HLA alleles and lung cancer in a Turkish population

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Background: The concept of genetic factors playing a role in the pathogenesis of lung cancer has gained increased attention. The present study was undertaken to examine the question of HLA association with lung cancer and to investigate the effects of HLA on survival time.

Methods: The distribution of HLA class I (A, B, C) antigens and class II (DR, DQ) alleles were studied in 81 unrelated Turkish patients with lung cancer. The HLA status of patients was compared with that of a control group consisting of 117 ethnically matched healthy donors. HLA class I antigens were studied by Terasaki's microlymphocytotoxicity test and HLA class II alleles were studied by polymerase chain reaction with the sequence specific primer (PCR-SSP) low resolution method.

Results: Only the frequencies of HLA-B51 and -DRB1*15 were lower in the lung cancer group compared with the healthy control patients. In a univariate analysis, age ($P=0.03$), Karnofsky Performance Status ($P=0.0001$), stage ($P=0.01$), HLA A24(9) ($P=0.008$), HLA B53 ($P=0.0006$), HLA B63(15) ($P=0.01$), HLA B64(14) ($P=0.01$), HLA B65(14) ($P=0.01$) and HLA CW5 ($P=0.01$) were significant prognostic factors. In a multivariate analysis, Karnofsky Performance Status ($P=0.001$), stage ($P=0.02$), HLA B53 ($P=0.03$) and HLA B64(14) ($P=0.03$) were independent prognostic variables.

Conclusions: This study demonstrates different HLA types among patients with lung cancer and healthy control subjects. Our results suggest that HLA antigens might affect the prognosis in lung cancer. Further investigations are warranted to delineate any possible role of the HLA system in the pathogenesis and prognosis of lung cancer.

Key words: Lung neoplasms, HLA antigens, survival, prognosis, Turkey

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Lung cancer is currently the leading cause of cancer-related death, and various genetic factors have been implicated in its pathogenesis. In recent years, there have been many reports of genetic predispositions for particular malignancies and numerous studies of genetic elements, such as that of the major histocompatibility complex (MHC), on the development of lung cancer.[1]

MHC is located on the short arm of chromosome 6, also termed the histocompatibility locus antigen (HLA) region. The MHC includes HLA class I (A, B, C) and HLA class II (DR, DQ) alleles, which code for HLA class I (A, B, C) and HLA class II (DR, DQ).[2] One important characteristic of the HLA alleles is that they are highly polymorphic (ie. several alleles exist at each locus), and this feature makes HLA an ideal marker for genetic studies.[3] In recent years, there have been many reports on the relationship between HLA and lung cancer occurrence, but the results are controversial.[1,4-8] These controversies may have resulted from the use of a limited number of HLA antigens as determined by serological tests in the past.[4-8] Some of these divergent results may also be attributable to variations in the study populations. Studies of pure ethnic

groups have proven to be of special interest in highlighting the associations between HLA antigens and lung cancer, as shown in two studies carried out in Japanese people.[1,9]

The identification of prognostic factors could potentially increase prognostic accuracy and might also be helpful for designing clinical trials of novel therapies. Several studies have described prognostic factors, such as age, pretreatment stage, performance status, weight loss and serum lactate dehydrogenase (LDH), and others associated with lung cancer.[10] These studies have indicated a relationship between HLA antigens and the prognosis of colon,[11] breast,[12] laryngeal carcinoma[13] and malignant melanoma.[14] According to the results of these studies, histocompatibility antigens in human tumors may directly influence the type of immune response and possible escape mechanisms. Thus, analysis of HLA may be important for determining not only the etiology, but also the appropriate therapy and prognosis of lung cancer. However, there are few reports investigating this relationship.

However, in a search of medical databases in English or Turkish languages, no single report with a reference to Turkish patients was noted on the possible association

between HLA and lung cancer. This study was therefore undertaken to investigate any association between HLA antigens and lung cancer in a Turkish population using serological and molecular techniques. As HLA type may also bear significance for prognosis as well as etiology, we also investigated any possible association between HLA and survival time with lung cancer.

Methods

We prospectively studied 81 unrelated Turkish patients (2 women and 79 men) with lung cancer, who were admitted to the Radiation Oncology Department of Medical Faculty Hospital at Ondokuz Mayis University, Samsun, between February 1999 and June 2001. All patients diagnosed with histologically defined carcinoma of the lung were under treatment with radiotherapy. These patients ranged in age from 35 to 78 years. The tumors were staged according to the American Joint Committee on Cancer (AJCC) staging system.[15] The performance status of each patient was scored according to the Karnofsky Performance Status (KPS) scale, and weight loss was defined by loss in the 3 months prior to diagnosis. Table 1 shows the pretreatment patient characteristics. One hundred and seventeen unrelated healthy donors (75 women, 42 men) without lung cancer living in the same geographic area were enrolled to serve as the control group. The controls ranged in age from 25 to 81. All patients and healthy donors were informed about the study and provided consent to participation.

