

HLA-A*02-B*46 Haplotype: an Adverse Prognostic Factor in Han Patients with Nasopharyngeal Carcinoma

Ruo-zheng WANG (王若铮)^{1#}, Dian-gang ZHANG (张典刚)^{1#}, Ran WU (吴冉)^{1,2}, Yun-hui HU (胡云辉)¹, Yan-chun PENG (彭彦春)³, Cheng CHANG (常成)¹, Tao DONG (董涛)^{1,3,4*}, Xi-yan WANG (王希彦)^{1*}

¹Department of Head and Neck Radiation Oncology, The Affiliated Tumor Hospital of Xinjiang Medical University, Urumqi, Xinjiang 830011, P.R. China

²Oncology Department, Bazhou People's Hospital of Mongolian Autonomous Prefecture, Kuerle, Xinjiang 841000, P.R. China

³MRC Human Immunology Unit, Radcliffe Department of Medicine, Oxford University, Oxford, OX3 9DS

⁴Nuffield Department of Medicine, Oxford University, Oxford, OX3 9DU

Ruo-zheng WANG, E-mail: ????; Dian-gang ZHANG, E-mail: ????

[#]The authors contributed equally to this work.

^{*}Corresponding authors, Tao DONG, E-mail: tao.dong@imm.ox.ac.uk; Xi-yan WANG, E-mail: wrz8526@163.com

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Conflicts of Interest Statement

The authors have no financial conflicts of interest.

Summary: Epidemiological studies have shown that human leukocyte antigen (HLA) allelic polymorphisms are closely correlated to susceptibility to nasopharyngeal carcinoma (NPC), and in a previous study, we showed that HLA-B*46 and HLA-A*02-B*46 haplotypes were strongly associated with NPC susceptibility. In this retrospective study, we investigated the phenotype of the HLA-A and HLA-B alleles and haplotypes and correlated these data to the clinical and pathological parameters of NPC to understand the role of HLA alleles and haplotypes in NPC prognosis. The cohort comprised 117 NPC patients from a Han population in Xinjiang. The local recurrence-free survival (LRFS), distant metastasis-free survival (DMFS), disease-free survival (DFS), and overall survival (OS) were analyzed. The 5-year DMFS of the HLA-A*02-B*46 haplotype carriers and

non-carriers was 66.4% and 90.3%, respectively. In addition, age was found to be a prognostic factor for LRFS, DFS, and OS ($P = 0.032$, 0.040 , and 0.013 , respectively). We found that the HLA-A*02-B*46 haplotype might be a prognostic marker in addition to the traditional TNM staging in patients with NPC.

Key words: human leukocyte antigens; phenotype; nasopharyngeal carcinoma; prognosis; metastasis

Nasopharyngeal carcinoma (NPC) is classified as a head and neck malignant cancer originating from the mucosal epithelium of the nasopharynx. The distribution of NPC varies markedly by geography, ethnicity, and familial aggregation. In addition to Epstein-Barr virus (EBV) infection and environmental factors, genetic factors have been recently shown to play a role in the pathogenesis of NPC^[1]. 下同 The human leukocyte antigen (HLA) system includes gene loci located on the short arm of chromosome 6 at 6p21.3. *HLA* genes play a vital role in antigen processing and presentation as well as the anti-tumor immune response.² Epidemiological studies have shown that HLA allelic polymorphisms are closely correlated with susceptibility to NPC.^{3–5} In a previous study, we investigated the HLA genotypes of 132 NPC patients and 168 normal controls of Han ethnicity in Xinjiang Province and found that the HLA-B*46 and HLA-A*02-B*46 haplotypes were strongly associated with NPC susceptibility.⁶ In this study, we further investigated the role of HLA alleles and haplotypes in NPC prognosis in a Han population in Xinjiang.

