Association of *HLA-B*51:01* with Papillary Thyroid Carcinoma in the Chinese Han Population of the Shandong Coastal Areas

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Background: A lot of work has been done to study the association between human leukocyte antigen (*HLA*) and papillary thyroid carcinoma (PTC) in various populations. But the results of the currently available studies are not consistent. The aim of this study was to evaluate the association of *HLA-A*, *-B*, *-C*, *-DRB1*, and *-DQB1* with PTC in the Chinese Han population of the coastal areas of Shandong Province with respect to age and sex.

Methods: A total of 154 patients diagnosed with PTC were analyzed for *HLA-A*, *-B*, *-C*, *-DRB1*, and *-DQB1* alleles by using a polymerase chain reaction sequence-based typing (PCR-SBT) method. Two hundred unrelated healthy individuals were typed as controls.

Results: Compared with the controls, the *HLA-B*51:01* (8.8% vs. 4.5%, $p=0.029$, OR 2.039 [CI 1.101–3.775]) and *HLA-C*07:06* (2.6% vs. 0.5%, $p=0.024$, OR 5.307 [CI 1.119–25.171]) allele frequencies were higher in the PTC patients, while the *HLA-C*07:01* (1.3% vs. 6.0%, $p=0.001$, OR 0.206 [CI 0.071–0.601]) allele frequency was lower in the PTC patients that did not persist after Bonferroni correction for multiple tests. This showed no statistically significant correlation of the *HLA-A*, *-DRB1*, and *-DQB1* alleles and PTC. The incidence of PTC was more frequent in females between 30 and 60 years old. There were no significant differences in the age and sex distributions between the total and the *HLA-B*51:01* positive PTC patients.

Conclusions: The *HLA* associations in this Chinese Han population differ markedly from studies done in Europeans and Caucasians. The results reveal that *HLA-B*51:01* is more likely to be a susceptible allele for PTC in addition to age and sex in the coastal areas of Shandong Province.

Introduction

PAPILLARY THYROID CARCINOMA (PTC), the most common type of thyroid malignancy, is increasing worldwide, and its specific cause remains partially unknown (1,2). It has been reported that PTC shows a sex predilection for young women (3). Oncogenesis is a multifactorial process influenced by genetic and environmental factors (4,5). However, only a few people fall ill when exposed to the same environment, so this phenomenon suggests that genetic susceptibility factors might play a more important role in tumorigenesis. The human leukocyte antigen (*HLA*) is the genetic system that has the most associations with diseases so far studied (5,6). *HLA* polymorphism determines the immune response and individual differences in disease susceptibility (5,6). It has been reported that *HLA* plays a key role in tumor occurrence and development (7). Recently, inconsistent results were obtained from several studies on the association of *HLA* and PTC in different ethnic groups. Some of the studies revealed that certain *HLA* alleles were responsible for PTC

susceptibility, whereas others showed that they were not (4,8–11). The above discrepancy is primarily due to the fact that the disease susceptibility factors depended on different *HLA* types in different ethnic groups (12,13). The incidence of PTC increased rapidly in the Chinese population, especially in coastal areas. Yet, up to now, the correlation between *HLA* alleles and PTC in the Chinese Han population has not been reported. So, in our study, we explored the association of the *HLA-A*, *-B*, *-C*, *-DRB1*, and *-DQB1* alleles with the risk of PTC in the Chinese Han population of the Shandong coastal areas, which could lay the foundation for early diagnosis, targeted therapies for personalized cancer medicine, and prognosis.

Materials and Methods

Study subjects

In this study, 154 Han Chinese patients with PTC from the coastal area of Shandong Province who underwent surgery were recruited from the Affiliated Hospital of Qingdao

University Medical College from September 2009 to May 2013. The average age $\pm$ standard deviation (*SD*) at the time of diagnosis was 47 ± 16 years. Two hundred healthy individuals with ethnic backgrounds similar to the PTC patients were enrolled as controls. All of the PTC patients and the controls were unrelated. Informed consent was obtained from all of the subjects participating in the study.

