

Analysis of HLA-A, HLA-B and HLA-DRB1 alleles in Chinese patients with lung cancer

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ABSTRACT. The primary function of the human leukocyte antigen (HLA) system is to regulate the immune response. Because of its important role in the immune response and its high degree of polymorphism, the HLA system is associated with many diseases. We examined the polymorphisms of HLA-A, B and DRB1 alleles in 100 unrelated patients with lung carcinoma and in 438 unrelated normal controls of Han nationality from North China, using sequence-based typing and PCR with sequence-specific primers. We found that the frequencies of HLA-A*0201, A*2601, B*1518, B*3802, DRB1*0401, DRB1*0402, and DRB1*1201 were higher in the lung carcinoma group than in the normal control group. The P values were 0.035, 0.040, 0.001, 0.017, 0.014, 0.004, and 0.019, respectively, and the odds ratio values were 1.052, 3.513, 4.047, 3.054, 4.237, 19.397, and 2.128, respectively. The frequency of HLA-DRB1*1302 was lower in the lung carcinoma group than in the normal control group (P = 0.046, odds ratio = 0.168). We concluded that patients with lung

cancer and healthy controls of Han nationality from North China differ in the frequencies of various HLA alleles.

Key words: Lung carcinoma; Human leukocyte antigen; Gene frequency

INTRODUCTION

Lung cancer is a common malignant tumor in the world, and its prognosis is poor. About 13,000,000 patients are diagnosed with lung cancer every year; the 5-year survival rate of lung cancer is only 10-15% (Parkin et al., 2005). In Europe, United States and China, lung cancer is currently the leading cause of cancer-related death (Ferlay et al., 2007; Zhang et al., 2008; Chen et al., 2008; Jemal et al., 2009). Despite the hard efforts that have been made, the etiology of lung cancer remains unclear. Recently, many researchers paid attention to the genetic susceptibility to lung cancer. With the improvement in molecular biotechnology, more and more genes associated with genetic susceptibility to lung cancer have been found. Most of them are DNA repair genes (such as *OGG1*, *XRCC1* and *APEX1*) and biotransformation genes (such as *CYP1* gene family and *EPHX1* gene) (Kiyohara et al., 2006; Gresner et al., 2007).

The human leukocyte antigen (HLA) system is the major histocompatibility complex in humans. The primary function of the HLA system is to regulate the immune response (Bjorkman et al., 1987). Because of its important role in the immune response and high polymorphism, the HLA system is associated with many diseases. Recently, many papers about the association between HLA and many types of tumor have been published, including cervical cancer (Schiff et al., 2005; Madeleine et al., 2008), leukemia (Dorak et al., 2002; Mundhada et al., 2004; Wu et al., 2007; Yari et al., 2008), gastric cancer (Wu et al., 2002; Quintero et al., 2005), ovarian cancer (Kübler et al., 2006), thyroid cancer (Haghpanah et al., 2009), and breast cancer (Cantú de León et al., 2009).

In 1998, Tokumoto studied the association between HLA and lung cancer. Thirty-six Japanese lung cancer patients and 90 controls were subjected to typing for HLA I and II antigens, and the results showed that the lung cancer patients had increased frequencies of HLA-DRB1*0901 and decreased frequencies of HLA-DRB1*1302 and DRB1*14-related alleles. In 2000, Yoshimura and co-workers showed similar results. As far as we know, there has been no report about the association between HLA polymorphism and Chinese lung cancer patients.

In our study, 100 Chinese lung cancer patients and 483 controls were subjected to typing for HLA I and II antigens by sequence-based typing (SBT) and polymerase chain reaction (PCR) amplification with sequence-specific primer (PCR-SSP) methods. Our results showed that some HLA allele frequencies were different between lung cancer patients and controls, suggesting that lung cancer may be associated with specific alleles.