1 MATERIALS AND METHODS

1.1 Study Subjects

In total, 117 (81 men, and 36 women) Han NPC patients (age range, 11–81; mean age, 48.03 years), who received intensity-modulated radiation therapy at the Department of Head and Neck Radiation Oncology of the Affiliated Tumor Hospital of Xinjiang Medical University between July 2007 and July 2011 were included in this study.⁶ The association of HLA-A/B alleles and haplotypes with the clinical outcome of NPC was investigated. Patients with distant metastasis and incomplete clinical history were excluded from the study. According to the World Health Organization pathological classifications, 5 patients had type I differentiation (squamous cell carcinoma), 43 had type II differentiation (non-keratinizing carcinoma), and 69 had type III differentiation (undifferentiated carcinoma). The Union for International Cancer Control–American Joint Committee on Cancer 2002 staging system was used to determine the clinical stage.

Follow-up was scheduled every 3 months in the first 2 years, every 6 months for 3–5 years, and annually thereafter. The last follow-up date was June 30, 2014. Six individuals were lost; thus, the follow-up rate was 94.9%. The clinical characteristics of the patients are listed in table 1.

1.2 Ethical Considerations

This study was approved by the institutional ethics committee of the Affiliated Tumor Hospital of the Xinjiang Medical University with approval number G-201103042. Written informed consent was obtained from each participant included in this study.

1.3 Genomic DNA Extraction

Venous blood was obtained from the patient before the start of therapy and stored at -20°C before analysis. Genomic DNA was extracted according to standard protocols⁷ by using a Genomic DNA Extraction kit (Bioteke, China) following the manufacturer's protocol. DNA concentration and purity were determined using an ultraviolet spectrophotometer (Thermo, Finland). The A260/280 ratio was 1.8–1.9 with an adjusted final concentration of 0.3–0.5 µg/µL. HLA-A and HLA-B genotyping was performed by the Beijing Institute of Genomics (BGI, China) by using high-throughput HLA sequence-based typing.

1.4 EBV Viral Capsid Antigen IgG Assay

Plasma (in EDTA) from the NPC patients was separated from the blood and stored at -20°C. The EBV viral capsid antigen IgG kit (Demeditec, Germany) was used for detection and quantization of human IgG antibodies against EBV viral capsid antigen in the plasma by following the manufacturer's protocol.

1.5 Statistical Analysis

Statistical analysis was performed using SAS 9.3 software. The 5-year overall survival (OS), local recurrence-free survival (LRFS), distant metastasis-free survival (DMFS), and disease-free survival (DFS) rates were evaluated using the Kaplan–Meier method. Univariate analysis was performed using the log-rank test. The Cox proportional hazards model was used to analyze the relationship between the different factors and prognosis for multivariate analysis.

2 RESULTS

2.1 Survival Rate of NPC Patients

The mean duration of follow-up was 61 months (7–84 months). The 5-year LRFS, DMFS, DFS, and OS were 84.9%, 80.1%, 69.2%, and 82.2%, respectively. Furthermore, 15 and 20 patients developed local recurrence and distant metastasis, respectively; 17 patients died.

2.2 Association of Clinical Characteristics and NPC Prognosis by Univariate Analysis

The effects of age, sex, and smoking on NPC prognosis were determined by dividing the study subjects into the corresponding groups: age, <50 and >50 years; men and women; and smoking and non-smoking groups. Our results showed that patients aged <50 years had a better survival rate in terms of LRFS, DFS, and OS than those aged >50 years ($P = 0.011$, 0.025 , and 0.006 , respectively). Furthermore, the impact of clinical characteristics, including World Health Organization pathological type, N and T stage, and clinical staging, on NPC patient survival rates was evaluated. Among all clinical factors, N stage was a potential prognostic factor for LRFS, DMFS, DFS, and OS ($P = 0.024$, 0.011 , 0.005 , and 0.017 , respectively). Furthermore, T stage had a marked impact on prediction of DMFS and DFS ($P = 0.048$ and 0.009 , respectively).

