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Frequency changes in HLA-I alleles: A marker to guide immunotherapy in lung adenocarcinoma patients and its relationship with tumor mutational burden and PD-L1 expression

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Abstract

Background: The aim of the study was to investigate differences in HLA-I alleles between lung adenocarcinoma patients and healthy controls and determine their association with PD-L1 expression and tumor mutational burden (TMB) to understand the mechanism underlying lung adenocarcinoma susceptibility.

Methods: Differences in HLA allele frequencies between the two groups were analyzed in a case–control study. PD-L1 expression and TMB in lung adenocarcinoma patients were determined and their relationships with HLA-I were analyzed.

Results: The lung adenocarcinoma group showed significantly higher HLA-A*30:01 ($p = 0.0067$, odds ratio [OR], 1.834; 95% confidence interval [CI]: 1.176–2.860), B*13:02 ($p = 0.0050$, OR, 1.855; 95% CI: 1.217–2.829), and C*06:02 ($p = 0.0260$, OR, 1.478; 95% CI: 1.060–2.060) and significantly lower B*51:01 ($p = 0.0290$, OR, 0.6019; 95% CI: 0.3827–0.9467), and C*14:02 ($p = 0.0255$, OR, 0.5089; 95% CI: 0.2781–0.9312) than the control group. Haplotype analysis results showed that HLA-A*30:01–B*13:02 ($p = 0.0100$, OR, 1.909; 95% CI: 1.182–3.085), A*11:01–C*01:02 ($p = 0.0056$, OR, 1.909; 95% CI: 1.182–3.085), A*30:01–C*06:02 ($p = 0.0111$, OR, 1.846; 95% CI: 1.147–2.969), and B*13:02–C*06:02 ($p = 0.0067$, OR, 1.846; 95% CI: 1.147–2.969) frequencies significantly increased and B*51:01–C*14:02 ($p = 0.0219$, OR, 0.490; 95% CI: 0.263–0.914) frequency significantly decreased in lung adenocarcinoma patients. Three-locus haplotype analysis showed that HLA-A*30:01-B*13:02-C*06:02 frequency ($p = 0.0100$, OR, 1.909; 95% CI: 1.182–3.085) significantly increased in patients.

Conclusion: HLA-A*30:01, B*13:02, and C*06:02 may be the susceptibility genes and HLA-B*51:01 and C*14:01 act as the resistance genes of lung adenocarcinoma. The changes in HLA-I allele frequencies had no association with PD-L1 expression and TMB among these patients.

KEYWORDS
allele, human leukocyte antigen, lung adenocarcinoma, PD-L1, TMB

INTRODUCTION

Lung cancer is a malignancy that seriously affects human life and health. Given its high incidence, lung cancer is a leading cause of cancer-related deaths.[1] In China, it causes a substantial amount of morbidity and mortality,[2] and exceeds breast, prostate, and colorectal cancer combined in terms of total number of deaths.[3]

Human leukocyte antigen (HLA), expressed on somatic and immune cells, is highly polymorphic at the gene level.

The group of genes encoding HLA known as the HLA gene complex is found on the short arm of chromosome 6 at locus 21(6p21).[4] HLA molecules are classified into class I, II, and III and are mainly involved in the processing and presentation of antigen molecules to control the occurrence and strength of an immune response. HLA-I, detected on nucleated cells such as cancer, somatic, and immune cells, serves as one of the recognition markers of killer $CD8^+$ T cells and participates in endogenous antigen presentation. HLA-I is an important arm of tumor antigen presentation and plays an indispensable role in mediating antitumor immune response and neoplastic progression.[5]