MATERIAL AND METHODS

Subjects

The study enrolled 100 unrelated patients with lung cancer of Han nationality from North China. All patients were admitted to our hospital between March 2009 and May 2009, and the diagnosis of lung cancer was made histologically. The subjects ranged in age from 36 to 77 years, and

68 of them were males and 32 females. Eighty-one patients had non-small cell lung cancer and 19 patients had small cell lung cancer. The 483 controls were healthy people of Han nationality from North China, and they were not related to each other or to the patients. All had undergone a chest X-ray, which showed no anomaly. All subjects gave informed consent to participate in this study.

HLA DNA typing

Genomic DNA from patients and controls was extracted by standard techniques from peripheral blood leukocytes (Davis et al., 1980; Miller et al., 1988). DNA was typed using the PROTRAN S1 sequence-based typing kit (Protran, Germany), following manufacturer instructions. If an ambiguous result was obtained, it was confirmed using the QIAGEN Olerup SSP typing kit (Qiagen, Netherlands).

Statistical analysis

The frequencies of alleles of HLA-A, HLA-B, HLA-DRB1 and linkage disequilibrium of the alleles were estimated using the Arlequin program, version 2.000. The frequency differences of alleles between patients and controls were estimated using the χ^2 test or the Fisher exact test where appropriate by the SPSS program, version 13.0. The level of significance was set at $P < 0.05$. If the frequency difference of an allele showed statistical significance, odds ratio (OR) and the 95% confidence interval (CI) were calculated.

RESULTS

Table 1 and Figure 1 show the HLA-A, B and DRB1 alleles whose frequencies were different between lung cancer patients and healthy controls. We found that the frequencies of 7 HLA alleles were higher in lung cancer patients than in healthy controls: HLA-A*0201 (0.2150 vs 0.1540, $P = 0.035$, OR = 1.502, 95%CI = 1.029-2.192), A*2601 (0.0250 vs 0.0072, $P = 0.040$, OR = 3.513, 95%CI = 1.183-10.429), B*1518 (0.0600 vs 0.0155, $P = 0.001$, OR = 4.047, 95%CI = 1.970-8.314), B*3802 (0.0400 vs 0.0135, $P = 0.017$, OR = 3.054, 95%CI = 1.463-7.127), DRB1*0401 (0.0300 vs 0.0072, $P = 0.014$, OR = 4.237, 95%CI = 1.535-11.693), DRB1*0402 (0.0200 vs 0.0010, $P = 0.004$, OR = 19.397, 95%CI = 4.102-91.729), and DRB1*1201 (0.0700 vs 0.0342, $P = 0.019$, OR = 2.128, 95%CI = 1.132-3.097). We found that the frequency of HLA-DRB1*1302 was lower in lung cancer patients than in healthy controls, where the frequencies in lung cancer patients and healthy controls were 0.005 and 0.0290, respectively ($P = 0.046$, OR = 0.168, 95%CI = 0.029-0.980). All alleles mentioned above were not in linkage disequilibrium with each other.

Table 1. Frequencies of HLA-A, B and DRB1 alleles whose frequencies were different in lung cancer patients and healthy controls.

Allele	Lung cancer	Normal	OR (95%CI)	P
A*0201	0.2150 (43/200)	0.1540 (149/966)	1.502 (1.029-2.192)	0.035
A*2601	0.0250 (5/200)	0.0072 (7/966)	3.513 (1.183-10.429)	0.040
B*1518	0.0600 (12/200)	0.0156 (15/966)	4.047 (1.970-8.314)	0.001
B*3802	0.0400 (8/200)	0.0135 (13/966)	3.054 (1.463-7.127)	0.017
DRB1*0401	0.0300 (6/200)	0.0072 (7/966)	4.237 (1.535-11.692)	0.014
DRB1*0402	0.0200 (4/200)	0.0010 (1/966)	19.397 (4.102-91.729)	0.004
DRB1*1201	0.0700 (14/200)	0.0342 (33/966)	2.128 (1.132-3.907)	0.019
DRB1*1302	0.0050 (1/200)	0.0289 (28/966)	0.168 (0.029-0.980)	0.046