HLA typing

HLA-A, *-B*, *-C*, *-DRB1*, and *-DQB1* gene typing was performed by a polymerase chain reaction sequence-based typing (PCR-SBT) method, using a BigDyeTM Terminator v3.1 Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA) on an ABI PRISM-3100 Sequencer (Applied Biosystems). The sequences were analyzed with SBT engine standard software (Genome Diagnostics BV, Utrecht, Netherlands), according to the IMGT/HLA Database (2012, www.ebi.ac.uk).

Statistical analysis

The calculation of the *HLA-A*, *-B*, *-C*, *-DRB1*, and *-DQB1* allele frequencies were estimated by Arlequin. To compare the differences between the allele frequencies in the PTC cases and controls, a 2×2 contingency table analysis was performed using a chi-square test and Fisher's exact test when the expected value for an *HLA* allele was <5 . The Bonferroni correction for multiple comparisons was applied, and the corrected *p*-value of <0.05 was considered statistically significant.

The total and the *HLA-B*51:01* positive PTC patients as well as the controls were categorized into groups according to age and sex. The age and sex proportional distribution differences were analyzed by a chi-square test and Fisher's exact test. Statistical significance was defined as values of $p < 0.05$. The strength of the correlation of the *HLA* alleles and PTC was estimated by odds ratios (ORs) with 95% confidence intervals (CIs). The SPSS v14.0 statistical package for Windows (SPSS, Inc., Chicago, IL) was used for the data analysis.

Results

Compared with the controls, the *HLA-B*51:01* (8.8% vs. 4.5%, $p=0.029$, OR 2.039 [CI 1.101–3.775]) and *HLA-C*07:06* (2.6% vs. 0.5%, $p=0.024$, OR 5.307 [CI 1.119–25.171]) allele frequencies were higher, and the *HLA-C*07:01* (1.3% vs. 6.0%, $p=0.001$, OR 0.206 [CI 0.071–0.601]) allele frequency was lower in the patients with PTC that did not persist after Bonferroni correction for multiple tests (Table 1). However, the *HLA-A*, *-DRB1*, and *-DQB1* alleles showed no significant difference in the frequencies between the patients and the healthy controls.

Table 2 shows the age and sex of the total and the *HLA-B*51:01* positive PTC patients as well as the controls. The age and sex distributions of the total PTC patient cohort and the controls are matching. The ages of the total PTC patients range from 17 to 88 years old. The statistics show that PTC was most prevalent in group II (30–60 years old), which was similar to that of the *HLA-B*51:01* positive PTC patients. The sex distribution in the PTC patients was significantly different ($p < 0.01$) and more frequent in females than in males. The female/male ratio of the total PTC patients

(5.16:1) was similar to that of the *HLA-B*51:01* positive PTC patients (3.7:1). In other words, the age and sex distributions of all of the participants and the *HLA-B*51:01* positive PTC patients demonstrated no significant differences in the study.

Discussion

HLA is the most complex and polymorphic gene in the human genome. It is responsible for the recognition and presentation of foreign antigens to the T lymphocytes and natural killer cells (NK), which is the starting point of the immune response (14). So far, it has been well documented that *HLA* participates, among others, in tumor immunity and transplant rejection (6). In recent years, the association of *HLA* with malignant disease has become a research focus. Some studies have shown that some *HLA* alleles play a protective role in tumorigenesis, whereas some were risk factors for some tumors. For example, *DQB1*03:01* can protect people from colon cancer and reduce the rate of deterioration (15), while *DRB1*04* indicates susceptibility for skin cancer (16).

Recently, a great deal of research had been done on the correlation of *HLA* and PTC in diverse populations (4,8–11). However, the *HLA* associations in this Chinese Han population differ markedly from studies done in Europeans and Caucasians. Genetic susceptibility to PTC was found to correlate with *HLA-DRB1*11/B*35* in Spaniards (4), *DRB1*01/B*35* in Italians (8), *DRB1*01* in Hungarians (9), *DRB1*05/B*62* in Germans (10), and *DRB1*08/DQB1*04* in Caucasians (11). Among the above results, the *HLA-DRB1* allele seems to be closely associated with PTC. Recently, Kamdi *et al.* (17) systematically reviewed and meta-analyzed the 13 available studies to investigate the association of the *HLA-DRB1* antigen expression with the risk of differentiated thyroid cancer. They found that the evidence that supports a significant association of *HLA-DRB1* and thyroid carcinoma is incomplete and uncertain. They suggested that *HLA-DRB1* typing as a prognosis or therapeutic marker may be premature at this time. Our results, which show that there is no significant association of the *HLA-DRB1* alleles with PTC, are consistent with this view.

