Loss of HLA Haplotype in Lung Cancer Cell Lines: Implications for Immunosurveillance of Altered HLA Class I/II Phenotypes in Lung Cancer

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ABSTRACT

Loss of expression of HLA class I antigens has been demonstrated in a wide variety of tumors and is considered to be one of the mechanisms whereby tumors escape T-cell surveillance. Genomic DNA of MHC class I/II molecules in seven lung cancer cell lines was investigated and compared with that in peripheral blood mononuclear cells. In three cell lines, OU-LC-A1, OU-LC-A2, and OU-LC-AS1, a loss of HLA haplotype was observed. Aberrations of HLA class I/II in tumor cell lines should be considered when MHC-restricted phenomena in vitro models are assessed and clinical use of tumor vaccination in vivo is considered.

INTRODUCTION

HLA class I molecules are transmembrane glycoproteins that consist of two polypeptide chains. The class I heavy chains are encoded by three principal genes (HLA-A, B, and C) within the HLA region of chromosome 6. The products of these genes are associated at the cell surface with a nonpolymorphic light chain, β2 microglobulin, and a cell-derived peptide to form a stable complex. Loss of expression of HLA class I antigens has been demonstrated in a wide variety of tumors. This may represent a mechanism whereby tumors escape T-cell surveillance (1–5).

Lung cancer represents the most frequent cause of cancer death among men worldwide, with an increasing overall incidence. The overall survival of lung cancer still remains ~10%. Especially, advanced non-small cell lung cancer has an extremely poor prognosis because it is resistant to currently available chemotherapy and radiotherapy regimens (6). Therefore, development of specific immunotherapy using tumor-specific CTLs or tumor antigens is important for improving treatment modalities.

In this study, we investigated MHC class I/II molecules on seven newly established non-small cell lung cancer cell lines using PCR of genomic DNA.

MATERIALS AND METHODS

Cell Lines. Seven lung cancer cell lines were newly established in our laboratory. One cell line, OU-LC-AS1, was obtained and established from supravacuicular lymph node metastasis of a male with adenosquamous carcinoma of the lung. Five cell lines, OU-LC-A1, OU-LC-KI, OU-LC-A2, OU-LC-A3, and OU-LC-A4, were established from pleural effusion of males with adenocarcinoma of the lung. EBC-2 was established from pericardial effusion of males with squamous cell lung cancer. The cells were cultured in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum.

DNA Samples. Genomic DNAs were prepared by the guanidine thiocyanate buffer [4 M guanidine thiocyanate, 25 mM sodium citrate (pH 7.0), 0.5% sodium N-lauroylsarcosinate, and 1% mercaptoethanol] lysis and phenol-chloroform extraction. First, leukocytes or cultured cells were mixed with guanidine thiocyanate buffer. After the solution was extracted twice with phenol and chloroform/isoamylalcohol (29:1), DNA was precipitated with ethanol and suspended in 100 ng/μl in 10 mM Tris-HCl-1 mM EDTA buffer.

HLA-A, -B, -C, -DR, and -DQ Locus-specific PCR. Genomic DNA from each lung cancer cell lines or autologous PBMCs was typed for HLA-A using the PCR-RFLP and PCR-sequence-specific primer method described previously (7), for HLA-B using the PCR-sequence-specific oligonucleotide probes method described previously (8), for HLA-C using the PCR-sequence-specific primer method described previously (9), and for HLA-DQ and -DR using the PCR-RFLP method described previously (10–12).

HLA Class I/II Serological Typing of Alleles. T lymphocytes were prepared by Ficoll-Hypaque centrifugation followed by nylon-wool separation. Serological HLA class I/II typing of the cell-enriched preparations was performed by a standard two-stage complement-dependent microcytotoxicity test using at least three monospecific alloantisera for the definition of each antigen (13).

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2 The abbreviation used is: PBMC, peripheral blood mononuclear cell.
Loss of HLA Haplotype in Lung Cancer

**RESULTS**

**Loss of HLA Haplotype in Non-Small Cell Lung Cancer Cell Lines.** Table 1 shows a summary of HLA-class I/II typing of PBMCs and lung cancer cell lines from seven patients. Typing of PBMCs from OU-LC-AS1 was performed by PCR of the genomic DNA. Typing of PBMCs from other patients was performed by microcytotoxicity assay. HLA antigens from both alleles were detected in PBMCs from all seven patients. MHC class I and II genes in the lung cancer cell lines derived from those patients were investigated by PCR of the genomic DNA. A haplotype loss was observed in the three cell lines, OU-LC-A1 (A*2402, B*4002, Cw*0303, DR8, DQ*0402), OU-LC-A2 (HLA-A*2601, B*1501, Cw*0401, DR14, DQ*0302), and OU-LC-AS1 (HLA-A*1101, B*5502, Cw*0102, DR4, DQ*0401).

**Loss of HLA Haplotype in OU-LC-AS1 Cells.** Fig. 1 shows OU-LC-AS1 as an example. As shown in Fig. 1, a and b, a 262-bp fragment from exon 2 and a 294-bp fragment from exon 3 were amplified from both the PBMCs and the tumor cell line by A*1101-specific primers (7). However, a 202-bp fragment from exon 2 and a 284-bp fragment from exon 3 were amplified from the PBMCs but not from the tumor cell line by A*2402-specific primers. As shown in Fig. 1, c and d, 263-bp fragments were amplified from both the tumor cell line and the PBMCs by DR4-specific primers. In contrast, 266-bp fragments were amplified from the PBMCs but not from the tumor cell line by DR52-associated group-specific primers (12). Similar results were observed in HLA-B, -C, and -DQ allele group-specific PCR. These findings indicated that HLA-A*2402, B*1511, Cw*0303, DR14, and DQ*0531 genes are deleted in OU-LC-AS1 cells.

