Expression of Monoclonal Antibody-defined Tumor Markers in Four Carcinomas

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ABSTRACT

The specificity of monoclonal antibody-based, CA 125, CA 15-3, CA 19-9 and carcinoembryonic antigen (CEA) immunoassays were evaluated by studying how these tumor markers are expressed, using serial serum specimens from patients with breast, ovarian, colorectal, and pancreatic carcinomas. It was found that none of the monoclonal defined markers was specific for any single malignant disease. Multiple markers of increased concentration were found in all serial specimens. However, only one dominant marker was associated with each type of malignant disease. Taking advantage of the different patterns of these tumor markers expressed among different carcinomas, it was demonstrated that the specificity of these monoclonal immunoassays for diagnosis and screening of cancers could be improved if ratios of the dominant marker to other markers were determined. For example, determining ratios of CA 15-3/CEA, CA 15-3/CA 125, and CA 15-3/CA 19-9 will improve the differentiation of elevated CA 15-3 of breast carcinoma from those of colon, ovarian, and pancreatic carcinomas, respectively.

Introduction

To date, immunoassays for tumor markers can be used only as an adjunct test and as a tool for monitoring therapy and detecting recurrence. None of the immunoassays has sufficient specificity for diagnosis or for screening the asymptomatic population.\(^1\)\(^,\)\(^9\) However, in most cases, increased concentrations of tumor markers may be detected several months ahead of other clinical evidences\(^9\)\(^,\)\(^17\) of cancer. If tests for tumor markers can be made more specific, early diagnosis for cancer, before metastasis, would greatly improve the chance of a successful treatment.

The recent advances in hybridoma technology\(^5\) have allowed the production of monoclonal antibodies directed against epitopes (antigenic determinants) on intact tumor cell surface.\(^3\)\(^,\)\(^7\) Most tumor marker tests have been changed from the competitive binding principle to a solid phase sandwich procedure.\(^6\)\(^,\)\(^7\)\(^,\)\(^13\)\(^,\)\(^14\) As a result, the newly developed monoclonal immunoassays are much improved in sensitivity and specificity. However, these improvements are due mainly to reduced interferences and decreased lot to lot and kit to kit variations.\(^13\) Like their polyclonal counterpart, none of the monoclonal immunoassays is specific for any single type of
and are being used in clinical laboratories for the management of cancer patients. They are CA 125 EIA, CA 19-9 RIA, and CA 15-3 RIA. All three kits use monoclonal immunoglobulins developed by somatic hybridization of spleen cells from mice immunized with intact tumor cells. In addition, several improved carcinoembryonic antigen (CEA) kits using monoclonal anti-CEA antibodies have also been introduced recently. Because monoclonal antibody-based immunoassay kits have higher sensitivity and can usually detect elevated concentration of tumor markers in a higher percentage of cancer patients, they are more useful than similar kits using polyclonal antibody for monitoring cancer patients.

![Breast](image)

**Figure 1.** Normalized serum concentration of tumor markers of one patient with metastatic breast carcinoma (case 1). 1A shows that CA 15-3 is the dominant marker for breast carcinoma. Serum concentration of CA 15-3 divided by its upper normal limit, 21, is compared with the normalized CEA level. H-CEA, CEA are determined by the Hybritech kit. 1B shows the rise and fall of normalized CA 15-3 and CEA during the clinical course of one patient in which 21, 3, 35, and 37 are upper normal limits for CA 15-3, H-CEA, CA 125, and CA 19-9, respectively. 1C similar to figure 1B in that it shows the rise and fall of normalized CA 19-9 and CA 125.

malignant disease. In other words, none of the epitopes defined by monoclonal antibodies is restricted to a single type of tumor.

Three monoclonal immunoassay kits recently became commercially available

![Breast](image)

**Figure 2.** Normalized concentration of tumor markers of a patient with metastatic carcinoma of the breast (case 2). See legend to figure 1 for other details.
have the potential to improve the specificity of the CA 125 immunoassay so that it can be used as a much needed diagnostic test for ovarian cancer. In this report the expression of all four monoclonal antibody-defined tumor markers were studied in four embryogenically, closely related carcinomas to determine whether or not the same approach can be applied to other monoclonal immunoassays to improve their specificity for other malignant diseases. Our study shows there is one dominant marker for each type of malignant disease, and ratios among markers can be used to help to differentiate various carcinomas.

Materials and Methods

Specimens

Seven sets of serial serum specimens were collected, two from breast carcinoma, two from nonmucinous ovarian cancer, one from pancreatic carcinoma, one from color carcinoma, and one from rectal carcinoma. All specimens were routine clinical specimens received by the laboratory for testing of different tumor markers. All patients were at cancer stage 3 or 4 with variable extent of metastases, and all were under chemotherapy.

