Lack and restoration of sensitivity of lung cancer cells to cellular attack with special reference to expression of human leukocyte antigen class I and/or major histocompatibility complex class I chain related molecules A/B

Tetsuro Baba, Takeshi Hanagiri, Yoshinobu Ichiki, Koji Kuroda, Yoshiki Shigematsu, Makiko Mizukami, Masakazu Sugaya, Mitsuhiro Takenoyama, Kenji Sugio and Kosei Yasumoto

Department of Surgery II, School of Medicine, University of Occupational and Environmental Health, 1-1 Iseigaoka, Yahatanishi-ku, Kitakyushu 807-8555, Japan

(Received May 30, 2007/Revised July 3, 2007/Accepted July 6, 2007/Online publication August 28, 2007)

Both cytotoxic T lymphocytes (CTL) and natural killer (NK) cells may play major roles in the host defense against cancer. However, their relationship against the same tumor remains to be elucidated. Among 26 human lung cancer cell lines established in our laboratory, 10 (38%) exhibited human leukocyte antigen (HLA)-class I haplotype loss and three (12%) lost HLA-class I expression totally by flow cytometry analysis. The two cell lines (E522L and C831L) that lost their expression of HLA-class I in vitro and in vivo were applied for further evaluations. Genetic abnormalities of β2-microglobulin gene were observed in both E522L (loss of mRNA) and C831L (point mutation). Transduction of the wild-type β2-microglobulin gene rendered them positive for class I expression. The CTL were induced from autologous peripheral blood mononuclear cells or regional lymph node lymphocytes by stimulation with wild-type β2-microglobulin transduced-E522L or -C831L, and they showed tumor-specific cytoxicity against wild-type β2-microglobulin-transductant, but not parental cells. In NK cell cytotoxicity, E522L showed high sensitivity to NK cells; however, C831L showed resistance despite loss of HLA-class I expression. E522L expressed MHC class I chain related molecules A/B, but C831L did not. The transduction of the MHC class I chain related molecule A gene from E522L rendered C831L positive for expression and sensitive to NK cell cytotoxicity. Reconstitution of HLA-class I and MHC class I chain related molecules A expression could abrogate evasion from cellular attack by CTL and NK cells, and it may lead to a breakthrough in the development of cancer immunotherapy. (Cancer Sci 2007; 98: 1795–1802)

As reported previously, many kinds of lung cancer associated antigens that are able to induce specific CTL have been identified.1–5) Tumor-specific-CTL are one of the important effectors in cellular immunity in patients with tumor expressing HLA-class I.6–9) NK cells also play an important role in the antitumor immune response by attacking particularly cancer cells with down-regulation or loss of the HLA-class I expression.10,11) However, in the past, the antitumor responses by CTL and NK cells have been analyzed independently12,13) and the relationship between them against the same tumor remains to be elucidated. CTL and NK cells showed a mutually complementary or exclusive antitumor activity against cancer cells, and their relationship should be important in considering the host defense against cancer.

Cancer cells have been reported to have several escape mechanisms from the host’s immunosurveillance.14-16) It is thought that one of the main escape mechanisms is HLA-class I abnormality.17–19) In non-small cell lung cancer, the rate of down-regulation of HLA-class I expression has been reported to range from 25 to 94%.20–22) CTL can not recognize cancer cells if they have lost HLA-class I expression. In a previous report, however, we showed that the precursors of tumor-specific CTL remained in vivo even though cancer cells lost their haplotype of HLA-class I, and that the CTL with strong cytotoxicity against cancer cells could be induced by reconstruction of the HLA-class I.23) Therefore, the first strategy to overcome the tumor escape mechanism is a restoration of the HLA-class I expression on such cancer cells.

In contrast, the HLA-class I molecule is well known to play an inhibitory role in NK cell cytotoxicity. NK cells usually show high cytolytic activity against cancer cells that have lost their HLA-class I expression.24) Therefore, the loss of HLA-class I expression is one tumor escape mechanism from CTL-mediated cytoxicity; at the same time HLA-class I deficient cancer cells might be suitable targets for NK cells. It is thought that the second strategy for anticancer immunotherapy against HLA-class I deficient cancer is to enhance the activity of NK cells.

The present study was conducted to elucidate the precise escape mechanisms from the host’s immunosurveillance using HLA-class I expression-deficient lung cancer cell lines, and to elucidate the method by which such escape mechanisms are overcome.

