Loss of Transporter in Antigen Processing 1 Transport Protein and Major Histocompatibility Complex Class I Molecules in Metastatic versus Primary Breast Cancer

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Abstract

We studied by immunohistochemistry the HLA-allelic, β2-microglobulin, and TAP-1 expression in primary breast carcinomas and related lymph node metastases. Thirty-three of the primary tumors and 44% of the lymph node metastases had a complete HLA class I loss. The higher incidence of antigenic loss in metastatic tumors suggests that recognition of HLA class I antigens by the host immunity could have an important role in the metastatic evolution of breast cancer. We observed a simultaneous defective expression of all three components involved in HLA class I expression. Since the controlling genes of heavy chain and TAP-1 are located in different chromosome than β2-microglobulin, it could be that a common factor exists regulating HLA class I antigen expression. Five of 25 (20%) primary and metastatic tumors from HLA-A2-positive individuals also had a selective loss. The high incidence of HLA class I loss in breast cancer patients shows that adjuvant immunotherapy to induce HLA class I expression could be of value in a subgroup of patients.

Introduction

HLA class I antigens are highly polymorphic transmembrane glycoproteins composed of one heavy chain encoded in the HLA-ABC region of chromosome 6 and a light chain (β2-m), encoded by a gene on chromosome 15. Their main function is to present endogenous antigenic peptides to cytotoxic T-lymphocytes. These peptides derive from the cytoplasmic protein pool and are transported into the pre-Golgi region by the MHC class II encoded proteins, TAP-1 and TAP-2 (1). Antigenic peptides are necessary for generating stable class I complexes in the endoplasmic reticulum. HLA class I antigenic expression is essential for T-cell-mediated cytotoxicity. Induction of HLA class I molecules in HLA class I negative cancer cells stimulates autologous T cells and cytoxicity. Some studies suggest that HLA class I antigens might function as restricting elements for T-cell recognition of tumor-specific antigens (2). To investigate the involvement of HLA class I antigenic loss in the evolution of clones that would escape immune surveillance and the role of the recently discovered TAP-1 gene, we studied the HLA-allelic, β2-m, and TAP-1 molecule expression immunohistochemically in primary breast carcinomas and related lymph node metastases. Histology, estrogen, and EGFR expression were also studied in an attempt to identify the relation of HLA class I antigenic loss to aggressive tumor behavior.

Materials and Methods

Representative samples of tumor specimens were collected following surgery from 63 patients with primary breast cancer and positive axillary lymphadenopathy confirmed by light microscopy. All of the above tissues were snap-frozen in liquid nitrogen and stored at −70°C. For 46 of these cases, quantitative ER and EGFR determination was carried out. Levels higher than 10 fm and 20 fm of specific binding sites/μg of cytosolic or membrane protein were considered to be positive for ER and EGFR, respectively. The receptor assays have been described previously (3).

In Table 1 are summarized the primary MABs used and their specificity. Immunohistochemical staining was performed using the APAAP method as described by Cordell et al. (4). Cytostatic tissue sections 5 μm thick were fixed in acetone for 10 min at room temperature, left to dry overnight, and stained immediately afterwards. The primary antibodies were added to the dry sections in a moist chamber for 30 min. Anti-mouse immunoglobulins (DAKO, Copenhagen, Denmark; 1:50 dilution with TBS [Tris hydroxymethylaminomethane buffered normal saline]) and APAAP complex (DAKO; 1:50 dilution with TBS) were added and incubated for 30 min each. This step was repeated twice (incubations of 10 min) to enhance the intensity of the final staining. The alkaline phosphatase substrate was applied afterwards for 20 min, and the alkaline phosphatase substrate was applied afterwards for 20 min, and the sections, after washing with TBS and tap water, were counterstained with hematoxyline and mounted in a suitable aqueous mounting medium. All antibody incubations were followed by 2 min washing in TBS. Paraffin sections were also studied using an avidin-biotin complex/horseradish peroxidase method for HCA2 MAb in all cases in which loss of W6/32 MAb was detected with the APAAP method in frozen sections.

Normal stromal and lymphocytes present within the stained section were studied in each case as a control. A particular allele was considered to be lost by the malignant cells only in that case where adjacent tissue was positively stained by the specific MAb. When both stromal and tumor cells were not stained by the MAB, the allele was considered not to be expressed by the patient.