Materials

The kits used were Abbott CEA-EIA One-Step (Monoclonal) and Abbott CA-125 EIA, * CA 19-9 RIA and CA 15-3 RIA, † and Hybritech Tandem-ECEA (Monoclonal). ‡

Methods

The expression of the monoclonal antibody-defined tumor markers in various

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* Abbott Laboratories, North Chicago, IL 60064.
† Amersham Co., Arlington Heights, IL 60005.
‡ Hybritech Inc., San Diego, CA 92121.
carcinomas was followed by assaying, in duplicate, the sera using monoclonal immunoassay kits. The assay procedures are described in the package insert for each kit. These kits are all immunoassays using polystyrene bead-conjugated monoclonal antibody to track tumor marker and enzyme or radioactive labeled monoclonal antibody for quantitation in a sandwich design. All washing steps and color reading are semiautomated.

Results

Dominant Markers

The concentrations of four, monoclonal antibody-defined, markers were measured in all specimens. In order to compare concentrations of different markers on the same scale, they were normalized by dividing the marker values by their individual upper normal limits. Marker concentrations expressed this way allow direct assessment of the extent of elevation. Therefore, a value greater than one indicated that the serum concentration of the marker was increased above the upper limit of normal. Our study indicated that one dominant marker could be identified for each type of malignant disease, even though multiple markers at increased concentrations were found in individual serial specimens. For each malignant disease, the normalized values of the dominant
markers were plotted in figures 1A, 3A, 4A, 5A and 6A in the bar form. Since the increase of the dominant marker was usually far above that of other markers, only the marker with the next highest value was plotted on the same scale with the dominant marker. Bars of other markers were usually too short to be visible at that scale. The outstanding markers for each type of malignant disease were: CA 15-3 for breast carcinoma (figures 1A, 2A), CA 19-9 for pancreatic carcinoma (figure 3A), CEA for colorectal carcinomas (figure 4A), and CA 125 for nonmucinous epithelial ovarian carcinomas (figures 5A, 6A). The only exception was found in figure 5A, in which CA 15-3 exceeded the value of CA 125 for the last portion of the serial specimens from an ovarian cancer patient. This could indicate that CA 125 was more sensitive to respond to chemotherapy than CA 15-3 in ovarian cancer. This area deserves further investigation.

**CO-ELEVATED MARKERS**

In all serial specimens, it was found that more than one marker was elevated. In other words, multiple markers in increased concentration could be observed in every single malignant disease. The number of elevated markers and the degree of elevation of each marker varied from case to case. In table I the dominant and other co-elevated markers are listed in association with each individual tumor. The frequency of elevation is also shown in figures 1 to 6. In addition to the dominant markers, concentrations of CA 125 and CEA were increased more frequently in breast carcinoma, as were CA 15-3 and CA 19-9 in ovarian carcinoma. It appears that a characteristic pattern of monoclonal antibody-defined tumor markers can be demonstrated not only for each type of malignant diseases but also may exist for each individual tumor.

**CONCURRENT CHANGES**

Although the concentration of the dominant marker in serial specimens was most sensitive to reflect the patient’s response to treatment, smaller but similar changes were usually observed in other tumor markers. For example, except for a few specimens, the change of CA 15-3 for breast cancer was far more
sensitive than that of other markers (figures 1B, 2A). Most of the values of CEA by the Hybritech kit (H-CEA), CA 125, and even CA 19-9, showed similar but smaller changes compared to CA 15-3 (figures 1B, 1C, 2A, 2B). Even the concentrations of CA 19-9, which are within normal range, fluctuate in parallel to other elevated markers. It appears that almost all detectable markers reflect the same tumor activity, and the major difference among markers is sensitivity. In other words, the dominant marker is most sensitive to changes of tumor activity.

Ratios Specific to Various Carcinomas

Although only one dominant marker is associated with one type of malignant disease, almost all monoclonal markers could be found at increased concentration in any of these four malignant diseases. To differentiate the elevated concentration of any marker of one carcinoma from the others, it was found that specific ratio could be applied which resulted in an improvement of the diagnostic specificity of the dominant marker. In Table II the different ratios are listed that may help to differentiate the four malignant diseases from each other. All the ratios calculated were plotted in figure 7. The same data were used in figures 1 to 6. Since a logarithmic scale was used, clear differentiation was obtained in every case. In general, the differences are from 10 to 1,000 fold. For example, a ratio of CA 15-3 to CA 125 clearly separates the elevated CA 15-3 of breast cancer from the increased CA 15-3 concentrations found in ovarian carcinoma (7A). The ratio of CA 15-3 to CEA clearly differentiate the elevated CA 15-3 of breast carcinoma from that of colorectal carcinoma. These ratios improve the specificity of CA 125 for nonmucinous ovarian cancer, CA 19-9 for pancreatic cancer, CA 15-3 for breast carcinoma, and CEA for colorectal carcinomas.