Studies have found that the frequencies of some HLA alleles in patients with lung cancer differ from those in healthy individuals, which indicates the plausible correlation between lung cancer and HLA.[6–9] HLA-I expression downregulation has been linked to an acquired resistance to immune checkpoint blockade (ICB) therapy in patients with advanced NSCLC,[10] and HLA-I mutations and heterozygosity loss have been deemed as contributing factors to cancer immune evasion.[5,11] These previous studies have confirmed the association of HLA with tumors. For instance, an increase in some HLA-A, HLA-B, and HLA-C allele frequencies is thought to enhance the susceptibility to lung cancer, while the frequencies of some alleles are lower in lung cancer patients than in healthy subjects where they might play a protective role against lung cancer. The normal HLA-I expression is associated with a good prognosis of non-small cell lung cancer (NSCLC). HLA-I expression is often lost in cancer cells, which allows them to escape the host immune recognition system. However, the relationship between HLA-I molecules and the degree of response to lung cancer immunotherapy remains unclear.[12] A previous study explored the mechanism of immune escape in lung cancer from the perspective of HLA LOH (loss of more than 50% of the genome sequence of one of the HLA pairs of alleles).[5] Another study revealed the link between supertype HLA-A02 and improved survival outcomes.[13] Thus, researchers are keen on exploring the relationship between HLA and lung cancer.

The paramount importance of the host immune response to render protection from different cancer types has been well acknowledged. Several studies have focused on the role of immune checkpoints and development of immune checkpoint inhibitors (ICIs) against CTLA-4, PD-1, or PD-L1.[14–16] The paradigm shift in lung cancer immunotherapy has already begun. PD-L1 expression and TMB serve as the most validated markers in randomized trials and are highly predictive of a patient's response to ICIs.[17–19] PD-L1 overexpression in NSCLC was correlated with higher response rates to anti–PD-1/PD-L1 therapy,[20,21] and PD-L1 level was positively associated with overall survival of ICB-treated NSCLC patients.[22] Tumor mutational burden (TMB) is a potential biomarker of immunotherapy response, as evident from its correlation with response rates to anti-PD-L1 treatment in several tumor types.[23] Higher TMB can highlight the increase in the number of tumor-associated neoantigens, which are thought to prompt immune recognition and mediate cancer cell killing.[24–26] Hence, tumors with higher TMB are more sensitive to ICI therapy.[27–31] Cuppens et al.[32] found that a combination of several biomarkers has greater predictive power than a single biomarker, which caught our attention.

A few studies have determined the association of changes in the frequencies of certain HLA alleles with PD-L1 expression and TMB in lung adenocarcinoma patients. Herein, we aimed to determine the frequency changes in HLA-I alleles and its association with PD-L1 expression and TMB to test their applicability as biomarkers for accurate diagnosis and immunotherapy of lung adenocarcinoma at the gene level.

METHODS

Subjects

All patients were admitted to the First Affiliated Hospital of Xi'an Jiaotong University from June 2019 to November 2020 and were diagnosed with lung adenocarcinoma by pathology. Overall, 337 patients were included in the analysis. A total of 446 healthy volunteers were randomly selected from the same period of health checkup people. The Ethics Committee of the First Affiliated Hospital of Xi'an Jiaotong University reviewed and approved the study protocol.

HLA-I data

Genetic testing data were sequenced and analyzed by Genecast Biotechnology Co., Ltd. The peripheral blood cells of healthy volunteers were collected for HLA analysis. HLA genotype for each subject was inferred using Optitype,[33] which uses a normal germline bam file as an

TABLE 1 Patient and control characteristics.

General information	Lung cancer (%)	Control (%)
Mean age ± SD	62 ± 9.97	45 ± 0
Sex		
Male	171 (50.7%)	229 (51.3%)
Female	166 (49.3%)	217 (48.7%)
TMB		
<10 mutations/Mb	108 (90%)	
≥10 mutations/Mb	12 (10%)	
PD-L1 TPS		
<1%	31 (70.5%)	
≥1%	13 (29.5%)	
≥50%	7 (15.9%)	

TABLE 2 Comparison of frequencies of HLA-I alleles in lung cancer patients and the control group.