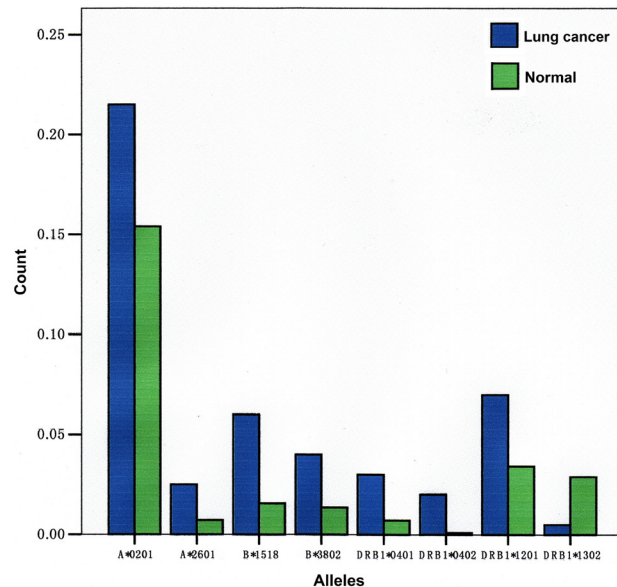


Figure 1. Frequencies of HLA-A, B and DRB1 alleles whose frequencies were different in lung cancer patients and healthy controls.

DISCUSSION

The first report about the association between HLA and susceptibility to a disease was published more than 40 years ago. Since then, such associations have been reported for more than 500 diseases, including some types of cancer. The association varies among the diseases and the populations studied, and there is generally a lack of a strong concordance between the HLA type and the disease.

There are many techniques used for typing HLA antigens. The complement-mediated microlymphocytotoxicity technique has been used as the standard for serologic typing of HLA I and HLA II antigens for many years (Terasaki and McClelland, 1964). Recently, some molecular typing techniques have been developed and used, including PCR and single-strand conformation polymorphism (Ainsworth et al., 1991; Young et al., 1993), PCR-restriction fragment length polymorphism (Bidwell et al., 1988), PCR and sequence-specific oligonucleotide probes (Dalva and Beksac, 2007), PCR-SSP (Olerup and Zetterquist, 1992), and SBT (Hoppe and Salama, 2007). Serologic typing can only yield results of 2 significant figures and 4 molecular typing techniques. According to HLA nomenclature, alleles that are the same in 4 significant figures have the same amino acid sequence (Marsh, 2003). So, in our study, SBT and PCR-SSP techniques were used for typing HLA antigens to get accurate 4-significant figure typing results for every subject.

We found that the frequencies of 4 HLA I alleles were higher in patients with lung cancer for the Han nationality of North China, and they were HLA-A*0201 (0.2150 vs 0.1540, $P = 0.035$, OR = 1.502, 95%CI = 1.029-2.192), A*2601 (0.0250 vs 0.0072, $P = 0.040$, OR = 3.5130, 95%CI = 1.183-10.429), B*1518 (0.0600 vs 0.0155, $P = 0.001$, OR = 4.047, 95%CI

= 1.970-8.314), B*3802 (0.0400 vs 0.01346, $P = 0.017$, OR = 3.054, 95%CI = 1.463-7.127). These results have not been previously reported. Tokumoto (1998) and Yoshimura et al. (2000) found that no frequency of HLA I allele was different between lung cancer patients and controls. However, they used a serologic method to type HLA I antigens, and the population that they studied was different from ours, where both could affect the results. Tokumoto and Yoshimura et al. found that the frequency of HLA-DRB1*1302 was lower in lung cancer patients than in controls. We obtained a similar result, where the frequency of HLA-DRB1*1302 was 0.0050 in lung cancer patients and 0.0290 in controls ($P < 0.05$, OR = 0.168, 95%CI = 0.029-0.980). They found that the frequency of HLA-DRB1*0901 was increased in lung cancer patients. Our results are not consistent with theirs, our results showed the following: HLA-DRB1*0401 (0.0300 vs 0.0072, $P = 0.014$, OR = 4.237, 95%CI = 1.535-11.693), *0402 (0.0200 vs 0.0010, $P = 0.004$, OR = 19.397, 95%CI = 4.102-91.729) and *1201 (0.0700 vs 0.0342, $P = 0.019$, OR = 2.128, 95%CI = 1.132-3.097) had higher frequencies in lung cancer patients, while the frequency of HLA-DRB1*0901 showed no difference between lung cancer patients and healthy controls. This discrepancy may represent population differences.

