Clinical Significance of Expression of Cancer/testis Antigen and Down-regulation of HLA Class-I in Patients with Stage I Non-small Cell Lung Cancer

TAKESHI HANAGIRI1, YOSHIKI SHIGEMATSU1, SHINJI SHINOHARA1, MASARU TAKENAKA1, SOHICH OKA1, YASUHIRO CHIKAIISHI1, YOSHIKA NAGATA1, TETSURO BABA1, HIDETAKA URAMOTO1, TOMOKO SO1 and SOHSUKE YAMADA2

1Second Department of Surgery and 2Department of Pathology and Cell Biology, School of Medicine, University of Occupational and Environmental Health, Kitakyushu, Japan

Abstract. Aim: The purpose of this study was to investigate the clinical significance of expression of cancer/testis (CT) antigen and down-regulation of HLA class-I in patients with stage I non-small cell lung cancer (NSCLC), which underwent complete surgical resection. Patients and Methods: The expression of HLA class-I molecules was evaluated in 136 resected NSCLC specimens by immunohistochemistry. The results were scored as the percentage of stained tumor cells and categorized into two groups: 0-79%, reduced expression; and >80%, normal expression. The expression of CT antigen was performed by reverse transcription-polymerase chain reaction (RT-PCR). Results: The expression of HLA class-I was normal in 49 tumors (36%), and there was reduced expression in 87 tumors (64%). The expression of Melanoma antigen (MAGE)-A3, MAGE-A4, and Kita-Kyushu lung cancer antigen-1 (KK-LC-1) was positive in 34 (25.0%), 22 (16.2%), and 42 (30.9%) patients, respectively. There was no significant difference in the proportion of HLA class-I expression associated with the expression of any of the CT antigens. Among the patients with positive expression of at least one of the CT antigens, the 5-year survival rate of the patients with the normal expression of HLA class-I was 87.5%; however, it was 63.4% in patients with the reduced expression of HLA class-I (p=0.0477). Conclusion: Reduced expression of HLA class-I was an unfavorable prognostic factor in patients with positive expression of CT antigen, and represents an important hurdle to antigen-based cancer immunotherapy.
or down-regulate expression of HLA class-I molecules on their surface, and this phenomenon is thought to be one means of tumor escape (10). The cancer immunoediting concept demonstrates that cancer cells sensitive for cellular attack should be easily eliminated, but the refractory variants having a deficiency of HLA class-I expression may escape from immunosurveillance and develop into clinical cancer (11). Therefore, the loss and down-regulation of HLA class-I expression may develop as consequence of the immunological failure of CTL attack. We reported that normal HLA class-I expression is correlated with favorable survival in patients with stage I disease (12). The evaluation of CT antigen and HLA expression may enable for the selection of individuals at a higher risk of disease relapse, and should help to determine antigen-based immunotherapy targeting minimal residual disease. The purpose of this study was to investigate the clinical significance of the expression of CT antigen and down-regulation of HLA class-I molecules in patients with stage I NSCLC that underwent complete surgical resection.

Patients and Methods

Patients and samples. The Institutional Review Board approved this study (No. 05-070), and informed consent was obtained from their patients for the use of the specimens and for the analysis. Two hundred and eighty-two patients underwent complete resection of stage I NSCLC at the Second Department of Surgery at the University of Occupational and Environmental Health between 2001 and 2006. Among them, adequate tissue specimens both for immunohistochemical staining and reverse transcription-polymerase chain reaction (RT-PCR) analysis of CT antigens were obtained from 136 patients. The preoperative assessments included chest roentgenography and computed tomography of the chest, upper abdomen and brain. Magnetic resonance imaging of the brain was routinely employed for assessment of distant metastasis. The use of positron-emission tomographic (PET) scans introduced from 2005 for the evaluation of staging, however, they were not routinely performed. Nineteen patients (14.0%) underwent PET scans before surgery in this study. The patients’ records, including their clinical data, preoperative examination results, details of any surgeries, histopathological findings, and the TNM stages of all patients were also reviewed. The histological diagnosis was performed based on examination of conventional hematoxylin and eosin-stained specimens. A complete mediastinal lymph node dissection was performed for patients that underwent lobectomy, or lymph node sampling was carried out following partial resection or segmentectomy to determine the pathological N status. The histopathological findings were classified according to the World Health Organization criteria, and the Union for International Cancer Control (UICC) TNM staging system (seventh edition) was also employed (13, 14).

Postoperative systemic chemotherapy was performed for patients with stage IB disease if they were able to tolerate such treatment after surgery, or unless the patients refused additional chemotherapy. Follow-up information was obtained from all patients through office visits or telephone interviews either with the patient, with a relative, or with their primary physicians. The patients were evaluated every three months by chest roentgenography, and chest computed tomography and bone scintigraphy were performed every six months for the first two years after surgery, and annually thereafter. The average period of follow-up after surgery was 48 months.