Alleles	Lung cancer (%)	Control (%)	*p*-value	OR	95% CI
A*01:01	3.86	5.27	0.1896	-	-
A*02:01	15.88	12.89	0.1073	-	-
A*02:03	2.67	3.36	0.4625	-	-
A*02:06	5.64	5.61	1.0000	-	-
A*02:07	6.53	6.50	1.0000	-	-
A*03:01	4.45	4.60	1.0000	-	-
A*11:01	18.84	19.28	0.8458	-	-
A*24:02	13.65	14.57	0.6096	-	-
A*26:01	3.26	3.48	0.8882	-	-
A*30:01	**7.12**	**4.04**	**0.0067**	**1.834**	**1.176–2.860**
A*31:01	2.67	4.26	0.1004	-	-
A*33:03	6.38	7.74	0.3231	-	-
B*07:02	2.82	3.03	0.8804	-	-
B*13:01	4.75	4.15	0.6194	-	-
B*13:02	**8.01**	**4.48**	**0.0050**	**1.855**	**1.217–2.829**
B*15:01	4.90	6.39	0.2286	-	-
B*15:02	1.93	3.48	0.0883	-	-
B*35:01	2.37	3.36	0.2913	-	-
B*40:01	7.27	6.28	0.4754	-	-
B*40:06	4.75	3.14	0.1111	-	-
B*44:03	3.41	3.70	0.7854	-	-
B*46:01	7.57	7.17	0.7701	-	-
B*48:01	3.26	2.80	0.6545	-	-
B*51:01	**4.30**	**6.95**	**0.0290**	**0.6019**	**0.3827–0.9467**
B*52:01	4.01	3.70	0.7910	-	-
B*54:01	3.41	2.47	0.2872	-	-
B*58:01	4.90	5.27	0.8169	-	-
C*01:02	12.76	12.11	0.6993	-	-
C*03:02	5.04	5.61	0.6521	-	-
C*03:03	6.82	7.06	0.9202	-	-
C*03:04	10.53	7.96	0.0911	-	-
C*04:01	5.64	7.74	0.1067	-	-
C*06:02	**11.87**	**8.41**	**0.0260**	**1.478**	**1.060–2.060**
C*07:02	12.46	14.01	0.4092	-	-
C*08:01	7.86	9.75	0.2109	-	-
C*12:02	3.86	4.04	0.8965	-	-
C*12:03	3.56	2.24	0.1248	-	-
C*14:02	**2.23**	**4.37**	**0.0255**	**0.5089**	**0.2781–0.9312**
xC*15:02	3.71	4.04	0.7928	-	-

Note: Bold indicates significant values $p < 0.05$.

input. The HLA-VBseq[34] tool was used to further confirm HLA genotyping.

HLA-I allele frequencies and haplotype frequencies

HLA-A, HLA-B, and HLA-C testing were carried out as per Hardy–Weinberg equilibrium using Arlequin software version 3.11 (http://cmpg.unibe.ch/software/arlequin 3).

Haplotype frequencies were calculated based on the EM algorithm using Arlequin. Haplotypes with frequencies <3% were excluded from analyses.

Assessment of PD-L1 expression using immunohistochemistry

Samples were analyzed for PD-L1 based on the FDA-approved Dako PD-L1 IHC 22C3 pharmDx kit (Agilent

TABLE 3 Comparison of haplotypes between lung cancer patients and control group.