HLA class I antigens were determined by the National Institutes of Health standard microcytotoxicity assay on peripheral blood mononuclear cells at the immunology unit of the microbiology and clinical microbiology department of Ondokuz Mayis University, Samsun.[16] Lymphocytes were isolated from heparinized peripheral blood by centrifugation over a Ficoll-Isopaque gradient, and HLA-A, -B, and -C typing was performed using a standard lymphocyte microcytotoxicity technique and Terasaki trays.

Genomic DNA was prepared from whole blood by denaturation/precipitation using trimethylammonium bromide salts for polymerase chain reaction (PCR) amplification. HLA genotyping was performed for HLA class II alleles using PCR amplification with DRB, and DQB low resolution typing by the PCR-SSP method. The PCR reaction mixtures contained 50ng/µL genomic DNA, 10xPCR buffer, dNTP, taq polymerase (Promega, USA), and specific primers. PCR amplifications were carried out in the GeneAmp PCR system 9700 (Perkin Elmer, Cetus, USA), and amplicons were then loaded in 3 mm wide slots in 2% agarose gels. Gels were examined under UV illumination and documented by photography.

Frequencies of HLA were compared by the chi-square ($\chi2$) test. The Fisher exact test was used where appropriate.

Table 1. Patient characteristics prior to treatment.

Characteristic	n (N=81)	%
Age		
<60	41	49
≥60	40	51
Histology		
Squamous cell	58	71
Adenocarcinoma	12	15
Small cell	11	14
KPS		
<70	21	26
≥70	60	74
Stage		
I	4	5
IIa	2	3
IIb	5	6
IIIa	18	22
IIIb	31	38
IV	21	26
LDH		
Normal	73	10
Elevated	8	90
Weight loss		
≥5%	55	68
>5%	26	32

Odds ratios (OR) with 95% confidence intervals (CI) were calculated. The level of significance was set at 0.05. The probability was corrected by the number of alleles studied. Survival was measured from the time of the diagnosis until the patient died or until the final analysis. Survival was analyzed using the Kaplan-Meier method. Differences between survival curves for each paramater were analysed by the log rank test. A multivariate analysis was performed by the Cox proportional hazard method. The parameters examined were age, histology, KPS, stage, LDH, weight loss and HLA antigens.

Results

There were significantly lower frequencies of HLA-B51 ($\chi2$=8.59, OR=0.35, CI=0.17-0.69, P=0.003, pc = NS) and HLA-DRB1*15 ($\chi2$=5.45, OR=0.35, CI=0.15-0.81, P=0.02, pc =NS) in the patients compared with the controls (Table 2). When the patients were subgrouped according to their histopathological features, no significant difference was found with regard to the HLA class I antigen

and class II alleles frequencies in squamous cell carcinoma cases compared with the controls. Adenocarcinoma (n=12) and small cell carcinoma (n=11) cases could not be analyzed separately due to their small number.

When we evaluated the factors that might affect survival by univariate analysis, age (P=0.03), KPS (P=0.0001), and stage (P=0.01) were significant prognostic factors (Table 3). The analyses of HLA-related parameters showed that A 24(9) (P=0.008), B53 (P=0.0006), B63 (15) (P=0.01), B64 (14) (P=0.01), B65 (14) (P=0.01) and CW5 (P=0.01) were associated with a shortened mean survival time (Table 4). On multivariate analysis KPS (P=0.001), stage (P=0.02), HLA B53 (P=0.03) (Figure 1) and HLA B64(14) (P=0.03) (Figure 2) were independent prognostic variables (Table 5).

Discussion

Associations between HLA antigens and susceptibility to various diseases have provided fresh insight into pathogenetic mechanisms by disclosing genetically controlled immunological bases that render certain subjects at greater risk of acquiring these diseases. Various genetic factors have been implicated in the pathogenesis of lung cancer.[5]

Studies of the association between HLA antigens and lung cancer have yielded conflicting results, but do suggest that several HLA antigens may be associated with a risk of developing lung cancer. An increased frequency has been reported for HLA -B40,[8] HLA-A1[9] and HLA-A29,[4] HLA-DR9[1,9] and a decreased frequency has been reported for HLA-A2, HLA-A3,[4] HLA-B40,[7] HLA-A31, HLA-B49, HLA-DR7,[5] HLA-A33, HLA-B44, HLA-B62, HLA-B75,[1] HLA-DR13 and HLA-DR14[1,9] in lung cancer patients as a whole. However, some investigators found no association between HLA antigens and the development of lung cancer.[6,17] Our study indicated the presence of significantly decreased frequencies of HLA-B51 and HLA-DRB1*15 in the patients when compared with healthy controls. However, all the p values become nonsignificant when multiplied by the number alleles studied, which implies that these observations need further study in a large, prospective study. This study provides the first available

data in a Turkish population on the association between HLA and lung cancer.