Immunohistochemistry. Immunohistochemical staining was conducted using serial sections from the same paraffin-embedded blocks by previously described methods (12). The expression of HLA class-I was assessed by immunohistochemistry using EMR8-5, a monoclonal antibody to HLA class-I heavy chain. Positive reactivity for the EMR8-5 antibody was confirmed by the staining of vascular endothelial cells and lymphocytes in sections of tumor specimens. The reactivity of EMR8-5 was determined by staining of the plasma membrane of tumor cells. Negative control sections were immunostained under the same conditions by substituting mouse IgG for the primary antibody. The expression of HLA class-I was assessed by two investigators that were blinded to the clinical status of the patients. The evaluation of the HLA class-I expression on cancer cells was defined as normal expression when ≥80% of cancer cells stained positively, or as reduced expression when <80% of cancer cells were positively stained in comparison to the HLA class-I expression on normal stromal cells.

Evaluation of CT antigen expression. The analysis used for the expression of CT antigens was previously described (9). Briefly, the cDNA converted from RNA extracted from each lung tumor served as a template for PCR. The gene-specific primers were: β-actin (internal control)-specific primers; forward primer, 5’-GGC ATC GTG ATG GAC TCC G-3’, and reverse primer, 5’-GCT GGA AGG TGG ACA GCG A-3’; MAGE-A3-specific primers; forward primer, 5’-TTA CTC CTA GCG AGC-3’ and reverse primer, 5’-ATG AAC TTC TAT GGA TTT CCG GTG AGG-3’. The PCR products were visualized with ethidium bromide staining under ultraviolet light after electrophoresis on 2% agarose gel.

Statistical analysis. Statistical significance was evaluated using the chi-square test or Fisher’s exact test. The Kaplan Meier method was used to estimate the probability of survival, and survival differences were analyzed by the log-rank test. Differences were considered to be statistically significant with p-values <0.05. All statistical analyses were performed with the StatView-J 5.0 software package (Abacus Concepts, Berkeley, CA, USA).

Results

The hospital records of 136 patients that underwent a complete resection of stage I NSCLC were retrieved. The patients included 82 males and 54 females. The mean age of the patients was 69.3 years (range, 43-87 years). The histological types included 96 adenocarcinomas, 33 squamous cell carcinomas, and seven other types of carcinomas. Bilobectomy was performed in four patients,
lobectomy in 117, segmentectomy in seven, and partial resection of the lung in eight patients. The pathological stage was diagnosed as stage IA in 96 patients, and stage IB in 40.

The immunohistochemical staining revealed 49 tumors (36%) with normal expression of HLA class-I, and 87 tumors (64%) with reduced expression (Table I). The percentage of male patients in the normal expression-group was 32%, and 68% in the reduced expression-group. For female patients, normal and reduced expression was observed in 43% and 57%, respectively. The proportion of patients in the normal expression-group with adenocarcinoma was 42%, and that in the reduced expression-group was 58%, whereas those for patients with squamous cell carcinoma were 24%, and 76%, respectively. The degree of HLA class-I expression did not correlate with the clinicopathological characteristics such as gender, histology, or pathological stage (Table I). The expression of HLA (24%) in squamous cell carcinoma tended to be lower than that (42%) of adenocarcinoma. The expression of MAGE-A3, MAGE-A4, and KK-LC-1 was positive in 34 (25.0%), 22 (16.2%), and 42 (30.9%) patients, respectively. There was no significant difference in the proportion of HLA class-I expression associated with the expression of each CT antigen (Table I).

The 5-year overall survival rates for the patients with positive expression for MAGE-A3 were 90.0%, and 65.8% in the groups with normal and reduced HLA expression, respectively (Figure 1a). The 5-year overall survival rates for the patients with positive expression for MAGE-A4 were 85.7% in the group with normal expression, and 60.0% in the reduced expression group (Figure 1b). The 5-year overall survival rates for patients with positive expression for KK-LC-1 were 78.6% in the group with normal expression, and 59.2% in the reduced expression-group (Figure 1c). No significant differences between the groups with normal and that with reduced expression of HLA were observed in the prognosis of patients with positive expression of any of the CT antigens. At least one of the three CT antigens was expressed in 70 patients (51.5%). Among the patients with positive expression of at least one of the CT antigens, the 5-year survival rate of the patients with normal expression of HLA was 87.5%; however, it was 63.4% in patients with the reduced expression of HLA (Figure 2). There was a significant difference in the survival rate of the patients between the group with normal HLA expression and that with reduced HLA expression ($p=0.0477$).

A univariate analysis of survival for patients with stage I NSCLC showed that gender (female vs. male, $p=0.0050$), age (<75 vs. ≥75 years, $p=0.1056$), T factor (T1 vs. T2, $p=0.0678$), histology (adenocarcinoma vs. others, $p=0.0362$), HLA expression (normal vs. reduced, $p=0.0645$) and any of CT antigens (negative vs. positive, $p=0.0238$) were significant prognostic factors. In a multivariate analysis using these significant variables (gender, histology, and expression of CT antigens), the expression of any CT antigen was not an independent prognostic factor for stage I NSCLC ($p=0.1148$).

