CA-549: Immunohistochemistry and Serum Levels in Breast Carcinoma and Other Neoplasms*

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ABSTRACT

CA-549 is a high molecular weight acidic glycoprotein found in the serum of breast cancer patients. Detection of CA-549 in serum using an immunoradiometric assay has [1] correlated with disease course in breast cancer patients and [2] aided in establishing the diagnosis of breast cancer in patients with metastatic disease. This study examines the expression of CA-549 using immunohistochemistry in normal breast, benign breast disease, breast carcinoma, and a variety of other carcinomas. In addition, in 29 patients both serum and tissue samples were available for correlation. CA-549 was constitutively expressed in normal breast tissue, but immunohistochemical positivity was restricted either to the luminal aspect or entire cell membrane. All patients with benign breast disease had normal levels of CA-549 despite immunohistochemical positivity. Nearly all (98 percent) of breast carcinomas showed reactivity for CA-549, with a majority (82 percent) of the cases showing cytoplasmic positivity. In patients with both serum and tissue studied, those with cytoplasmic staining of the breast carcinomas had mean serum level of 174 U per ml (range 1.9 to 785), compared to 37.3 U per ml (range 2.1 to 86.8) in those with only membrane or luminal staining of tumor (p = 0.0578). Immunoreactive CA-549 was found in many normal epithelia and in other types of carcinomas. CA-549 is [1] constitutively expressed on the cell membrane of normal breast epithelium, [2] commonly present in the cytoplasm of breast carcinomas, and [3] found often in a variety of carcinomas.

Introduction

CA-549 is a high molecular weight acidic glycoprotein detected by a new

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monoclonal antibody, BC4E549, raised by immunizing mice with a partially purified membrane preparation from T417 human breast tumor grown in nude mice.² CA-549 was adapted to an immunoradiometric serum assay and is a new marker for breast cancer.¹⁻³ It was demonstrated by us earlier that CA-549 serum assay is a relatively specific marker for breast cancer and that it was superior to carcinoembryonic antigen (CEA) in monitoring disease progression.¹ Elevated serum levels were found in a minority of patients with other epithelial malignancies, but they tended to be lower than the levels seen with patients with breast carcinoma.¹⁻³

In light of the relative specificity and sensitivity of CA-549 as a serum marker for breast carcinoma, the potential utility of CA-549 has been evaluated as an immunohistochemical marker for mammary carcinoma in formalin-fixed, paraffin embedded tissue. In addition, both immunohistochemical positivity and the pattern of positivity were correlated with serum levels of CA-549 when available.

Materials and Methods

Case Selection

Serum samples were obtained from patients with benign breast disease and malignant neoplasms of breast, colon, lung, prostate, ovary, and endometrium. These randomly selected patients had participated in a previous study to evaluate CA-549 serum levels.¹ Of the 301 patients in the previous study, 84 had surgical pathology material available for immunohistochemical study. Both serum levels and tissue immunoreactivity were correlated in this population which consisted of 26 cases of benign breast disease, and 29 breast, 16 colon, eight prostate, and five lung carcinomas. To study further the immunohistochemistry of CA-549, a variety of malignant epithelial tumors with available paraffin blocks, were chosen from the surgical pathology files of The Johns Hopkins Hospital.

Monoclonal Antibodies

**BC4N154** is a murine IgM monoclonal antibody produced in fusion using splenic lymphocytes from a mouse immunized with human milk fat globule membranes. This antibody was covalently linked to polystyrene beads and used only in the immunoradiometric (IRMA) assay.²

**BC4E549** is a murine IgG1 monoclonal antibody derived from fusion using splenic lymphocytes from a mouse immunized with purified membranes from the T417 human breast tumor cell line grown in nude mice.³ This antibody was used as the labelled antibody in the IRMA serum assay and as the primary antibody for the immunohistochemical studies.

Serum Assay

Serum analyses were performed using an immunoradiometric assay (IRMA) as previously described.¹⁻² Any CA-549 level >11 Units per ml was considered elevated.¹

Immunohistochemistry

Immunohistochemical staining was adapted to a Code-On immunohistochemical stainer.† Briefly, 6μ sections were cut from routinely processed 10 percent buffered formalin-fixed and paraffin embedded tissue blocks, deparaffinized, rehydrated in Tris saline buffer (50 mM Tris, 150 mM NaCl), and treated with three percent H₂O₂ in absolute methanol for five min to block endogenous peroxidase activity. Following

* Hybritech, Inc., San Diego, CA.
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pronase treatment‡ 0.5 mg per ml for 30 min at 37°C, slides were rinsed with Tris buffer and blocked with non-immune horse serum, 1:100. BC4E549 monoclonal antibody diluted 1:400 in Tris-saline was applied for 30 min at 37°C, followed by biotinylated horse anti-mouse, 1:100§ and preformed, prediluted avidin-peroxidase complex‖. Aminoethylcarbazole (Biomeda) was utilized as the chromogen. Non-immune mouse serum served as a negative control. The counterstain was Mayer’s hematoxylin.