Results

Positive labeling for HLA class I antigens was detected by the W6/32 MAb in all normal breast epithelia, stroma, and lymphoid cells. The staining was strong, uniform, and more evident on the cell membrane than in cytoplasm. Of 63 patients, 41 were HLA-A2 positive, with the positivity being detected with the MA2.1 MAb (Table 1).

For 21 of 63 cases, the primary tumor cells did not stain with W6/32, thus complete loss of HLA class I antigenic expression was observed in 33% of our cases. For all of these cases, staining for β2-m (BBM1 MAb) and TAP-1 transport protein (MA2.1 MAb) was also negative, showing a coordinate loss of expression for different genetic loci within tumor cells. There was no case HLA class I antigenic loss, which was exclusively attributed to defective expression of heavy chains, β2-m, or TAP-1 defective expression alone (Fig. 1 a and b).

Of 41 patients that were HLA-A2 positive, 25 of them had tumors bearing HLA class I antigen detected by the W6/32. In 5 of these 25 cases, tumor cells did not stain with the MA2.1 MAb (but did with the
LOSS OF TAP 1 AND MHC CLASS 1 IN METASTATIC BREAST CANCER

Table 1 MAbs used, specificity, and results obtained in 63 primary tumors and related lymph node metastases

<table>
<thead>
<tr>
<th>MAbs</th>
<th>Specificity</th>
<th>Normal expression</th>
<th>Pr. a Tumor(-)/stroma(+)</th>
<th>Met. tumor(-)/stroma(+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>W6/32</td>
<td>β2-m/hc</td>
<td>63/63</td>
<td>21/63</td>
<td>28/63</td>
</tr>
<tr>
<td>HCA2</td>
<td>hc/A-locus</td>
<td>63/63</td>
<td>21/63</td>
<td>28/63</td>
</tr>
<tr>
<td>BBM1</td>
<td>β2-m</td>
<td>63/63</td>
<td>21/63</td>
<td>27/63</td>
</tr>
<tr>
<td>AK1.7</td>
<td>TAP-1</td>
<td>63/63</td>
<td>21/63</td>
<td>27/63</td>
</tr>
<tr>
<td>MA2.1</td>
<td>A2/B17</td>
<td>41/63</td>
<td>26/41</td>
<td>33/41</td>
</tr>
<tr>
<td>Total loss</td>
<td></td>
<td>21/63</td>
<td>28/63</td>
<td></td>
</tr>
<tr>
<td>A2 allele selective loss</td>
<td>5/25</td>
<td>5/25</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Pr., primary; Met., metastatic.

Selective loss refers to HLA-A2 allelic loss observed in 5 of 25 HLA-A2-positive individuals (MA2.1-positive individuals) suffering from W6/32-positive tumors (bearing β2-m/hc complex).

Loss of TAP 1 and MHC class 1 in metastatic breast cancer metastases had a complete loss of HLA class I molecule expression versus 21 of 63 (33%) of the primary tumors (P = NS, χ² test).

HLA class I loss was not related to T-stage, grade, or histology. ER and EGFR expression was also analyzed in 46 cases. Of 22 ER+/EGFR+ primary tumors, only 5 (5 of 22, 22%) had a total loss of the HLA class I molecules. Nine of 19 (47%) of EGFR-positive cases had a total HLA class I antigenic loss (P = NS).

Discussion

Since the ability of cytotoxic T-lymphocytes to recognize antigens is mediated by the MHC class I molecules, loss of expression of HLA class I led to the suggestion that host immune response might be important in the control and elimination of malignant cells (5). The prognostic significance of HLA antigenic loss from breast or other cancer cells remains controversial. Expression of class I and II molecules were found to be related to a better differentiation and prognosis in 94 breast tumors (6). A study on 32 node-negative breast carcinomas showed that HLA-DR was related to a better 5-year, disease-free survival, although multivariate analysis failed to show that HLA-DR expression was an independent prognostic factor. Wintzer et al. (7) found that HLA class I antigenic expression had no apparent influence on disease-free and overall survival of breast cancer patients. However, our observation that lymph node metastasis has a higher incidence of HLA class I antigenic loss suggests that clinical studies on the prognostic role of HLA expression in primary tumors could be compromised by the eventual antigenic loss in metastatic sites as compared to the examined primaries.

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W6/32, showing a selective loss of the HLA-A2 allele (Fig. 2, a and b). Thus, 5 of 25 (20%) HLA-A2-positive individuals with primary tumors bearing HLA class I molecules had a selective loss of the HLA-A2 allele. Sixteen of 41 had complete loss (39%).