**Discussion**

CT antigens are expressed in variable proportions of a wide range of different types of tumors such as melanoma, lung, esophagus, stomach, colon and breast carcinomas, but not in normal tissues except for the testis, ovary and placenta,
which do not express HLA class-I (6). Many CT antigens, including MAGE-A3, MAGE-A4 and KK-LC-1, are antigenic proteins that are able to bind HLA class-I molecules and elicit cell-mediated immune responses.

Therefore, CT antigens are theoretically ideal targets for antigen-based cancer immunotherapy. The expression of CT antigens [MAGE, B-Melanoma antigen (BAGE), B-Melanoma antigen (GAGE), KK-LC-1 and New York-esophageal squamous cell carcinoma-1 (NY-ESO-1)] has been reported in lung cancer (7, 9, 15). Several investigators reported that expression of CT antigens is associated with advanced disease and poor outcome (9, 15, 16). The function of the genes coding for CT antigens in the development of cancer has not been fully understood. CT antigens are thought to play a role in embryonic development and tumor transformation or progression. Marcar et al. reported that MAGE-A antigens inhibit p53 function by blocking its interaction with chromatin, resulting in cancer progression (17). Some MAGE proteins have been reported to play roles in the regulation of cell survival, proliferation, and sensitivity to apoptosis (18, 19).

Loss or down-regulation of HLA class-I expression has been demonstrated in a variety of solid tumors (20). The reduced expression of HLA class-I molecules in tumor cells is one of the mechanisms of immune escape from recognition by CTLs. The theory of cancer immunoediting proposes that the majority of tumor cells with normal HLA class-I expression bearing a tumor antigen are attacked and eradicated by CTLs during the early phase of carcinogenesis, and thereafter, only tumor cells with abnormal expression of HLA class-I antigens escape from the host’s immune surveillance system and develop to become a clinically relevant cancer (21). The down-regulation rate of HLA class-I expression ranges from 33 to 94% in NSCLC (22-24). Kikuchi et al. reported that a high rate of negative expression of HLA class-I was observed in squamous cell carcinomas in smoking male patients, in comparison to adenocarcinoma in non-smoking female patients (24). Our previous study
found that the proportion of patients with normal expression of HLA was significantly higher in those with adenocarcinoma, but a significant correlation was observed between the degree of HLA class-I expression and patient gender, T factor, N factor, and pathological stage (12). The correlation between the expression of HLA class-I molecules on cancer cells and the prognosis of patients with cancer is still controversial (22-24). Our previous study revealed that patients with stage I disease with normal expression have a more favorable prognosis in comparison to patients with heterogeneous expression, whereas there are no differences in patient survival associated with the HLA expression in those with stage II-III disease (12).

The present study investigated patients with stage I NSCLC with special reference to the clinical significance of the expression of CT antigen and down-regulation of HLA class-I molecules. No significant difference in survival was observed due to the expression of HLA among patients with positive expression of MAGE-A3, MAGE-A4 and KK-LC-1. However, a reduced expression of HLA class-I molecules negatively affected the survival of patients with positive expression of at least one of the CT antigens ($p=0.0477$). In a multivariate analysis, the expression of any CT antigen was not an independent prognostic factor. It is considered that other confounding factors might influence their prognosis more strongly, because the previous study showed both of MAGE-A3 and MAGE-A4 were expressed more frequently in male patients, and more highly expressed in squamous cell carcinoma than in adenocarcinoma (9).

The present findings suggest that CTL activity might play a more important role in patients with positive expression of CT antigens because the normal expression of HLA was associated with a favorable prognosis. The down-regulation of HLA class-I expression in tumor cells is the major mechanism of escape from attack by CTL, resulting in disease progression. A previous study demonstrated that the CTL response can be restored by gene transduction of HLA class-I molecules. The antigens that cause the loss of HLA class-I should be critical to the survival of cancer cells, and such antigens could trigger a strong and effective immune response in patients with NSCLC. HLA restoration in tumor cells has the potential, as a new strategy, to improve tumor-specific immunotherapy (11, 25).

Recent clinical studies on immunotherapy showed favorable therapeutic effects and suggest the possibility of an alternative treatment approach to NSCLC (26, 27). MAGRIT is a clinical trial which investigates the efficacy of MAGE-A3 antigen-specific cancer immunotherapeutic agents in an adjuvant setting for patients with MAGE-A3-positive NSCLC (27). The characterization of antigen and HLA profiles may lead to customized immunotherapy for this disease, and these analyses should more accurately determine which individuals are at a higher risk of relapse after surgical resection. It is necessary to evaluate the efficacy of antigen-based adjuvant immunotherapy for the selection of patients according to HLA expression pattern in future clinical trials.

Conflicts of Interest

None declared.

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References


