Correlation Between HLA Alleles and EGFR Mutation in Japanese Patients with Adenocarcinoma of the Lung

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Introduction: The identification of activating mutations in the epidermal growth factor receptor (*EGFR*) gene is one of the most intriguing recent discoveries in the field of lung cancer research, and they are more commonly found in adenocarcinoma occurring in females, never/light smokers, and East Asian patients. Why such certain patients are susceptible to the development of *EGFR*-mutant tumors is currently unknown.

Methods: This study evaluated the medical records of 437 patients with adenocarcinoma of the lung who underwent a surgical resection. The genetic status of the *EGFR* gene was investigated by polymerase chain reaction-based analyses. The serological typing of histocompatibility leukocyte antigen (HLA) class I was performed using a microcytotoxicity test of lymphocytes or polymerase chain reaction-sequence-specific oligonucleotides, and the correlation between the *EGFR* mutation and HLA alleles was analyzed.

Results: An *EGFR* mutation was found more frequently in females and never/former smokers than their counterpart. In females, the incidences of *EGFR* mutation were 61.0% and 41.7% in HLA-A2 (+) and A2 (−) patients with adenocarcinoma of the lung, respectively ($p = 0.008$). The *EGFR* mutation was found more frequently in female patients with HLA-A2. However, no significant correlation was identified between the frequencies of other HLA alleles and *EGFR* mutations in the same patients group.

Conclusions: *EGFR* mutations are associated with HLA-A2 in female patients with adenocarcinoma of the lung. Further research was needed to elucidate the other relevant factors in the histogenesis of lung cancer with an EGFR mutation.

Key Words: HLA, EGFR, Adenocarcinoma, Surgical resection.

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Lung cancer is the most common cause of cancer death in the majority of developed countries.[1] The absolute number of patients with lung cancer is still increasing worldwide.[2] Despite advances in therapeutic modalities, the prognosis of lung cancer is poor.[3,4] In particular, the patients with advanced-stage disease experienced a high mortality rate despite multidisciplinary treatment.[4] Therefore, the development of new modalities such as molecular target therapy is needed. Recently, epidermal growth factor receptor (*EGFR*)-tyrosine kinase inhibitor has shown dramatic clinical response in a significant proportion of patients harboring an EGFR mutation.[5–8] Such *EGFR* mutations occur more frequently in females, nonsmokers, Asians, and adenocarcinoma than in their counterparts.[9,10] However, there is no reliable information concerning carcinogens or molecules that affect the EGFR mutation in lung cancer susceptibility to make a paradigm shift from a preventive point of view.

The major histocompatibility complex, namely, the histocompatibility leukocyte antigen (HLA) in humans, is specific to each individual and has some hereditary features such as haplotype inheritance, allele polymorphism, and linkage disequilibrium. HLA class I molecules play a key role in the immune response by presenting endogenous antigens to CD8 positive T cells. The natural killer cells also specifically recognize HLA class I molecules, and this recognition inhibits the killing function of natural killer cells.[11] The alleles of HLA are involved in the immune response, and some particular HLA types influence the susceptibility to several diseases, especially autoimmune disease.[12] HLA-A2 and -A24 are frequent in the Japanese population, and several HLA alleles influence the prognosis of lung cancer patients.[13,14] Therefore, they may have many features in common with the *EGFR* mutation among the Japanese. These findings led to the speculation that the *EGFR* mutation may occur in the tumor harboring a particular HLA type. This study investigated the relationship between the EGFR mutation and antigen frequencies of HLA-A and -B loci among patients with adenocarcinoma of the lung.

PATIENTS AND METHODS

Patients

Patients provided written informed consent to participate in this study and to donate blood, and a signed consent

form was also obtained from each patient before collection of the blood samples. The institutional review board-approved informed consent for the use of the tumor specimens was obtained either from all the patients or from the patient's legal guardians. Tumor samples were obtained from 623 patients with primary lung adenocarcinoma who had undergone a surgical resection between April 1996 and February 2009. Forty-seven patients were excluded from the analysis because of insufficient blood samples (anemia or viral infections), and the tumor samples from 139 patients were too small to extract sufficient DNA for all the analyses that include the determination of the EGFR mutation. Finally, a total of 437 patients (247 males and 190 females) were included in the analysis. The patients ranged in age from 18 to 91 years (average age: 67.8 years). There were 167 never smokers, 109 former smokers, and 161 current smokers. Former smokers were defined as those who quit smoking at least 3 years before the time of surgery. According to the international pathologic staging system,[15] the pathologic stages were diagnosed as stage I in 283 patients (IA: 198, IB: 85), stage II in 45 (IIA: 10, IIB: 35), stage III in 91 (IIIA: 51, IIIB: 40), and stage IV in 18. The patient characteristics are shown in Table 1.