Materials and Methods

The study protocol was approved by the human and animal ethics review committee of University of Occupational and Environmental Health, Kitakyushu, Japan, and a signed consent form was obtained from each subject before obtaining the tissue samples used in this study.

Lung cancer cell lines. Lung cancer cell lines have been established from pericardial effusion or surgically resected samples, respectively, as described previously.23) E522L was established from pericardial effusion of patient E522 who was a 53-year-old male with...
primary lung adenocarcinoma in the right lower lobe. C831L was established from resected primary lung cancer of patient C831 who was a 54-year-old male with large cell carcinoma of the right lung. To identify their HLA genotypes, PCR was performed by Shionogi Biomedical Laboratories (Osaka, Japan). HLA genotypes were HLA-A*0207/2402, -B*4006/5901, -Cw*0102/0801 in C831L, -Cw*0702/0801 in E522L and HLA-A*0206/2601, -B*0702/3501, -Cw*0702/0801 in C831L.\(^{(23)}\)

### Table 1. Lung cancer cell lines established in our laboratory and human leukocyte antigen (HLA)-class I abnormality

<table>
<thead>
<tr>
<th>Histology</th>
<th>Cell lines</th>
<th>Source of primary culture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>A110L</td>
<td>Primary tumor</td>
</tr>
<tr>
<td></td>
<td>A129L</td>
<td>Primary tumor</td>
</tr>
<tr>
<td></td>
<td>A925L</td>
<td>Primary tumor</td>
</tr>
<tr>
<td></td>
<td>B203L</td>
<td>Primary tumor</td>
</tr>
<tr>
<td></td>
<td>B901L</td>
<td>Primary tumor</td>
</tr>
<tr>
<td></td>
<td>C422L</td>
<td>Primary tumor</td>
</tr>
<tr>
<td></td>
<td>D611L</td>
<td>Primary tumor</td>
</tr>
<tr>
<td></td>
<td>ES22L</td>
<td>Pericardial effusion</td>
</tr>
<tr>
<td></td>
<td>F112L</td>
<td>Primary tumor</td>
</tr>
<tr>
<td></td>
<td>G821L</td>
<td>Primary tumor</td>
</tr>
<tr>
<td></td>
<td>H1224L</td>
<td>Primary tumor</td>
</tr>
<tr>
<td></td>
<td>K420L</td>
<td>Primary tumor</td>
</tr>
<tr>
<td></td>
<td>L619L</td>
<td>Primary tumor</td>
</tr>
<tr>
<td></td>
<td>LB04L</td>
<td>Primary tumor</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>C1026L</td>
<td>Primary tumor</td>
</tr>
<tr>
<td></td>
<td>H1215L</td>
<td>Primary tumor</td>
</tr>
<tr>
<td></td>
<td>L1023L</td>
<td>Primary tumor</td>
</tr>
<tr>
<td>Large cell carcinoma</td>
<td>A904L</td>
<td>Primary tumor</td>
</tr>
<tr>
<td></td>
<td>C311L</td>
<td>Primary tumor</td>
</tr>
<tr>
<td></td>
<td>C831L</td>
<td>Primary tumor</td>
</tr>
<tr>
<td></td>
<td>J206L</td>
<td>Primary tumor</td>
</tr>
<tr>
<td>Pleomorphic carcinoma</td>
<td>G603L</td>
<td>Primary tumor</td>
</tr>
<tr>
<td>Adenosquamous carcinoma</td>
<td>A529L</td>
<td>Primary tumor</td>
</tr>
<tr>
<td>Small cell carcinoma</td>
<td>D1008L</td>
<td>Normal</td>
</tr>
<tr>
<td>Unclassified carcinoma</td>
<td>F1012L</td>
<td>Subcutaneous metastasis</td>
</tr>
</tbody>
</table>