2.3 Association between HLA Alleles and Haplotypes and NPC Prognosis by Univariate Analysis

In total, 14 and 25 alleles on HLA-A and HLA-B loci, respectively, were studied, and 123 haplotypes on HLA-A/B alleles were detected. HLA-A24 and HLA-B46 alleles had an insignificant effect on NPC survival parameters. The A2-B46 haplotype, a known NPC risk factor, was also found to be strongly associated with 5-year DMFS in NPC patients ($\chi^2 = 8.362$, $P = 0.003$). Furthermore, 33 patients belonged to HLA-A*02-B*46 (28.2%); among them, 15 (45.5%) developed distant metastasis. The 5-year DMFS of the HLA-A*02-B*46 haplotype carriers and non-carriers was 66.4% and 90.3%, respectively (table 1). The association between different clinical stages such as stages I–II and III–IV and NPC outcomes was also analyzed. The HLA-A*02-B*46 haplotype was found to be markedly correlated with the 5-year DMFS in NPC patients at stage III–IV ($\chi^2 = 6.276$, $P = 0.012$). The 5-year DMFS of the HLA-A*02-B*46 haplotype carriers and non-carriers for clinical stage III–IV NPC patients was 62.5% and 87.8%, respectively (fig. 1).

2.4 Multivariate Analysis

Multivariate analysis indicated that age was an independent prognostic factor for LRFS, DFS, and OS ($\chi^2 = 4.613, 4.231, \text{ and } 6.210$; $P = 0.032, 0.040, \text{ and } 0.013$, respectively) in the NPC population. N stage can be used as a potential predictive factor for DMFS, DFS, and OS ($\chi^2 = 4.258, 5.943, \text{ and } 5.634$; $P = 0.039, 0.014, \text{ and } 0.018$, respectively). The HLA-A*02-B*46 haplotype was also strongly associated with DMFS ($\chi^2 = 5.027, P = 0.024$) in the NPC patients (table 2).

3 DISCUSSION

EBV infection is known to be closely associated with the development of NPC by contributing to the pathogenesis of poorly differentiated and undifferentiated pathological types of carcinoma. NPC has unique pathological characteristics, has distinctive sensitivity to radiotherapy, and is highly invasive, metastatic, and malignant.⁸ Some NPC patients in complete remission develop distant metastasis and local recurrence, leading to treatment failure. Hence, identifying new prognostic molecular markers for the treatment and prognosis of NPC patients is important.

The EB virus-specific immune response after infection plays an important role in the anti-tumor process of humans. HLA genes are responsible for the regulation of the major gene-specific immune system in humans and can be divided into HLA class I (A, B, C), class II (DR, DQ, DP), and class III (complement component coding genes). HLA class I genes are located on the surface of nucleated cells *in vivo*; they recognize and present endogenous antigens and participate in the CD8⁺ cytotoxic T lymphocyte (CTL)-mediated response via the endogenous processing pathway. They exert their biological effects via the cell-mediated immune response and play an essential role in T-cell-mediated anti-tumor immunity.⁹ Despite recent advances in understanding the role of HLA alleles and haplotypes in NPC susceptibility, little is known regarding their prognostic value or association with patient outcome. Yuan et al. observed that HLA-I down-regulation is associated with tumor progression and a poor prognosis in clear cell renal cell carcinoma (CCRCC) patients.¹⁰ In particular, they showed a significant correlation of HLA-I expression with TNM stage, lymph node metastasis, and Fuhrman grade in CCRCC. Patients with tumors displaying down-regulation of HLA-I showed significantly shorter overall survival ($P = 0.021$).

Anti-cancer immunotherapy is considered a promising treatment modality for metastatic renal cell carcinoma (mRCC), as up-regulation of erythropoietin (Epo) and its receptor (EpoR) is involved in tumorigenesis in RCC. Minami et al. found that the novel EpoR-peptides EpoR52-60 could be promising candidates for a peptide-based anti-cancer vaccine for mRCC patients carrying HLA-A24.¹¹

Tertipis *et al* studied 425 patients with tongue and tonsil cancer and found that HLA-A*02 non-carriers had a higher DFS rate than HPV-positive patients.¹² Simons et al. also showed that NPC patients with HLA antigen A2 in the absence of B-Sin 2 (BW46) or BW17 had a better prognosis.¹³ In this study, the HLA-A*02-B*46 haplotype was found to be an independent prognostic factor for DMFS, and HLA-A*02-B*46 haplotype carriers had lower DMFS than non-carriers. However, no associations between HLA-A and HLA-B alleles and disease prognosis were found in the NPC patients in this study. This discrepancy in results may have resulted from insufficient sample size, genetic differences, or other factors related to the frequency distribution of HLA alleles.