The patients were followed up every month within the first postoperative year and at approximately at 2- to 4-month intervals thereafter. The evaluations included a physical examination, chest roentgenography, an analysis of blood chemistry, and measurements of tumor markers. Chest and abdominal computed tomography, brain magnetic resonance imaging, and a bone scintiscan were performed every 6 months for 3 years after surgery. Additional examinations were performed if any symptoms or signs of recurrence were detected. The median follow-up periods were 47.4 months. At the last follow-up, 257 patients were alive and free of cancer, whereas 55 patients had died of other causes without evidence of cancer, 63 patients were alive with recurrent cancer, and 62 patients had died of cancer.

TABLE 1. Characteristics of the Adenocarcinoma Patients in This Study

Characteristics	No. of Patients (%) $n = 437$
Gender	
Male	247 (56.5)
Female	190 (43.5)
Smoking	
Never	167 (38.2)
Former	109 (24.9)
Current	161 (36.8)
Pathological stage	
IA	198 (45.3)
IB	85 (19.5)
IIA	10 (2.3)
IIB	35 (8.0)
IIIA	51 (11.7)
IIIB	40 (9.2)
IV	18 (4.1)

Detection of EGFR Mutations

Genomic DNA was extracted from each tumor. The EGFR mutations were examined by previously described methods.[9] Briefly, the exon 19 deletion of *EGFR* was detected by a simple screening method, which was the detection of shorter band than 147 bp of the polymerase chain reaction (PCR) product from wild-type allele on agarose gel electrophoresis. The exon 21 L858R point mutation was detected by mutant-allele-specific amplification. The 3′ ends of 22-bp oligonucleotides changed as the PCR primers corresponded to G for T of *EGFR* codon 858. Namely, the sense primer sequence for wild type was 5′-TCAAGATCACAGATTTTGGGCT and that for L858R mutation was 5′-TCAAGATCACAGATTTTGGGCG. The antisense primer sequence for both wild type and mutant type was 5′-CATCCTCCCCTGCATGTGTTAAAC. The PCR products were separated by electrophoresis on a 2% agarose gel.

HLA Serological Typing

The serological typing for HLA class I antigens was performed using a microcytotoxicity test of the lymphocytes[16] from April 1996 to June 2003. From July 2003 to February 2009, HLA-A and -B loci, but not HLA-C locus, were examined using PCR-sequence-specific oligonucleotides by SRS Inc., Tokyo, Japan. Each examination was performed using fresh peripheral blood lymphocytes of patients. PCR-sequence-specific oligonucleotides was developed as a new method with a high specificity using a small amount of DNA. The 26 samples from the lung cancer patients were checked using these two methods, and all the results were completely consistent. HLA-B5 is a broad antigen serotype that was divided into the heterogeneous subgroups of HLA-B51 and -B52 in 2004.[17] Therefore, the samples expressing B5 were rearranged for B51 or B52 in this study.

Statistical Analysis

Statistical associations were determined by the χ^2 test. The Kaplan-Meier method was used to estimate the probability of survival, and survival differences were analyzed by the log-rank test. The statistical difference was considered to be significant if the p value was less than 0.05. The data were analyzed with the use of the Abacus Concepts; Survival Tools for the StatView software package (Abacus Concepts, Inc., Berkeley, CA).

RESULTS

Relationship Between EGFR Mutation and Clinicopathological Factors

EGFR mutations were detected in 165 patients (37.8%) among 437 patients. Ninety-four (21.5%) patients exhibited the L858R point mutation in exon 21 and 71 (16.2%) had an inframe deletion in exon 19. An *EGFR* mutation was found more frequently in females and never/former smokers than their counterparts (Table 2). No significant correlation was identified between the *EGFR* mutations and age, pathologic stage, pathologic T status, and pathologic N status. Next, we investigated the relationship between allele frequencies of HLA and EGFR mutation to interpret the phenomenon showing a high frequency of *EGFR* mutation in females and never/former smokers.