Our results demonstrate that *HLA-C*07:06* is likely to be a susceptibility gene and *HLA-C*07:01* might be a protective gene for PTC. Rios *et al.* (18) reported a relationship between the lower frequency of the *HLA-C*07* allele and the presence of differentiated thyroid carcinoma. However, Vahid *et al.* (19) found that *HLA-C*04* and *HLA-C*15* contributed to the risk for the development of PTC and that there was no association between *HLA-C*07* and PTC. A correlation between the *HLA-C*07* allele and many other tumors has already been established as a factor involved in tumor pathogenesis (20,21). Therefore, *HLA-C*07* is not likely to have a specific association with PTC.

Our results reveal that the *HLA-A* and *-DQB1* allele frequencies show no significant difference between the PTC patients and the healthy controls. This confirms the previous findings in other populations (4,8–10).

It is known that the cytotoxic T lymphocytes (CTL) and NK cells are the principle effector cells for killing tumors (22–24). The NK cells and part of the active CTL cells can express immunoglobulin-like receptors (KIR), whose anti-tumor activity is regulated by inhibiting and activating the

TABLE 1. DISTRIBUTION OF *HLA-B* AND *-C* ALLELES
IN 154 PATIENTS WITH PAPILLARY THYROID CARCINOMA AND 200 CONTROLS

<i>HLA-B</i>			<i>HLA-C</i>		
<i>Allele</i>	<i>PTC % (n)</i>	<i>Control % (n)</i>	<i>Allele</i>	<i>PTC % (n)</i>	<i>Control % (n)</i>
0702	2.6 (8)	2.3 (9)	0102	11.7 (36)	9 (36)
0705	1 (3)	1.8 (7)	0103	0.6 (2)	1 (4)
0801	1 (3)	3 (12)	0202	1 (3)	1 (4)
1301	2.3 (7)	4 (16)	0302	2.9 (9)	3.8 (15)
1302	14.9 (46)	12.5 (50)	0303	11.4 (35)	8.8 (35)
1401	0.3 (1)	0.5 (2)	0304	5.8 (18)	6.3 (25)
1402	0.3 (1)	0.5 (2)	0401	1.9 (6)	4.5 (18)
1501	4.2 (13)	1.8 (7)	0403	0.3 (1)	0 (0)
1502	2.3 (7)	1.8 (7)	0501	0.3 (1)	0.5 (2)
1507	1 (3)	1.5 (6)	0602	10.7 (33)	15.5 (62)
1511	1.6 (5)	3 (12)	0701	1.3 (4)*	6 (24)
1512	0.3 (1)	0.5 (2)	0702	12.7 (39)	11.3 (45)
1517	0.3 (1)	0.5 (2)	0704	1.6 (5)	0.5 (2)
1518	1.6 (5)	0.5 (2)	0706	2.6 (8)*	0.5 (2)
1558	0.3 (1)	0 (0)	0801	12.7 (39)	10.3 (41)
2704	1.3 (4)	0.5 (2)	0802	0.6 (2)	1 (4)
2705	1.3 (4)	1 (4)	0803	1 (3)	1.3 (5)
3501	4.2 (13)	2.8 (11)	0822	0.3 (1)	0 (0)
3503	0.3 (1)	1.8 (7)	1202	2.6 (8)	3 (12)
3701	1 (3)	0.5 (2)	1203	3.9 (12)	2.8 (11)
3801	0.3 (1)	1.5 (6)	1402	5.5 (17)	3.5 (14)
3802	1.6 (5)	2.5 (10)	1403	1.9 (6)	2.8 (11)
3901	1.3 (4)	2 (8)	1502	6.2 (19)	5.5 (22)
3905	0.3 (1)	0 (0)	1505	0.3 (1)	1.5 (6)
4001	7.8 (24)	7.3 (29)			
4002	1.3 (4)	2 (8)			
4006	1.9 (6)	3.8 (15)			
4402	0 (0)	0.5 (2)			
4403	7.1 (22)	5.3 (21)			
4601	7.8 (24)	6.8 (27)			
4801	4.2 (13)	2 (8)			
4901	0.3 (1)	0.5 (2)			
5001	0.6 (2)	0.5 (2)			
5101	8.8 (27)*	4.5 (18)			
5102	0.3 (1)	2.5 (10)			
5201	2.3 (7)	3 (12)			
5401	2.3 (7)	4 (16)			
5501	0.3 (1)	0.5 (2)			
5502	1.3 (4)	2.5 (10)			
5701	1.3 (4)	2.5 (10)			
5801	3.9 (12)	3 (12)			
6701	0.6 (2)	1.5 (6)			
8101	0 (0)	1 (4)			
8102	0.3 (1)	0 (0)			