Haplotype	Lung cancer (%)	Control (%)	*p*-value	OR	95% CI
A-B					
A*02:07-B*46:01	4.84	4.34	0.6281	1.126	0.700–1.810
A*11:01-B*40:01	2.76	3.06	0.8804	0.929	0.512–1.686
A*30:01-B*13:02	**6.23**	**3.36**	**0.0100**	**1.909**	**1.182–3.085**
A*33:03-B*58:01	3.68	4.14	0.6963	0.890	0.530–1.494
A-C					
A*02:07-C*01:02	4.45	4.27	0.9006	1.047	0.642–1.708
A*11:01-C*01:02	**3.81**	**1.62**	**0.0056**	**2.516**	**1.303–4.858**
A*11:01-C*07:02	3.25	5.16	0.0794	0.621	0.369–1.042
A*30:01-C*06:02	**6.22**	**3.48**	**0.0111**	**1.846**	**1.147–2.969**
A*33:03-C*03:02	3.85	4.23	0.7968	0.902	0.542–1.501
B-C					
B*07:02-C*07:02	2.82	3.03	0.8804	0.929	0.512–1.686
B*13:01-C*03:04	4.14	3.80	0.7939	1.094	0.656–1.823
B*13:02-C*06:02	**7.86**	**4.48**	**0.0067**	**1.818**	**1.190–2.776**
B*15:02-C*08:01	1.93	3.48	0.0883	0.546	0.284–1.052
B*40:01-C*07:02	2.78	3.16	0.7663	0.895	0.494–1.617
B*46:01-C*01:02	6.23	6.50	0.9169	0.956	0.634–1.441
B*51:01-C*14:02	**2.08**	**4.15**	**0.0219**	**0.490**	**0.263–0.914**
B*52:01-C*12:02	3.26	3.25	1.0000	1.004	0572–1.764
B*54:01-C*01:02	3.41	2.13	0.1543	1.623	0.877–3.006
B*58:01-C*03:02	4.90	5.27	0.8169	0.926	0.586–1.462
A-B-C					
A*02:07-B*46:01-C*01:02	4.28	4.12	0.8994	1.039	0.632–1.708
A*30:01-B*13:02-C*06:02	**6.23**	**3.36**	**0.0100**	**1.909**	**1.182–3.085**
A*33:03-B*58:01-C*03:02	3.70	4.14	0.6963	0.890	0.530–1.494

Note: Bold indicates significant values $p < 0.05$.

Technologies). A TPS ≥1% was considered as positive PD-L1 and that of ≥50% was deemed as PD-L1 high.

Assessment of TMB

TMB was evaluated by the TruSight Oncology 500 assay (Illumina Inc.). An integrated workflow was used to determine variants in 523 cancer-associated genes as well as for assessment of TMB. Results were expressed as number of nonsynonymous variants per Mb. Samples with 10 variants/Mb or more were considered as TMB high.

Statistical analysis

Differences in HLA-A, HLA-B, and HLA-C allele frequencies between lung adenocarcinoma and controls were compared using a chi-square test. All alleles with low frequencies (<3%) were excluded from the analyses.

Odds ratio (OR) and 95% confidence intervals (95% CI) were calculated for each allele. Statistical analyses were performed using SPSS (SPSS Inc.) and GraphPad Prism (GraphPad software Inc.).

RESULTS

Patient clinical characteristics

The general information of the 337 patients enrolled in this study is provided in Table 1. Their mean ± SD age was 62 ± 9.97 years, and there was a small difference in the number of male and female patients. Of the 120 patients that had TMB results, the score was low in 108 (90%) and high in 12 (10%) patients. Among the 44 patients with PD-L1 TPS results, 31 (70.5%) patients were negative and 13 (29.5%) were positive for PDL-1. Among the patients with positive PDL-1 expression, seven (15.9%) had a TPS of ≥50%.

Alleles in lung adenocarcinoma patients and controls

The number and frequency diversity of alleles were observed in both patients and controls. The lung adenocarcinoma group had significantly higher HLA-A*30:01 ($p = 0.0067$, OR, 1.834; 95% CI: 1.176–2.860), B*13:02 ($p = 0.0050$, OR, 1.855; 95% CI: 1.217–2.829), and C*06:02 ($p = 0.0260$, OR, 1.478; 95% CI: 1.060–2.060) and significantly lower HLA-B*51:01

($p = 0.0290$, OR, 0.6019; 95% CI: 0.3827–0.9467) and C*14:02 ($p = 0.0255$, OR, 0.5089; 95% CI: 0.2781–0.9312) than the control (Table 2). Differences in the frequencies of other alleles were not statistically significant.