Some investigators divided lung cancer patients into subgroups according to histology. Ford et al found[8] that there was an increased frequency of HLA-B5 in small cell carcinomas, HLA-B15 in anaplastic tumours, HLA-B40 in stage III patients and a decreased frequency of HLA-B12 in adenocarcinomas. Toumbis et al[4] found that there was a significant increase in frequency of HLA-Aw19 and a decreased frequency of HLA-A2 in squamous cell carcinomas; a decreased frequency of HLA-A3 in small cell carcinomas; and no significant change in adenocarcinoma. In the present study no significant difference was found with regard to the HLA class I antigen and class II alleles frequencies in squamous cell carcinoma cases compared with the controls. Adenocarcinoma (n=12) and small cell carcinoma (n=11) cases could not be analyzed separately due to their small number.

Some of the divergent results described in the literature may be attributable to the use of different populations with different tumor types and risk factors. HLA antigen frequencies vary for different ethnic groups and in populations separated by geographical boundaries.[5] For example, DRB1*09 was found to be the most frequent allele (21.3%) in the Japanese population, whereas it was rare in the Turkish population (6.8%).[1,18] In addition, study methods could also account for these discrepancies. Most studies in lung cancer have been assessed only by serological techniques.[4-8] Yoshimura et al.[1] used both molecular and serological techniques, but their study population was different from the Turkish population.

After establishing the diagnosis of lung cancer, it is desirable to determine the likely clinical course and survival outcome. This is important for both patients and the treating physician, because this estimate is essential to guide the choice of therapy. Several retrospective analyses have evaluated traditional and newer prognostic factors for patients with lung cancer. Age, pretreatment stage, performance status, weight loss, and LDH are important factors.[10,19] The findings of the present study showed that age, KPS and pretreatment stage were significant prognostic

Table 2. Frequency of HLA-B51 and HLA-DRB1*15 in Turkish patients with lung cancer and healthy controls.

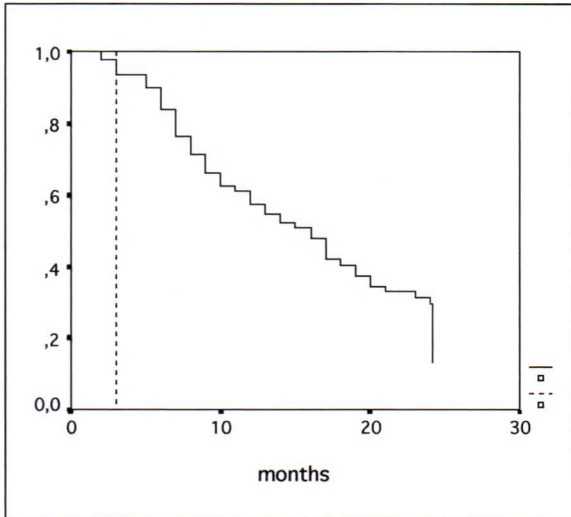| Antigens	Lung Cancer (n=81)		Control (n=117)		χ^2	OR	95% CI	P	P_c
	n	%	n	%					
B51	14	17.3	44	37.6	8.59	0.35	0.17-0.69	0.003	NS
DRB1*15	8	9.9	28	23.9	5.45	0.35	0.15-0.81	0.02	NS

P_c= corrected P value

Figure 1. Survival curves in cancer patients with HLA B53 (---) and without HLA B53 (—)(*P*=0.03).
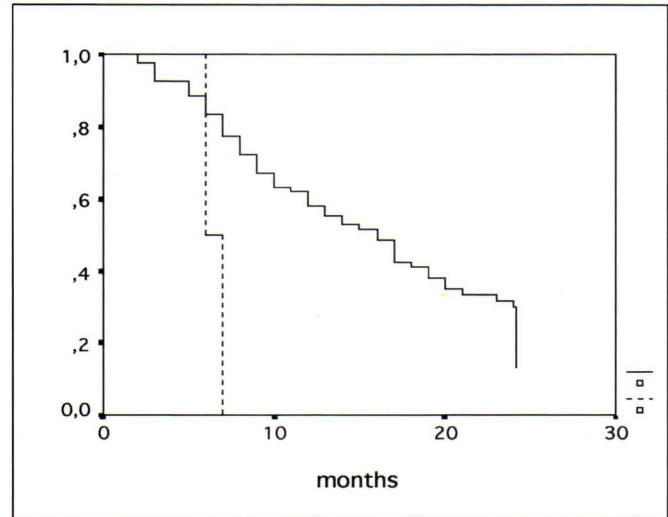

Figure. 2. Survival curves in cancer patients with HLA B64(14)(---) and without HLA B64(14) (—) (*P*=0.03).