For all 63 cases, the involved lymph nodes were also stained with the same MAbs. In all 26 cases with total or selective HLA class I allelic loss, the metastatic tumor to the lymph nodes showed the same features. However, 7 out of 37 cases in which the primary tumor expressed the whole range of the tested HLA class I antigens showed a total loss of HLA class I antigenic expression (Fig. 3 a and b). In one of these seven cases, the β2-m and the TAP-1 molecule remained expressed (Table 1). Twenty-eight of 63 (44%) of the lymph node
The HLA class I expression in primary tumor and related lymph node metastases has not been studied extensively. Cromme et al. (8) in a recent study showed that lymph node metastases from cervical carcinomas display a significant decrease of HLA class I expression compared with the primary tumor. Our study is the first that reports a comparative interpretation of HLA class I antigenic loss in primary breast cancer and related metastatic tumor deposits in the axillary lymph nodes. Forty-four % of the tumoral lymph node metastases had a complete HLA class I antigenic loss compared to the 33% antigenic loss of the primary tumors. The fact that 5 of 25 (20%) primary and metastatic tumors from HLA-A2-positive individuals had a selective loss of the allele shows that the combined total and selective HLA class I antigenic loss in primary breast cancer and related nodal metastases is at least 53 and 64%, respectively. It is likely that if other specific HLA class I epitopes were studied, this would be higher.

IFN-α and IFN-γ were potent inducers of HLA class I in a variety of breast cell lines (9, 10), and the differentiating agent all-trans-retinoic acid enhanced HLA-DR expression (11). Our observation that metastatic spread to lymph nodes is associated with HLA class I antigenic loss in 7 of 42 (17%) primary breast cancers bearing HLA class I antigens shows a possible role of HLA loss in the metastatic evolution of breast cancer. Adjuvant immunotherapy with IFN-γ and retinoic acid could be of value in a subgroup of breast cancer patients with negative tumor, HLA class I antigenic expression.

The relation of hormone and other growth factor receptors to the HLA class I antigenic expression in breast cancer has not been studied thoroughly. Hormones could have an influence on the expression of HLA antigens and, therefore, could indirectly be involved in tumor behavior regulation (12). Solary et al. (13) showed in breast cell lines that IFN-γ, which is a potent HLA class I antigen inducer, exerts its antiproliferative action through an inhibition of estradiol effects and reduction of the formation of an estrogen-induced protein (13). In the present study, we examined the ER and EGFR relation to HLA class I molecule expression in 46 node-positive breast cancer patients. Although the number of cases was too small for a statistically significant conclusion, it seems that positive EGFR receptors are related to an HLA antigenic loss in nearly 50% of cases, while only 20% of ER+/EGFR− cases suffer from HLA expression alterations. The poorer prognostic outcome (14) of ER− and EGFR− cases may be partially attributed to HLA-related escape of tumoral cells from immune surveillance.

In all 21 primary tumors with total loss of the HLA class I molecules, a defective expression of all three components (heavy chain, β2-m, and TAP-1 molecule) involved in the HLA antigenic presentation was noticed. Since the β2-m gene is located to a different chromosome to that encoding the heavy chain and TAP-1, it is possible that a common factor exists regulating these genes. The activation of an unknown oncosuppressor HLA class I-related genes located in different chromosomes could be an explanation for this finding. c-myc, an oncogene expressed in breast cancer, is found to down-regulate the expression of HLA class I molecules in melanomas (15) but not in non-small cell lung carcinomas (16). In our study, EGF gene expression was related to a higher incidence of HLA class I antigenic loss. It would be interesting to study whether over-expressed oncosuppressors in breast cancer, such as EGF, c-myc, c-erbB-2 or others are involved in HLA down-regulation. Recently, Danioz et al. (17) identified a new gene conserved in the mouse MHC, encoding a putative GTP-binding protein that could have a regulatory role.

The recent study from Nistico et al. (18) where two human α/β T-cell clones recognizing autologous breast tumor cells through HLA- and TCR/CD3-independent pathways were characterized, shows that expression defects other than HLA class I antigenic ones could be also involved in breast cancer escape from T-cell-mediated immune surveillance. However, our finding that 64% of tumors metastasized to the lymph nodes have complete or selective loss of the HLA class I antigens requires further investigation. In particular, whether HLA class I molecules can be induced in vivo in breast cancer patients by cytokines and enhance the immune response are questions that issue from the present study.

Acknowledgments

We thank Alain Townsend for supplying the AK1-7 antiserum and Nick Murray, Ann Hill, and Peter Krausa for supplying the antibodies used in this study.

References

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