The molecular mechanisms of associations between HLA and diseases are still poorly understood or not known, even though a number of hypotheses have been proposed and examined for many diseases. We determined the amino acid sequences of the alleles from the IMGT/HLA sequence database and analyzed them, but could not draw a reasonable conclusion. Further studies need to be done in this area to explain the mechanisms of associations between HLA and lung cancer.

In conclusion, we studied the association between HLA and lung cancer. Our results suggest that HLA-A*0201, A*2601, B*1518, B*3802, DRB1*0401, DRB1*0402, and DRB1*1201 are associated with lung cancer susceptibility in the Han nationality of North China and that HLA-DRB1*1302 is associated with resistance. However, because of the relatively small number of patients examined and the possibility of racial difference, this study should be considered preliminary, and further studies are needed in this area to clarify the association between HLA and lung cancer.

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REFERENCES

- Ainsworth PJ, Surh LC and Coulter-Mackie MB (1991). Diagnostic single strand conformational polymorphism (SSCP): a simplified non-radioisotopic method as applied to a Tay-Sachs B1 variant. *Nucleic Acids Res.* 19: 405-406.
- Bidwell JL, Bidwell EA, Savage DA, Middleton D, et al. (1988). A DNA-RFLP typing system that positively identifies serologically well-defined and ill-defined HLA-DR and DQ alleles, including DRw10. *Transplantation* 45: 640-646.
- Bjorkman PJ, Saper MA, Samraoui B, Bennett WS, et al. (1987). The foreign antigen binding site and T cell recognition regions of class I histocompatibility antigens. *Nature* 329: 512-518.
- Cantú de León D, Perez-Montiel D, Villavicencio V, Garcia CA, et al. (2009). High resolution human leukocyte antigen (HLA) class I and class II allele typing in Mexican mestizo women with sporadic breast cancer: case-control study. *BMC Cancer* 9: 48.
- Chen WQ, Zhang SW and Kong LS (2008). Cancer mortality report of 34 registries in China, 2004. *Bull. Chin. Cancer* 17: 913-916.