**Histology Cell lines**

**HLA-class I**

**Surface expression**

**Genetic analysis**

**Source of primary culture**

**Histology**

**Cell lines**

**Surface expression**

**Genetic analysis**

**Source of primary culture**

**Histology**

**Cell lines**

**Surface expression**

**Genetic analysis**

**Source of primary culture**

**Histology**

**Cell lines**

**Surface expression**

**Genetic analysis**

**Source of primary culture**

**Histology**

**Cell lines**

**Surface expression**

**Genetic analysis**

**Source of primary culture**

**Histology**

**Cell lines**

**Surface expression**

**Genetic analysis**

**Source of primary culture**

**Histology**

**Cell lines**

**Surface expression**

**Genetic analysis**

**Source of primary culture**

**Histology**

**Cell lines**

**Surface expression**

**Genetic analysis**

**Source of primary culture**

**Histology**

**Cell lines**

**Surface expression**

**Genetic analysis**

**Source of primary culture**

**Histology**

**Cell lines**

**Surface expression**

**Genetic analysis**

**Source of primary culture**

**Histology**

**Cell lines**

**Surface expression**

**Genetic analysis**

**Source of primary culture**

**Histology**

**Cell lines**

**Surface expression**

**Genetic analysis**

**Source of primary culture**

**Histology**

**Cell lines**

**Surface expression**

**Genetic analysis**

**Source of primary culture**

**Histology**

**Cell lines**

**Surface expression**

**Genetic analysis**

**Source of primary culture**

**Histology**

**Cell lines**

**Surface expression**

**Genetic analysis**

**Source of primary culture**

**Histology**

**Cell lines**

**Surface expression**

**Genetic analysis**

**Source of primary culture**

**Histology**

**Cell lines**

**Surface expression**

**Genetic analysis**

**Source of primary culture**

**Histology**

**Cell lines**

**Surface expression**

**Genetic analysis**

**Source of primary culture**

**Histology**

**Cell lines**

**Surface expression**

**Genetic analysis**

**Source of primary culture**

**Histology**

**Cell lines**

**Surface expression**

**Genetic analysis**

**Source of primary culture**

**Histology**

**Cell lines**

**Surface expression**

**Genetic analysis**

**Source of primary culture**

**Histology**

**Cell lines**

**Surface expression**

**Genetic analysis**

**Source of primary culture**

**Histology**

**Cell lines**

**Surface expression**

**Genetic analysis**

**Source of primary culture**

**Histology**

**Cell lines**

**Surface expression**

**Genetic analysis**

**Source of primary culture**

**Histology**

**Cell lines**

**Surface expression**

**Genetic analysis**

**Source of primary culture**

**Histology**

**Cell lines**

**Surface expression**

**Genetic analysis**

**Source of primary culture**

**Histology**

**Cell lines**

**Surface expression**

**Genetic analysis**

**Source of primary culture**

**Histology**

**Cell lines**

**Surface expression**

**Genetic analysis**

**Source of primary culture**

**Histology**

**Cell lines**

**Surface expression**

**Genetic analysis**

**Source of primary culture**

**Histology**

**Cell lines**

**Surface expression**

**Genetic analysis**

**Source of primary culture**

**Histology**

**Cell lines**

**Surface expression**

**Genetic analysis**

**Source of primary culture**

**Histology**

**Cell lines**

**Surface expression**

**Genetic analysis**

**Source of primary culture**

**Histology**

**Cell lines**

**Surface expression**

**Genetic analysis**

**Source of primary culture**

**Histology**

**Cell lines**

**Surface expression**

**Genetic analysis**

**Source of primary culture**

**Histology**

**Cell lines**

**Surface expression**

**Genetic analysis**

**Source of primary culture**

**Histology**

**Cell lines**

**Surface expression**

**Genetic analysis**

**Source of primary culture**

**Histology**

**Cell lines**

**Surface expression**

**Genetic analysis**

**Source of primary culture**

**Histology**

**Cell lines**

**Surface expression**

**Genetic analysis**

**Source of primary culture**

**Histology**

**Cell lines**

**Surface expression**

**Genetic analysis**

**Source of primary culture**

**Histology**

**Cell lines**

**Surface expression**

**Genetic analysis**

**Source of primary culture**

**Histology**

**Cell lines**

**Surface expression**

**Genetic analysis**

**Source of primary culture**
weekly at a tumor-to-lymphocyte ratio of 1:10 in culture medium with 20 U/mL of rIL2 (donated by Takeda Chemical Industries, Osaka, Japan) for 3 weeks. The CTL activity was assessed at 7 days after the last stimulation. To generate the CTL clone, a limiting dilution method was performed as reported previously.(1)