Haplotype analysis in lung adenocarcinoma cases and controls

According to the results of two-locus haplotype analysis, the frequencies of HLA-A*30:01–B*13:02 ($p = 0.0100$, OR, 1.909; 95% CI: 1.182–3.085), A*11:01–C*01:02 ($p = 0.0056$, OR, 2.516; 95% CI: 1.303–4.858), A*30:01–C*06:02 ($p = 0.0111$, OR, 1.846; 95% CI: 1.147–2.969), and B*13:02–C*06:02 ($p = 0.0067$, OR, 1.818; 95% CI: 1.190–2.776) significantly increased and that of HLA-B*51:01–C*14:02 ($p = 0.0219$, OR, 0.490; 95% CI: 0.263–0.914) significantly decreased in lung adenocarcinoma patients as compared with the control group. Three-locus haplotype analysis revealed the significantly higher frequency of HLA-A*30:01–B*13:02-C 06:02 ($p = 0.0100$, OR, 1.909; 95% CI: 1.182–3.085) in lung adenocarcinoma patients than in the control (Table 3).

Relationship between frequency changes in HLA-I alleles and PD-L1 expression and TMB

We investigated the relationship of frequency changes in HLA-I alleles with PD-L1 expression and TMB at loci with statistically significant frequency changes in HLA-I alleles. However, no statistical association was observed at these loci (Table 4).

HLA-B/-C amino acid residues that mediate risk and protection in lung adenocarcinoma

For HLA-B and -C, we found six (positions 24, 41, 63, 103, 145, 163) and three (positions 99, 114, 163) key residues that significantly differed in amino acid frequencies between lung adenocarcinoma patients and controls (Tables 5 and 6).

For HLA-B, among the three amino acid residues at position 24, threonine (T) indicated risk ($p = 0.0009$, OR, 1.442; 95% CI: 1.166–1.784) of lung adenocarcinoma, alanine (A) exhibited protection ($p = 0.0365$, OR, 0.8069; 95% CI: 0.6604–0.9860), and serine (S) had no significant effect. At position 41, alanine (A) mediated protection ($p = 0.0027$, OR, 0.7174; 95% CI: 0.5776–0.8909) and threonine (T) had no significant effect. Of the amino acid residues at position 63, glutamic acid (E) was associated with the risk of lung adenocarcinoma ($p = 0.0134$, OR, 1.302; 95% CI: 1.056–1.606) and asparagine (N) mediated protection ($p = 0.0134$, OR, 0.7678; 95% CI: 0.6226–0.9468). At position 103, leucine (L) and valine (V) showed a risk ($p = 0.0413$, OR, 1.258; 95% CI: 1.009–1.569) and protective effect ($p = 0.0413$, OR, 0.7949; 95% CI: 0.6374–0.9912) for lung adenocarcinoma, respectively. At position 145, leucine (L) mediated the

TABLE 4 The relationship of HLA-I allele frequencies with PD-L1 expression and tumor mutational burden (TMB).

Biomaker	A*30:01	B*13:02	B*51:01	C*06:02	C*14:02
TPS (44 cases)	0.438	0.310	0.302	0.127	0.153
CPS (44 cases)	0.053	0.116	0.835	0.053	0.882
TMB (120 cases)	0.446	0.374	0.579	0.224	0.143

Note: The numbers in the table represent *p* values.

TABLE 5 HLA-B amino acid variants that exhibited a strong association with lung cancer.