- Dalva K and Beksac M (2007). HLA typing with sequence-specific oligonucleotide primed PCR (PCR-SSO) and use of the Luminex technology. *Methods Mol. Med.* 134: 61-69.
- Davis RW, Thomas M, Cameron J, St John TP, et al. (1980). Rapid DNA isolations for enzymatic and hybridization analysis. *Methods Enzymol.* 65: 404-411.
- Dorak MT, Oguz FS, Yalman N, Diler AS, et al. (2002). A male-specific increase in the HLA-DRB4 (DR53) frequency in high-risk and relapsed childhood ALL. *Leuk. Res.* 26: 651-656.
- Ferlay J, Autier P, Boniol M, Heanue M, et al. (2007). Estimates of the cancer incidence and mortality in Europe in 2006. *Ann. Oncol.* 18: 581-592.
- Gresner P, Gromadzinska J and Wasowicz W (2007). Polymorphism of selected enzymes involved in detoxification and biotransformation in relation to lung cancer. *Lung Cancer* 57: 1-25.
- Haghpanah V, Khalooghi K, Adabi K, Amiri P, et al. (2009). Associations between HLA-C alleles and papillary thyroid carcinoma. *Cancer Biomark.* 5: 19-22.
- Hoppe B and Salama A (2007). Sequencing-based typing of HLA. *Methods Mol. Med.* 134: 71-80.
- Jemal A, Siegel R, Ward E, Hao Y, et al. (2009). Cancer statistics, 2009. *CA Cancer J. Clin.* 59: 225-249.
- Kiyohara C, Takayama K and Nakanishi Y (2006). Association of genetic polymorphisms in the base excision repair pathway with lung cancer risk: a meta-analysis. *Lung Cancer* 54: 267-283.
- Kübler K, Arndt PF, Wardelmann E, Krebs D, et al. (2006). HLA-class II haplotype associations with ovarian cancer. *Int. J. Cancer* 119: 2980-2985.
- Madeleine MM, Johnson LG, Smith AG, Hansen JA, et al. (2008). Comprehensive analysis of HLA-A, HLA-B, HLA-C, HLA-DRB1, and HLA-DQB1 loci and squamous cell cervical cancer risk. *Cancer Res.* 68: 3532-3539.
- Marsh SG (2003). HLA nomenclature and the IMGT/HLA sequence database. *Novartis Found. Symp.* 254: 165-173.
- Miller SA, Dykes DD and Polesky HF (1988). A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res.* 16: 1215.
- Mundhada S, Luthra R and Cano P (2004). Association of HLA Class I and Class II genes with bcr-abl transcripts in leukemia patients with t(9;22) (q34;q11). *BMC Cancer* 4: 25.
- Olerup O and Zetterquist H (1992). HLA-DR typing by PCR amplification with sequence-specific primers (PCR-SSP) in 2 hours: an alternative to serological DR typing in clinical practice including donor-recipient matching in cadaveric transplantation. *Tissue Antigens* 39: 225-235.
- Parkin DM, Bray F, Ferlay J and Pisani P (2005). Global cancer statistics, 2002. *CA Cancer J. Clin.* 55: 74-108.
- Quintero E, Pizarro MA, Rodrigo L, Pique JM, et al. (2005). Association of *Helicobacter pylori*-related distal gastric cancer with the HLA class II gene DQB1*0602 and cagA strains in a southern European population. *Helicobacter* 10: 12-21.
- Schiff MA, Apple RJ, Lin P, Nelson JL, et al. (2005). HLA alleles and risk of cervical intraepithelial neoplasia among southwestern American Indian women. *Hum. Immunol.* 66: 1050-1056.
- Terasaki PI and McClelland JD (1964). Microdroplet assay of human serum cytotoxins. *Nature* 204: 998-1000.
- Tokumoto H (1998). Analysis of HLA-DRB1-related alleles in Japanese patients with lung cancer - relationship to genetic susceptibility and resistance to lung cancer. *J. Cancer Res. Clin. Oncol.* 124: 511-516.
- Wu MS, Hsieh RP, Huang SP, Chang YT, et al. (2002). Association of HLA-DQB1*0301 and HLA-DQB1*0602 with different subtypes of gastric cancer in Taiwan. *Jpn. J. Cancer Res.* 93: 404-410.
- Wu Y, Liu B, Lin W, Xu Y, et al. (2007). Human leukocyte antigen class II alleles and risk of cervical cancer in China. *Hum. Immunol.* 68: 192-200.
- Yari F, Sobhani M, Sabaghi F, Zaman-Vaziri M, et al. (2008). Frequencies of HLA-DRB1 in Iranian normal population and in patients with acute lymphoblastic leukemia. *Arch. Med. Res.* 39: 205-208.
- Yoshimura C, Nomura S, Yamaoka M, Ohtani T, et al. (2000). Analysis of serum ErbB-2 protein and HLA-DRB1 in Japanese patients with lung cancer. *Cancer Lett.* 152: 87-95.
- Young NT and Darke C (1993). Allelic typing of the HLA-DR4 group by polymerase chain reaction-single-strand conformation polymorphism analysis. *Hum. Immunol.* 37: 69-74.
- Zhang SW, Chen WQ and Lei ZI (2008). A report of cancer incidence from 37 cancer registries in China, 2004. *Bull. Chin. Cancer* 17: 909-912.