Assay of CTL and NK cell activity. The cytotoxicity of CTL and NK cells was assessed using a standard 51Cr release assay as described previously.(27) The CTL were co-cultured with target tumor cells at different effector : target ratios (1:1, 3:1, 10:1, 30:1). NK cell activity was evaluated at an indicated effector : target ratio (10:1, 20:1, 40:1, 80:1) for 4 h at 37°C. The CTL activity was also assessed by the cytokine production. In brief, CTL (3 × 105) was co-cultured with stimulator tumor cells (3 × 104) for 8 h, and the amount of IFN-γ in the supernatant was measured using a human IFN-γ ELISA test kit (Biosource, Camarillo, CA, USA).(2) In a blocking assay of CTL, one-quarter of the diluted culture supernatant of hybridomas (W6/32 or L243) was added to the co-culture of CTL and tumor cells. In a blocking assay of NK cell cytotoxicity, anti-NKG2D mAb (1D11) was added in the co-culture of NK cells and targets.

Measurement of soluble MICA in culture supernatant of E522L and C831L. Soluble MICA was measured using a DuoSet ELISA Development System (R&D systems, Minneapolis, MN, USA). Culture supernatants were obtained after 48 h-culture of 1 × 10⁶ cells in a 25 cm² flask and applied for the ELISA kit. The difference between the two groups was statistically analyzed using Student’s t-test.

Results

HLA-class I expression status of lung cancer cell lines. Twenty-six permanent lung cancer cell lines have been established in our laboratory since 1994, as shown in Table 1. Three cell lines (E522L, C831L, C1026L) lost their whole HLA-class I expression (12%) based on the finding of a flow cytometry analysis, as shown in Table 1. Ten cell lines had haplotype loss of HLA (38%) by comparing the HLA genes between cancer cells and normal lymphocytes. In total, 13 cell lines (50%) showed an abnormal expression of HLA-class I.

The results of flow cytometry analysis, staining with anti-HLA-class I mAb against E522L and C831L, are shown in Fig. 1A. HLA-class I expression in these cell lines could not be induced even after treatment with IFN-γ (200 U/mL) for 48 h (Fig. 1A). Genetic analysis of β2m in E522L and C831L. Because the abnormalities of the β2m gene have been reported to cause the loss of HLA-class I expression,(13,17) we genetically analyzed β2m genes in E522L and C831L. RT-PCR showed a total loss of the β2m
gene in E522L (Fig. 1B). In C831L, mRNA of the \( \beta_2m \) gene was normally expressed. However, the sequence analysis of \( \beta_2m \) revealed a point mutation in the ORF (Fig. 1C). Cytosine at 64 base from the start position of ORF was substituted for thymine, and this change resulted in the formation of an early stop codon.

Restoration of HLA-class I expression by transduction of \( \omega_\beta_2m \).

The total loss of HLA-class I expression in both the cell lines was thus ascribed to genetic abnormalities of \( \beta_2m \). Therefore, the \( \omega_\beta_2m \) gene was transduced into E522L and C831L, used in order to restore HLA-class I expression, as described in the Materials and Methods. After selecting by the antibiotics, a flow cytometry analysis revealed that E522L-\( \omega_\beta_2m \) and C831L-\( \omega_\beta_2m \) restored the expression of HLA-class I on their cell surface (Fig. 1D).

HLA expression in original cancer cells or cancer tissue.

To confirm the negative expression of the HLA-class I of original cancer cells in vivo, purified cancer cells using the Ficoll–Hypaque gradient method (approximately 90%) from pericardial effusion were examined by flow cytometry in patient E522, and showed that 92% of the cells had lost their HLA-class I (Fig. 2A). The primary lung cancer tissue of patient C831 was evaluated using immunohistochemical staining with anti-HLA-class I mAb, EMR8-5. As shown in Fig. 2B, interstitial cells strongly expressed HLA-class I on their cell surfaces, but HLA-class I was not expressed on cancer cells at all. The control cancer tissue (A110) expressed HLA-class I on the surfaces of cancer cells. These findings indicated that most cancer cells in both patient E522 and patient C831 had already lost their HLA-class I expression of tumor cells in vivo.

CTL response against cancer cells with or without HLA-class I expression.

The CTL of patient E522 were induced from autologous PBMC by stimulations with \( \omega_\beta_2m \) transduced autologous tumor cells (E522L-\( \omega_\beta_2m \)). The CTL showed cytotoxicity to E522L-\( \omega_\beta_2m \), but not to parental E522L, E522 PHA blasts or K562 (Fig. 3A).