PTC, papillary thyroid carcinoma. * $p < 0.05$.

TABLE 2. AGE AND SEX DISTRIBUTION OF THE PATIENTS WITH PAPILLARY THYROID CARCINOMA AND THE CONTROLS

<i>Variables</i>	<i>Total PTC patients (N=154) % (n)</i>	<i>Total controls (N=200) % (n)</i>	<i>HLA-B*51:01 positive PTC patients (N=27) % (n)</i>	<i>HLA-B*51:01 positive controls (N=18) % (n)</i>
Age (years)				
Group I (<30)	7.8 (12)	8 (16)	3.7 (1)	5.6 (1)
Group II (30–60)	69.5 (107)	72 (144)	66.7 (18)	77.8 (14)
Group III (>60)	22.7 (35)	20 (40)	29.6 (8)	16.7 (3)
Sex				
Female	83.8 (129)	87 (174)	77.8 (21)	72.2 (13)
Male	16.2 (25)	13 (26)	22.2 (6)	27.8 (5)

KIR interactions with the HLA Class I (HLA-I) ligands. The HLA and KIR molecules are encoded by two of the most diverse gene families in the human genome. Evidence suggests that even a single amino acid change can influence structural and functional properties of HLA and KIR. Receptor–ligand relationships between HLA and KIR are allele-type specific. So it is no surprise that the extremely diverse character of the *HLA-I* and *KIR* genes, along with diverse HLA-I/KIR combinations, may contribute to susceptibility to, or protection against, tumors (25). *KIR* genes encode receptors that differ in specificity for HLA-I ligands and signaling potential; the specificities of KIR2DL1, 2DL2, 2DL3, and 2DS1 for HLA-C, KIR3DL1 for HLA-B, and KIR3DL2 for HLA-A are well recognized (26–28). The inhibitory receptor KIR3DL1 specifically recognizes HLA-B molecules that have the serologically defined Bw4 motif (amino acid positions 77–83), and KIR3DL1 exhibits a stronger inhibitory effect in the presence of HLA-B Bw4 subtypes that have isoleucine at position 80 (Bw4-80I) (*B*51:01*, *B*27:05*, *B*15:02*) (29). The ranking of the inhibitory ability is *B*51:01* > *B*27:05* > *B*15:02* from greatest to least (27,30). Our study shows a high frequency of *HLA-B*51:01* in the PTC patients. Thus, we speculate that a possible immune escape mechanism involved in the pathogenesis of PTC consists in the combination of *HLA-B*51:01* and KIR3DL1. They may inhibit the cytotoxic activity of the NK and CTL cells, which helps the tumor cells escaping from the host's immune response. Therefore, *HLA-B*51:01* might be a contributor risk for the development of PTC.

Age and sex are important risk factors for PTC. This study reveals that the incidence of PTC in females was significantly higher than in males, and it was highest in patients between 30 and 60 years old. The distribution characteristics of age and sex did not significantly differ between the total and the *HLA-B*51:01* positive PTC patients. Therefore, *HLA-B*51:01* may be a risk factor for PTC in addition to age and sex.

HLA association studies will help us understand the underlying mechanisms in PTC further. Because the *HLA* allele distributions are ethnically and geographically restricted, more comparative studies with larger sample sizes are needed to investigate the correlation of *HLA* and PTC in different geographical areas in order to lay the foundation for early diagnosis, treatment, and prognosis.

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Author Disclosure Statement

The authors declare no competing financial interests and no other conflicts of interest.

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