Amino acid position	Amino acid variants	Lung cancer (AA-F%) $2n = 674$	Control (AA-F%) $2n = 892$	*p*-value	OR	95% CI
24	**A**	**316 (46.88)**	**466 (52.24)**	**0.0365**	**0.8069**	**0.6604–0.9860**
	S	107 (15.88)	166 (18.61)	0.1785		
	T	**251 (37.24)**	**260 (29.15)**	**0.0009**	**1.442**	**1.166–1.784**
41	**A**	**442 (65.58)**	**648 (72.65)**	**0.0027**	**0.7174**	**0.5776–0.8909**
	T	232 (34.42)	244 (27.35)			
63	**E**	**452 (67.06)**	**544 (60.99)**	**0.0134**	**1.302**	**1.056–1.606**
	N	**222 (32.94)**	**348 (39.01)**	**0.0134**	**0.7678**	**0.6226–0.9468**
103	**L**	**210 (31.16)**	**236 (26.46)**	**0.0413**	**1.258**	**1.009–1.569**
	V	**464 (68.84)**	**656 (73.54)**	**0.0413**	**0.7949**	**0.6374–0.9912**
145	**L**	**86 (12.76)**	**77 (8.63)**	**0.0081**	**1.548**	**1.118–2.143**
	R	**588 (87.24)**	**815 (91.37)**	**0.0081**	**0.6460**	**0.4666–0.8944**
163	**E**	**253 (37.54)**	**263 (29.48)**	**0.0008**	**1.437**	**1.162–1.777**
	L	**331 (49.11)**	**494 (55.38)**	**0.0138**	**0.7775**	**0.6361–0.9502**
	T	90 (13.35)	135 (15.13)	0.3197	0.8642	0.6480–1.152

Note: Bold indicates significant values $p < 0.05$.

TABLE 6 HLA-C amino acid variants that showed a strong association with lung cancer.

Amino acid position	Amino acid variants	Lung cancer (AA-F%) $2n = 674$	Control (AA-F%) $2n = 892$	*p*-value	OR	95% CI
99	C	94 (13.95)	114 (12.78)			
	F	**57 (8.46)**	**133 (14.91)**	**0.0001**	**0.5272**	**0.3798–0.7319**
	S	84 (12.46)	126 (14.13)	0.3391		
	Y	**439 (65.13)**	**519 (58.18)**	**0.0052**	**1.343**	**1.092–1.651**
114	**D**	**546 (81.01)**	**677 (75.90)**	**0.0154**	**1.355**	**1.059–1.733**
	N	**128 (18.99)**	**215 (24.10)**	**0.0154**	**0.7382**	**0.5771–0.9443**
156	D	10 (1.48)	10 (1.12)			
	L	356 (52.82)	470 (52.69)			
	Q	2 (0.30)	3 (0.33)			
	R	**164 (24.33)**	**269 (30.16)**	**0.0107**	**0.7447**	**0.5936–0.9343**
	W	**142 (21.07)**	**140 (15.70)**	**0.0061**	**1.434**	**1.107–1.857**

Note: Bold indicates significant values $p < 0.05$.

risk ($p = 0.0081$, OR, 1.548; 95% CI: 1.118–2.143) and arginine (R) showed a protective effect ($p = 0.0081$, OR, 0.6460; 95% CI: 0.4666–0.8944). At position 163, glutamic acid (E) showed a risk effect ($p = 0.0008$, OR, 1.437; 95% CI: 1.162–1.777), leucine (L) showed a protective effect ($p = 0.0138$, OR, 0.7775; 95% CI: 0.6361–0.9502), and threonine (T) had no significant association with lung adenocarcinoma.

For HLA-C, among the four possible amino acid residues at position 99, tyrosine (Y) showed a risk effect ($p = 0.0052$, OR, 1.343; 95% CI: 1.092–1.651) and phenylalanine (F) provided protection ($p = 0.0001$, OR, 0.5272; 95% CI: 0.3798–0.7319) from lung adenocarcinoma; the other two residues had no significant effect. At position 114, aspartic acid (D) was related to the risk of lung adenocarcinoma ($p = 0.0154$, OR, 1.355; 95% CI: 1.059–1.733) and asparagine (N) showed a protective effect ($p = 0.0154$, OR, 0.7382; 95% CI: 0.5771–0.9443). At position 156, tryptophan (W) mediated risk ($p = 0.0061$, OR, 1.434; 95% CI: 1.107–1.857) and arginine (R) mediated protection ($p = 0.0107$, OR, 0.7447; 95% CI: 0.5936–0.9343); the other three residues had no significant effect on lung adenocarcinoma.