In patient C831, one CTL clone was established from autologous RLNL, as described in the Materials and Methods, and showed tumor-specific reactivity. The CTL clone killed C831L-\( \omega_\beta_2m \), but not parental C831L, C831EBV-B or K562 (Fig. 3B). Moreover, the reactivity of the CTL clone was elucidated by IFN-\( \gamma \) production in response to C831L-\( \omega_\beta_2m \) and the response was inhibited by the addition of anti-HLA-class I mAb (Fig. 3C).

NK cell cytotoxicity against E522L or C831L.

NK cell-sensitivities of E522L and C831L were examined using NK cells produced by PBMC from a normal healthy donor. E522L was as sensitive to NK cells as K562. However, the NK cell-sensitivity was completely abrogated by the restoration of the expression of HLA-class I with the transduction of \( \omega_\beta_2m \) (Fig. 4A). In contrast, C831L was insensitive to NK cells despite the loss of the HLA-class I expression (Fig. 4B). These findings indicated that E522L and C831L clearly have different pathways to react with NK cells.

Analysis of the difference in sensitivity to NK cells between E522L and C831L.

To explore the mechanisms regarding the difference in the sensitivity against NK cells between E522L and C831L, we examined the surface expression of NK cell-activating ligands on both cancer cells. MICA/B is a ligand for NK cell-activating receptor, NKG2D. Flow cytometry revealed that C831L did not express MICA/B, whereas E522L highly expressed it (Fig. 5A). A DNA sequence analysis of the MICA gene in these cell lines
Fig. 3. The activity of cytotoxic T lymphocytes (CTL) induced from autologous lymphocytes. (A) In patient E522, CTL were induced from autologous peripheral blood mononuclear cells (PBMC) by weekly stimulation with irradiated E522L-wβ2m. The cytotoxicity of CTL was assessed by a standard 51Cr release assay. The CTL killed E522L-wβ2m, but not parental E522L without either human leukocyte antigen (HLA)-class I expression, E522-PHA blasts or K562. (B) In patient C831, CTL were induced from autologous regional lymph node lymphocyte (RLNL). The CTL showed a cytoytic activity against C831L-wβ2m, but not against parental C831L, C831 EBV-B or K562. (C) Reactivity of the CTL clone induced from patient C831 was analyzed by the interferon (IFN)-γ production in response to stimulators. The restriction of the CTL clone was analyzed by the addition of anti-HLA-class I or anti-HLA-DR monoclonal antibody (mAb). The CTL produced IFN-γ in response to C831L-wβ2m and the response was inhibited by the addition of anti-HLA-class I mAb, but the CTL did not show any response against parental C831L, C831EBV-B or K562.

Fig. 4. The natural killer (NK) cell activity against E522L and C831L. (A) To examine the sensitivity of E522L to NK cell activity, NK cells derived from healthy donor peripheral blood mononuclear cells (PBMC), was applied for 51Cr release assay. K562, sensitive to NK cell activity, was used as a positive control. E522L was as sensitive as K562 to NK cell activity. The restoration of HLA-class I expression completely abrogated the sensitivity. (B) Cytotoxic assay of NK cells against C831L. C831L exhibited resistance to the NK cell activity.
revealed that the allele of TM was different. The allele for MICA in E522L was MICA-A008/5 (extracellular domain/TM), and the allele in C831L was MICA-A008/5.1. The MICA gene (MICA-A008/5) was cloned from E522L and transduced into C831L using retrovirus vector, as described in the Materials and Methods. After selecting by the antibiotics, a flow cytometry analysis revealed that MICA(A008/5)-transduced C831L (C831L-MICA-A5) expressed MICA on the cell surface (Fig. 5B). An NK cell cytotoxic assay showed that the MICA transduction rendered C831L sensitive to NK cells (Fig. 5C). The sensitivity of C831L-MICA-A5 was thus inhibited by the addition of anti-NKG2D mAb.

Measurement of soluble MICA in culture supernatants of E522L and C831L. To elucidate whether soluble MICA was associated with the resistance of C831L against NK cell cytotoxicity, NK cells were pretreated with culture supernatant of C831L or E522L, and cytotoxic activities against E522L and K562 were evaluated. However, the activity was not affected by the presence of culture supernatant of C831L and E522L (data not shown). The amount of soluble MICA in the culture supernatant of C831L was as low as that of E522L (Fig. 5D).

