesions are generally found through the use of skeletal surveys and/or bone scintigraphy, but even their diagnostic accuracy and prognostic role have been questioned (4). Others have pointed to the value of bone marrow biopsies and their morphological examination as aids to breast cancer metastatic detection. The incidence of histological evidence of marrow metastases in patients without radiological evidence of disease has ranged from 1.6 to 14% (9, 16). While these differing incidences may reflect sampling errors or the stage of the disease when the marrow was examined, it is also highly possible that it is a reflection of the difficulty in detecting small amounts of tumor tissue in the marrow. Hence, additional techniques to identify tumor cells may be advantageous.

The present pilot study has revealed the ancillary role of immunocytochemical histological techniques, with antibodies directed towards tumor cell components, in the detection of micrometastases. Indeed, many of these deposits take the form

<table>
<thead>
<tr>
<th>Patient</th>
<th>Conventional histology</th>
<th>Immunocytochemical demonstration of EMA</th>
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<tbody>
<tr>
<td>1</td>
<td>N</td>
<td>Negative</td>
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<tr>
<td>2</td>
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</tr>
<tr>
<td>12</td>
<td>N</td>
<td>Positive</td>
</tr>
</tbody>
</table>

Total positive: 8

*N, no metastatic cells detected.

Bone scintigraphy and/or skeletal survey indicated the presence of metastases involving the skeleton but not necessarily the pelvic bones.
of foci of single tumor cells which go unrecognized in conventionally stained preparations. Our study also suggests that this approach complements the cytological examination of marrow aspirates (Table 1). The present incidence of detecting micrometastases in the iliac crest, however, remains relatively low. It may be that a high detection rate will be achieved by sampling the bone marrow at multiple sites and extending the immuno-cytochemical technique to the study of marrow smears. Preliminary studies to demonstrate EMA in such smear preparations are under way, but certain methodological problems need to be solved prior to this approach if it is to have a routine application. The prospective application of those principles to patients without overt metastases but who are considered to have a poor prognosis, as judged by tumor histological grade, receptor content, and axillary nodal status, may help to delineate a subset in whom active therapy should be instituted earlier.

The success of this approach depends on a knowledge of the sites to which the tumor under study metastasizes. With recent evidence suggesting that the patterns of metastases may alter with therapy and length of survival (12), other techniques to assist in selecting the appropriate site for biopsy are needed. It is hoped that the use of radioantibodies may assist in this context. While newer detection technologies may help to achieve this goal, the development of monoclonal antibodies (10) to tumor cell surface components, both known, such as CEA and presently unrecognized ones, may provide a battery of reagents with the necessary selectivity or specificity to increase the sensitivity of both the in vivo and immunocytochemical approach to cancer detection.

References

Fig. 2. Human breast carcinoma. Immunocytochemical demonstration of EMA on the luminal aspect and in the cytoplasm of some but not all of the tumor cells. × 360.
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