DISCUSSION

Lung cancer is caused by environmental variables, genetic susceptibility, and other factors. Considering its rapid progression, high metastasis rate, and poor prognosis, lung cancer has become the world's highest cause of cancer-related mortality. The rapid development in molecular biology technology has propelled the research at the genetic level on the association between lung cancer and HLA. Considering its importance in the human immune system, HLA participates in antigen presentation, tumor cell recognition and killing by immune cells, and antitumor immune response. Endogenous cancer cells present antigenic peptides on their cell membranes that are recognized by HLA-I. This phenomenon serves as the molecular basis for human T lymphocytes to recognize and kill cancer cells. HLA gene polymorphism dictates the polymorphism of HLA antigen molecules and determines the complexity and diversity of immune responses involved in the HLA system. HLA gene polymorphisms at multiple genetic loci can increase the risk of predisposition to lung cancer. The function of the HLA-I system of exposing foreign antigens to the host immune system renders it a crucial role in the modern immunotherapy era.[35] In this study, we analyzed the differences in the expression of HLA-I gene between lung adenocarcinoma patients and healthy controls, trying to find HLA-I gene that can be used as a biomarker. PD-L1 and TMB are widely concerned as potential biomarkers, but few studies have been conducted to explore the relationship between HLA-I gene expression and PD-L1 and TMB. Therefore, we investigated the possible association between the above three potential biomarkers of lung adenocarcinoma. In addition, we also identified the specific amino acids and elucidated structural features that mediated lung adenocarcinoma risk or protective effect. As the available references are limited, we conducted this study from a new point of view to provide a comprehensive reference for clinical immunotherapy. Studies have confirmed the role of several HLA genes in cancer pathogenesis. The susceptibility to lung cancer and patient clinical characteristics may be related to certain amino acid sequences encoded by various HLA genes.[36] We conducted genetic studies on lung adenocarcinoma patients, and found that HLA-A*30:01, B*13:02, and C*06:02 frequencies were significantly higher in patients than in healthy controls ($p < 0.05$), suggesting that these alleles may increase the susceptibility to lung adenocarcinoma. These alleles might perform important regulatory functions by activating oncogenes, promoting carcinogenesis and, thus, contributing to occurrence of lung adenocarcinoma. B*51:01 and C*14:02 frequencies were significantly lower in adenocarcinoma patients than in healthy controls ($p < 0.05$),

suggestive of their role as resistance genes in lung adenocarcinoma. In addition, haplotype analysis showed that the frequencies of HLA-A*30:01–B*13:02, A*11:01–C*01:02, A*30:01–C*06:02, and B*13:02–C*06:02 significantly increased ($p < 0.05$) and that of HLA-B*51:01–C*14:02 significantly decreased in the patient group. In addition, HLA-A*30:01-B*13:02–C*06:02 frequency significantly increased among patients as compared with the control ($p < 0.05$), suggesting that these haplotypes may act as markers of lung adenocarcinoma. We also analyzed the relationship between the frequency changes in HLA-I alleles and PD-L1 expression and TMB at the loci with significant changes in HLA-I allele frequencies but failed to observe any statistical association. Perea et al.[1] reported similar results.

Many studies have revealed the abnormal expression of HLA-I genes in lung cancer patients. Hurkmans et al.[37] observed downregulation of 48 and 41% in HLA-A and HLA-B/C expression among 30 NSCLC patients. Perea et al.[1] reported HLA-I antigen downregulation in 44% of patients. Baba et al.[38] found 10 types of HLA-I gene haplotype deletion and three types of HLA-I antigen expression deletion in 26 lung cancer cell lines. Kikuchi et al.[39] studied 161 NSCLC tissues and found that 68.9% had low or no expression of HLA-I antigens and that the $CD8^{+}$T cell population in these cancer tissues significantly decreased as compared with that in the cancer tissues with strong HLA-I antigen expression. Yoshimura et al.[40] suggested that HLA-A33, -B44, -B62, and -B75 frequencies were lower in lung cancer patients than in controls and that these changes may contribute to lung cancer susceptibility. A previous study found that the frequencies of four HLA-I alleles, including HLA-A*0201, A*2601, B*1518, and B*3802, were higher in lung cancer patients from the Han ethnicity from North China.[41] Considering the current study results, the expression of HLA-I in lung cancer tissues is not completely consistent with previous studies, and the underlying mechanism needs to be studied further.