**Results**

**IMMUNOHISTOCHEMICAL DETECTION OF CA-549**

*Cellular Patterns:* CA-549 was expressed to a varying degree by a wide variety of normal epithelial tissues (table I) and epithelial neoplasms (table II). Within individual cells two patterns of staining were identified: a luminal/membranous pattern or cytoplasmic pattern. With the luminal/membranous pattern, CA-549 immunoreactivity was found in either the luminal aspect of cells occupying a gland, or rimming the entire cell membrane; there was a concomitant absence of cytoplasmic positivity. With the cytoplasmic pattern diffuse staining of the cytoplasm usually occurred in the majority of the cells. In both staining patterns, nuclei were always negative. For classification purposes, tissues that showed any degree of unequivocal immunoreactivity were considered as “positive.” Expression of any appreciable degree of cytoplasmic reactivity resulted in classification as the cytoplasmic pattern.

*Non-neoplastic Tissue:* Normal epithelium from a variety of organs, when positive, tended to show uniform staining intensity and exclusively the luminal/membranous pattern of cellular positivity. In figure 1A is illustrated this pattern of uniform luminal/membranous staining in the normal breast. A similar staining pattern was seen in benign breast disease (figure 1B).

*Neoplastic Tissue:* In contrast to the relatively uniform staining of non-neoplastic tissue, tumors showed marked variation in staining intensity reflecting tumor heterogeneity as seen with other tumor markers.7,8 In breast carcinoma, immunohistochemical staining for CA-549 was seen in 57/58 (98 percent) of cases. In these cases the majority of the tumor cells showed cytoplasmic staining.

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*All normal epithelia showed only luminal/membranous pattern of staining.

**TABLE I**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Positive Staining* (Percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>26/26 (100)</td>
</tr>
<tr>
<td>Colon</td>
<td>13/28 (46)</td>
</tr>
<tr>
<td>Lung</td>
<td>6/6 (100)</td>
</tr>
<tr>
<td>Prostate</td>
<td>13/20 (65)</td>
</tr>
<tr>
<td>Endometrium</td>
<td>5/5 (100)</td>
</tr>
</tbody>
</table>

*Cytoplasmic Pattern.
†Luminal/Membranous Pattern.
The cytoplasmic staining pattern predominated, occurring in 47/57 (82 percent) of cases.

The incidence of CA-549 immunohistochemical positivity in the other carcinomas ranged from 8 to 100 percent with cytoplasmic staining in 0 to 80 percent of the tumors depending on their primary site (table II).

**Correlation of CA-549 Levels and Immunohistochemistry:** All 26 patients with benign breast disease had normal serum levels of CA-549 and showed exclusively the luminal/membrane staining of normal breast structures or fibrocystic disease.

Of the 29 cases of breast carcinoma with both serum levels and immunoperoxidase staining, 28 (96 percent) showed positive immunohistochemical staining for CA-549. 18/28 (64 percent) had cytoplasmic staining and 10/28 (36 percent) the luminal membranous pattern.

CA-549 serum levels were elevated in 23/29 (79 percent). The mean serum level for the entire group was 121.9 U per ml (range 1.9 to 785.0). The mean serum level for the cases that showed the cytoplasmic staining pattern on immunohistochemistry was 174.5 U per ml (S.D. = 263.6, range 1.9 to 785.0), while the mean
Figure 2. (A): Low power view illustrating the difference in immunohistochemical staining pattern for CA-549 between the centrally located normal breast duct with faint luminal staining, and the surrounding infiltrating duct carcinoma (200×). (B): Immunohistochemical staining for CA-549 in a case of infiltrating duct carcinoma of breast. Note the presence of intense cytoplasmic staining, and contrast to exclusive luminal staining seen in figure 1 (600×).

serum level for the cases with the luminal/membranous pattern of immunohistochemical staining was 37.3 U per ml (S.D. = 29.8, range 2.1 to 86.8). Although these results show that there is a tendency for higher serum levels among the cases with the cytoplasmic pattern of staining as compared to those with luminal/membranous pattern, the difference was of borderline statistical significance (student's t-test p = 0.0578).