**DISCUSSION**

In this study, we showed that the allelic loss was caused by a deletion of a complete HLA haplotype in a high frequency (42.7%) in newly established lung cancer lines.

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**Table 1** HLA typing of seven lung cancer cell lines and autologous PBMCs

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Histology</th>
<th>Material</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>DR</th>
<th>DQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>OU-LC-A1</td>
<td>Adenocarcinoma</td>
<td>PBMCs</td>
<td>A24, 11</td>
<td>B61, 62</td>
<td>Cw4, 9</td>
<td>DR4, 8</td>
<td>DQ3, 4</td>
</tr>
<tr>
<td>OU-LC-A2</td>
<td>Adenocarcinoma</td>
<td>PBMCs</td>
<td>A*2402</td>
<td>B*4002 (61)</td>
<td>Cw*0303 (9)</td>
<td>DR8, DQ*0402</td>
<td></td>
</tr>
<tr>
<td>OU-LC-AS1</td>
<td>ACC*</td>
<td>PBMCs</td>
<td>A*1101, *2402</td>
<td>B*5502, *1511</td>
<td>Cw*0102, *0303</td>
<td>DR4, DQ*0302</td>
<td></td>
</tr>
<tr>
<td>OU-LC-K1</td>
<td>Adenocarcinoma</td>
<td>PBMCs</td>
<td>A2</td>
<td>B39, 51</td>
<td>Cw7, 10</td>
<td>DR4, 9</td>
<td>DQ3, 4</td>
</tr>
<tr>
<td>EBC-2</td>
<td>SCC*</td>
<td>PBMCs</td>
<td>A31, 33</td>
<td>B35, 44</td>
<td>Cw9</td>
<td>DR8, 13</td>
<td>DQ1, 0302</td>
</tr>
<tr>
<td>OU-LC-A3</td>
<td>Adenocarcinoma</td>
<td>PBMCs</td>
<td>A*1101, *2402</td>
<td>B*51, 54</td>
<td>Cw*0303 (9), *1403</td>
<td>DR4, DQ0401</td>
<td></td>
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<tr>
<td>OU-LC-A4</td>
<td>Adenocarcinoma</td>
<td>PBMCs</td>
<td>A24, 31</td>
<td>B7, 51</td>
<td>Cw7</td>
<td>DR4, DQ0403</td>
<td></td>
</tr>
</tbody>
</table>

* ACC, adenosquamous cell carcinoma; SCC, squamous cell carcinoma.

Loss of expression of HLA class I antigens has been demonstrated in a variety of tumors (1–3). In some tumor cells, all class I molecules are lost, whereas in other cases, selective loss of expression of class I alleles takes place (14–16). Because these alterations lead to the removal of a restriction element that would otherwise present peptides as tumor rejection antigens, they may represent a mechanism whereby tumors escape T-cell surveillance. In fact, it was shown that some tumors fail to present antigens to T cells because of reduced expression of MHC class I molecules and may modulate their susceptibility to cytotoxic T cells.

Several mechanisms appear to be involved in the loss of MHC class I expression in transformed cells (17). These include absence or altered binding of trans-acting factors involved in MHC class I regulation (18, 19), loss of transporter molecules (20), mutation localized within the β2 microglobulin gene (21), and overexpression of proto-oncogene products (22). Alterations that probably lead to a general down-regulation of MHC class I molecules have been reported in cell lines derived from tumors.

Loss of a HLA haplotype has been reported in a few tumor cell lines, including three melanoma cell lines, MZ2MEL, 533-MEL, and 1195-MEL (23), and a pancreas cell line, IMIM-PC-2 (24). However, there has been no report concerning lung cancer. Our results have shown, for the first time, a haplotype loss of HLA in lung cancer cells. This information represents a useful background to design effective strategies for clinical use of tumor vaccination. However, here, the original tumor tissue was not investigated; this raises a possibility that HLA antigen alterations might have developed due to the selection of a specific population during in vitro culture. Because this provides critical points concerning tumor immunoevasive, further evaluation in vivo should be needed. Whether directly or indirectly, the loss of heterozygosity in the HLA genes may be important in progression of the disease and more detailed molecular stud-
ies are necessary to determine their real prevalence and significance in human cancers.

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REFERENCES


Fig. 1 Group-specific amplification of the HLA-A (a and b) and the HLA-DR (c and d) genes. Genomic DNA extracted from PBMCs (a and c) and OULC-AS1 (b and d) were used. The amplified products were subjected to electrophoresis on a 10% polyacrylamide gel. Lanes 1 (a and b), HLA-A1101-selective amplification; Lanes 3 (a and b), HLA-A2402-selective amplification; Lanes 4 (c and d), HLA-DR4-selective amplification; Lanes 52 (c and d), HLA-DR14-selective amplification; Lanes M (a–d), marker φX174 DNA digested with HinfI.


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