Discussion

In patient E522 and C831, the total loss of the HLA-class I expression was observed not only in cancer cell lines cultured in vitro (Fig. 1), but also in malignant cells from the original pericardial effusion of E522 and primary cancer tissue of C831 (Fig. 2). In previous reports, 25–94% of non-small cell lung cancers had a loss or down-regulation of HLA-class I expression in immunohistochemical staining.(20,21) However, the correlation between the expression of HLA-class I of cancer cells and the prognosis of cancer patients remains unclear. An HLA-class I loss or down-regulation on lung cancer and breast cancer has been reported to be associated with favorable survival,(24,28) but a down-regulation in colorectal cancer has been suggested to be a poor prognostic factor.(29) CTL attack against normal HLA-expressing tumor cells might cause an in vivo selection of cancer cells showing an abnormal expression of HLA as reported previously.(2)

In E522L, mRNA of the β2m gene was not expressed at all (Fig. 3A). In melanoma cell lines, the loss of β2m RNA was reported to be ascribed to two mutational events, a loss of heterozygosity and a microdeletion of the β2m gene in exon 1.(18,30) A mutation hotspot was suggested to be located in the CT repeat region (at position from 37 base to 44 base of the ORF) in exon 1 of the β2m gene, and mutations of this region have been identified in more than 75% of cancer cells with HLA-class I loss.(18,19) However, the mutation site in C831L (Fig. 3B) was not consistent with the reported hotspot.

Previous reports have indicated that cancer cells with a deficiency of β2m molecules were able to escape from a CTL attack; however, the CTL could attack the cancer cells, which thus restored the HLA expression by β2m transduction.(13) In our experiment, autologous CTL were induced by E522L-β2m and C831L-β2m, and such CTL could not respond to parental cancer cells without an HLA expression (Fig. 3). These results indicated the existence of precursor CTL against normally HLA-class I expressed cancer cells in vivo. These findings may suggest that cancer cells can thus evade CTL attack by means of loss of HLA-class I expression due to genetic changes of β2m.

An HLA-class I expression of tumor cells is a pivotal condition for the recognition of specific CTL. However, the absence of HLA-class I on target cancer cells may cause an activation of NK cells. Such a phenomenon is well known as the ‘missing-self’ hypothesis.(10,31) In fact, E522L exhibited a high sensitivity to
NK cell, and transduction of wβ2m rendered them resistant by restoration of HLA-class I as shown in Fig. 4A. However, C831L exhibited resistance to NK cell despite the loss of HLA-class I expression, as shown in Fig. 4B. According to previous reports, there are several check points of target tumor cells for NK cell sensitivity, the surface expression of HLA-class I molecules as a ligand for NK cell inhibitory receptor, the surface expression of ligands for activating NK cell receptors, and the surface expression of non-HLA-specific inhibitory receptors. Not only HLA-class I, but also HLA-E and CD1d, may protect target cells from NK cell killing. However, neither HLA-E nor CD1d was expressed on C831L, because wβ2m is also essential for their expression on the cell surface. Therefore, we examined the expression of ligands for the activating NK cell receptors.

MICA/B has recently been reported as a ligand to activate NK cells by means of activating receptor, NKG2D. The down-regulation of MICA/B has been reported to be one of the mechanisms for evasion from NK cell killing. Therefore, we examined the expression of MICA/B as a ligand for NK cell inhibitory receptor, in our experiments.

In the present study, we analyzed the escape mechanisms from immunosurveillance in lung cancer cells, and revealed that: (i) 50% of lung cancer cell lines had abnormalities in their HLA-class I expression; (ii) an abnormality of the β2m gene caused lack of HLA-class I expression and transduction of wβ2m induced restoration of HLA-class I expression; (iii) HLA-class I deficient cells were exempted from an attack by CTL and reconstitution of HLA-class I expression induced CTL recognition against such cancer cells; (iv) cancer cells that lost their HLA-class I expression did not always show sensitivity to NK cell cytotoxicity; and (v) reconstitution of the MICA expression, the NK cell-resistant cancer cells restored sensitivity to NK cell cytotoxicity.

Acknowledgments

This study was supported in part by a Grant-in-Aid from the Ministry of Education, Culture, Sports, Science, and Technology, Japan and by High-Altitude Research Grant from the University of Occupational and Environmental Health, Japan. We thank Takashi Fukuyama, Yoshika Nagata, Misako Fukushima, Kahoru Noda, Yukari Oshibuchi, Yuki Goto, and Aya Katayama for their technical assistance.

References