Additionally, serum levels and immunohistochemical staining data were available on 16 colon, eight prostate, and five lung carcinomas. 15/16 (94 percent) of the colon adenocarcinomas were positive for CA-549 in a luminal/membranous pattern by immunohistochemistry. Only two of these patients (13 percent) had modest elevations of the serum levels (11.6 and 15.1 U per ml).

Six (75 percent) of eight prostate adenocarcinoma specimens had immunohis-
tochemical staining for CA-549 with only one case showing a cytoplasmic pattern; 5/8 (63 percent) had elevated serum levels with a mean of 14.7 U per ml (range 11.5 to 29.4 U per ml). The highest serum level, 29.4 U per ml, was seen in the patient with cytoplasmic staining.

All five (100 percent) of the lung carcinomas were immunoreactive with one case showing a cytoplasmic pattern. None of these patients had elevated serum levels.

Discussion

The development of non-invasive techniques to monitor disease progression in breast cancer patients remains an important clinical goal. Serum levels of CA-549 have been shown to be both a sensitive and specific marker for tumor progression in patients with breast carcinoma.1 In addition, markedly high serum levels of CA-549 were strongly suggestive of breast carcinoma in patients with metastatic disease.1,3 Our immunohistochemical studies sought to determine if CA-549 was a breast-specific marker in tissue and if serum levels correlated with tissue reactivity.

Correlation of immunohistochemical studies and serum levels have been performed for carcinoembryonic antigen (CEA),4 alpha-fetoprotein (AFP), and human chorionic gonadotrophin (HCG).6,9 Despite the subjectivity of assessing immunohistochemical staining, these studies found that immunohistochemical positivity for these substances correlated with serum levels and were helpful in both tumor classification and monitoring of metastatic disease. Differentiation of embryonal carcinoma, endodermal sinus tumors, choriocarcinoma, and seminoma was enhanced using tissue and serum correlation.9 Studies with CEA showed that tumors with strong CEA immunohistochemical positivity were more likely to have elevated serum levels. Thus, tissue staining could be used to predict which patients would benefit from long term follow-up of serum CEA levels for assessment of metastatic disease.4

Our results show that although serum CA-549 levels were elevated in only 1.5 percent of healthy women,1 nearly all epithelial structures in the normal breast expressed CA-549. Similarly, in patients with breast cancer, elevated CA-549 levels ranged from 63 percent in partial remission to 83 percent with disease progression (1); yet, 98 percent of breast cancers demonstrated immunohistochemical positivity for CA-549. However, despite the fact that normal breast structures reacted as frequently as carcinoma, it was the pattern of CA-549 reactivity which appeared to differ in the benign and malignant breast tissue and to correlate with the likelihood of elaboration into the serum. Normal breast and benign breast disease showed exclusively membranous or luminal positivity with concomitant normal serum levels, whereas the carcinomas showed predominantly cytoplasmic positivity and elevated serum levels. The pattern of immunohistochemical reactivity seen in this study is similar to the observations of Demers et al3 on the immunohistochemistry of CA-549, and to those reported with other related glycoproteins.5,10 Among the breast cancer patients with both CA-549 immunohistochemistry and serum levels, those whose tumor biopsies had cytoplasmic reactivity tended to have higher serum levels than those with luminal or membranous positivity, although in this small population of patients there was not statistical significance (p = 0.0578).

Though markedly elevated serum levels of CA-549 strongly suggested the diagnosis of metastatic breast carcinoma in a patient with metastatic disease of unknown primary,1 tissue immunoreactivity is not limited to the breast. A wide
variety of normal epithelia and carcinomas express CA-549 epitopes (table II).

As a serum test, CA-549 has been shown to be helpful in monitoring patients with breast carcinoma and may be a helpful adjunct in establishing the diagnosis of breast carcinoma. The diagnostic utility of CA-549 immunohistochemistry, however, remains limited. It is not a breast specific marker and the high frequency of positivity in breast carcinoma makes it an unlikely candidate as a prognostic indicator.

The mechanisms responsible for the cellular localization of CA-549 and its secretion into the serum are unknown. Further studies aimed at understanding the processing of the CA-549 antigen and its subcellular localization are warranted as they could provide clues to the biochemistry of secretory proteins and differentiation of human carcinomas.

Acknowledgment

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References