Discussion

It is important to note that since only small number of cases have been used in
this study, the results should be considered provisional. A more extensive investigation involving more cases is needed for confirming the current results before recommending ratio determinations for cancer diagnosis. Nevertheless, by using four monoclonal immunoassay kits on specimens from four embryogenically closely related carcinomas, it was possible to identify a relatively specific epitope (or tumor marker defined by monoclonal antibody) for each neoplasm. Although more than one marker was elevated in any single serial specimen, the concentration of the dominant marker was usually far above that of other markers.

There are some advantages of using serial specimens to study the specificity of tumor markers. The specific increase and decrease of the serum concentration of any given marker responding to the treatment is better appreciated in serial specimens. In random specimens, any low marker concentration detected in certain cancer patients could be mistaken as a lack of sensitivity or specificity of the kit used. How much increase and decrease is noted of a tumor marker in serial specimens may also indicate the relative specificity and sensitivity of a tumor marker when elevated concentrations of several markers are found. Such distinction would be impossible in random specimens. Use of serial specimens from closely related carcinomas was especially useful in comparing the specificity of these four monoclonal immunoassays and led to the idea of using ratios to improve further their specificity.

The success of using ratios to differentiate among neoplasms when they all contain increased concentrations of the same marker relies on the identification of a dominant marker for each neoplasm and the presence of a large difference in concentration between the dominant marker and other markers in each malignant disease. For example, when elevated concentration of marker A was detected in two malignant diseases (A and B), the ratio of markers A to B will help to differentiate neoplasm A from B—provided that markers A and B are dominant markers for neoplasm A and B, respectively. A good example is how CA 15-3 to CA 125 would differentiate breast carcinoma from ovarian carcinoma when both show elevation in CA 15-3. In this case, CA 15-3 and CA 125 are dominant markers for breast and ovarian cancers, respectively. The same rationale applies to the selection of other ratios. However, a more extensive study is needed in the future to establish the means and ranges of ratios for each type of malignant disease and ratio for normal reference range. It is hoped that by identifying dominant markers as well as how frequently other markers may also be elevated for each carcinoma, it may be possible to simplify the number of different ratios required for each neoplasm in order to improve the specificity for their diagnosis. In our earlier studies concerning ovarian cancer, it was found that the ratio of CA 125 to CEA alone provided sufficient specificity to separate nonmucinous epithelial ovarian cancer from

<table>
<thead>
<tr>
<th>Malignant Disease (Carcinoma)*</th>
<th>CA 15-3‡</th>
<th>CA 125</th>
<th>CA 19-9</th>
<th>CEA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>All‡</td>
<td>125/15-3</td>
<td>19-9/15-3</td>
<td>CEA/15-3</td>
</tr>
<tr>
<td>Ovarian</td>
<td>15-3/125§</td>
<td>All</td>
<td>19-9/125</td>
<td>CEA/125</td>
</tr>
<tr>
<td>Colorectal</td>
<td>15-3/CEA</td>
<td>125/CEA</td>
<td>19-9/CEA</td>
<td>All</td>
</tr>
<tr>
<td>Pancreatic</td>
<td>15-3/19-9</td>
<td>125/19-9</td>
<td>All</td>
<td>CEA/19-9</td>
</tr>
</tbody>
</table>

* Serum specimens were obtained from patients with these malignant diseases. Ratios are designed to differentiate between them when specimens contained increased marker concentrations.

† Dominant markers whose diagnostic specificity could be improved by ratio determinations.

‡ "All" means that all the ratios listed are in the same vertical column.

§ 15-3, 19-9, and 125 are abbreviated forms of CA 15-3, CA 19-9 and CA 125, respectively.
other nonovarian cancers, even though they all contain elevated serum CA 125 concentrations.

It appears that identification of the dominant marker and appropriate ratio for different carcinomas would help to identify the unknown primary site of a metastatic cancer. Determining the ratio may provide a more accurate classification and staging of carcinomas, similar to the use of alpha-fetoprotein (AFP) and human chorionic gonadotropin (HCG) in germ cell tumors.\textsuperscript{11} It is necessary to investigate what type of molecules in the serum and in the tumor tissues are associated with these tumor markers. Information concerning the extent and frequency of expression of these tumor markers, in relation to the general tumor activity and particularly with various
Pancreatic

10^4

10^3

10^2

10^1

10^-1

10^-2

10^-3

10^-4

199/CEA Pan

199/CEA Rec

199/125 Pan

199/125 Ov

199/153 Pan

199/153 Ov

Figure 7. 7G to 7I show improved differentiation of pancreatic carcinoma from other carcinomas when ratios of CA 19-9 to other markers were used. 7J to 7L show the improved differentiation of colorectal carcinomas from others when ratios of CEA to other markers were used.

Colorectal

10^3

10^2

10^1

10^-1

10^-2

10^-3

10^-4

CEA/125 Re

CEA/125 Ov

CEA/199 Re

CEA/199 Pan

CEA/153 Re

CEA/153 Br

References


4. CHAN, D. W., BRUZEK, D. J., OESTERLING, J. E., et al.: Prostate-specific antigen as a marker