Table 3. Univariate analysis of possible prognostic factors in patients with lung carcinoma.

Characteristics	Median survival	Log Rank	Standard error	95% CI	*P* value
Age					
<60	12	4.67	3.12	5.89-18.11	0.03
60	19		3.36	12.42-25.58	
Histology					
Squamous cell	16	0.31	1.89	12.29-19.71	0.8
Adenocarcinoma	14		2.60	8.91-19.09	
Small cell	19		7.18	4.93-33.07	
KPS					
<70	7	35.55	0.76	5.52-8.48	0.00001
70	20		2.63	14.85-25.15	
Stage					
I	—	14.09	—	—	0.01
IIa	7		0.00	0.00-0.00	
IIb	21		0.00	0.00-0.00	
IIIa	18		4.37	9.44-26.56	
IIIb	14		2.70	8.71-19.29	
IV	9		0.89	7.26-10.74	
LDH					
Normal	17	2.36	2.40	12.31-21.69	0.1
Elevated	10		2.36	5.38-14.62	
Weight loss					
5%	18	2.97	2.86	12.40-23.60	0.08
>5%	10		2.98	4.16-15.84	

Table 4. Univariate analysis of HLA class I antigens and class II alleles in patients with lung carcinoma.

HLA Antigens		Median survival	Log Rank	Standard error	95% CI	P value
A 24(9)	+	11	3.99	3.12	4.88-17.12	0.008
	-	19		2.07	14.95-23.05	
B53	+	3	11.85	—	0-0	0.0006
	-	16		1.87	12.34-19.66	
B63(15)	+	8	5.72	2.45	3.20-12.8	0.01
	-	16		1.78	12.51-19.49	
B64(14)	+	6	5.56	0.0	0-0	0.01
	-	16		1.79	12.49-19.51	
B65(14)	+	6	5.87	0.82	4.40-7.60	0.01
	-	16		1.78	12.51-19.49	
CW5	+	9	5.77	1.10	6.85-11.15	0.01
	-	17		1.68	13.70-20.30	

* Median survival time in cancer patients with HLA (+) and without HLA (-).

Table 5. Cox analysis of prognostic factors and HLA antigens in patients with lung carcinoma.

HLA Antigens	ß	Wald	Standard error	Relative Risk	P value
Stage	0.297	5.058	0.132	1.346	0.02
KPS	-1.213	12.026	0.350	0.297	0.001
B53	-2.377	4.394	1.134	0.93	0.03
B64(14)	-1.580	4.247	0.767	0.206	0.03

factors. In addition, KPS and pretreatment stage were also significant independent prognostic factors.

While investigating new prognostic factors associated with lung cancer, some authors investigated HLA and survival time. Rogentine et al.[6] studied seventy patients with squamous carcinoma or adenocarcinoma of the lung and found that possession of HLA-Aw19 and -B5 was significantly correlated with 2-year disease-free survival. Markman et al.[20] studied 50 patients with small cell carcinoma of the lung and found that patients possessing the HLA-A1 phenotype had a significantly poorer 1-year survival rate than individuals who did not possess this allele. Mottironi et al.[17] showed that patients exhibiting A3 and Aw33 antigens had a prolonged survival time. Prazak et al.[7] studied 162 patients with various histological types of lung carcinoma and found that patients with HLA-A10, A11 and B27 had a shortened mean survival time, but patients

with HLA-B12 had a prolonged survival time. We found that patients with HLA-A 24(9), B53, B63(15), B64(14), B65(14), and CW5 had a shortened mean survival time in univariate analyses, and that HLA B53 and HLA B64(14) were significant independent negative prognostic factors in patients with lung cancer, by univariate and multivariate survival analyses.

In conclusion, this study demonstrates different HLA types among patients with lung cancer and healthy control subjects. Our results suggest that HLA antigens might affect the prognosis in lung cancer. Our results show some differences when compared to international findings. Also, we found that patients with HLA- B53, and B64(14) had a shortened mean survival time. The small number of patients and ignorance of multiple confounding factors could weaken this association, but they are the first available data in a Turkish population on the association

between HLA and lung cancer and HLA effects of survival time. We suggest that further investigations be conducted to delineate any possible role of the HLA system in the pathogenesis and prognosis of lung cancer.

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