As a transmembrane protein expressed on different cells, PD-L1 exhibits inhibitory functions and prevents T cell activation.[42] Tumor cells are thought to escape the inhibitory effect of the host immune system through PD-L1 overexpression.[43] Previous studies have reported a better response rate of NSCLC patients with higher PD-L1 expression to anti-PD-1/PD-L1 therapy. Although PD-L1 has a predictive value, various shortcomings restrict its ability to accurately predict the antitumor response to ICIs.[44,45]

TMB corresponds to the total somatic or acquired mutations per coding area of a tumor genome that encodes for tumor-specific neoantigens. These molecules subsequently activate the host T-cell immune response against tumor.[46] TMB is a valuable prognostic biomarker for different solid tumors, but its importance in NSCLC treatment decision-making is still debatable.

Herein, we investigated the amino acid sequences encoded by HLA-B and HLA-C alleles in relation to lung adenocarcinoma. We identified the specific amino acids and elucidated structural features that mediated lung adenocarcinoma risk or protective effect. The amino acids Thr^{24}, Glu^{63}, Leu^{103}, Leu^{145}, and Glu^{163} at HLA-B showed susceptible associations, while Ala^{24}, Ala^{41}, Asn^{63}, Val^{103}, Arg^{145}, and Leu^{163} exhibited protective associations. At HLA-C, the amino acids Tyr^{99}, Asp^{114}, and Trp^{156} indicated susceptibility and Phe^{99}, Asn^{114}, and Arg^{156} exhibited protective effects.

The knowledge of the genetic susceptibility mechanism underlying lung adenocarcinoma can reveal the pathogenesis of lung cancer and facilitate development of targeted interventions for the high-risk population. The above results suggest that the occurrence of lung cancer may be associated at least in part with autoimmunity. The specific mechanism of HLA in lung cancer and the relationship of HLA-I with PD-L1 expression and TMB remain unclear. Uncovering the molecular mechanism can open up new horizons for treatment.

This study had a few limitations. First, it adopted a case–control method. The patients were all from the hospital; hence, there was a selection bias. In addition, the sample size of this experiment was limited, and the conclusions drawn still need to be verified in a large-scale cohort study.

In conclusion, although there were still uncertainties in this study, several potential susceptibility and resistance gene loci of lung adenocarcinoma were confirmed. Future studies should determine the association between changes in HLA-I gene frequencies and PD-L1 expression and TMB. HLA-I heterozygosity and diversity have attracted attention as potential predictive biomarkers.[47,48] The roles of several biomarkers in the diagnosis and treatment of lung cancer patients have been elucidated. This study provided additional ideas and clues for pathogenesis of lung cancer and immunotherapy. PD-L1, TMB, or HLA-I diversity as biomarkers might reveal the exact patient population that may benefit the most and encourage development of new agents and treatment combinations. We believe that a deeper understanding of these biomarkers and their mutual relationship as well as the development of ICIs will provide durable benefits to a growing number of patients.

AUTHOR CONTRIBUTIONS

Wu Xuanpeng compiled the data and wrote the draft. Wang Hao, Xue Fei and Jiang Tao designed the test plan. Wang Tianju analyzed the data. Chen Nanzheng and Zhang Yong provided resources. Zhang Guangjian, Fu Junke and Wu Qifei provided guidance and edited the draft. All authors have reviewed this article.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing financial and personal interests in this study.

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