Numerous studies have shown that HLA-A*02-B*46 is the most common NPC-associated haplotype and shows significant linkage disequilibrium in NPC populations from southern China, Taiwan, and other Asian countries.^{5, 14, 15} Our previous studies have also confirmed that HLA-A*02-B*46 haplotype is correlated with NPC susceptibility in Xinjiang.⁶ Nevertheless, whether or not HLA-A*02-B*46 is associated with disease prognosis remains largely unknown. Linkage disequilibrium theory, antigen presenting theory, and immune escape theory have been suggested to underlie the association of HLA with NPC susceptibility.^{9, 16, 17} However, whether or not these mechanisms contribute to the impact of HLA alleles and haplotypes on NPC prognosis warrants further investigation. HLA-A*02-B*46-expressing cells have been hypothesized to show weak antigen-presentation capacity and thus evade the cytotoxic T lymphocyte-mediated anti-tumor immune response in the body, leading to the adverse clinical progression in HLA-A*02-B*46-positive NPC individuals.

Consistent with the findings of a previous study by Han *et al*, our data also showed that age and N stage were the predominant prognostic factors in Han NPC patients in Xinjiang.¹⁸ Hence, traditional TNM staging combined with HLA-A*02-B*46 haplotype and other prognostic molecular markers might be helpful to better predict NPC prognosis. Phenotyping of HLA-A and

HLA-B alleles and haplotypes in NPC patients can allow HLA-A*02-B*46 carriers to be treated with targeted interventions to reduce the rate of distant metastasis; thus, genetic and immunological findings can help develop novel prognosis and treatment strategies for NPC.

With recent advances in microsatellite loci genotyping technology, genetic statistics, and molecular biology, elucidating the association between HLA and NPC prognosis might become possible. Future large-scale prospective studies with multi-center clinical research collaboration need to be performed to further investigate the impact of other HLA alleles, haplotypes, or recessive genes in the HLA region on NPC prognosis.

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Table 1 Univariate analysis of the prognostic role of various factors in the 117 NPC patients

Variables	Item	<i>n</i>	LRFS			DMFS			DFS			OS (%)	χ^2	<i>P</i>
			(%)	χ^2	<i>P</i>	(%)	χ^2	<i>P</i>	(%)	χ^2	<i>P</i>			
Age	≤ 50	72	92.4	6.451	0.011	84.2	0.032	0.857	76.1	5.020	0.025	87.6	7.465	0.006
	>50	45	72.1			83.6			58.6			61.5		
Sex	Male	81	84.2	0.006	0.938	83.5	0.870	0.351	68.0	0.306	0.580	73.8	0.357	0.550
	Female	36	85.8			85.9			72.9			83.1		
Smoking history	Yes	54	80.5	0.317	0.573	83.2	0.432	0.511	63.4	1.529	0.216	66.0	2.998	0.083
	No	63	91.6			84.3			77.7			93.1		
Pathological type	Non-keratinizing	43	81.3	7.120	0.130	86.0	0.502	0.973	74.1	2.358	0.670	84.7	3.888	0.421
	Undifferentiated	69	88.9			80.0			60.9			83.6		
T stage	T1	9	100.0	3.260	0.353	100.0	7.904	0.048	100.0	11.554	0.009	100.0	3.685	0.298
	T2	23	90.9			93.9			89.7			94.1		
	T3	44	86.8			85.8			79.0			76.0		
	T4	41	76.8			69.3			41.2			68.3		
N stage	N0	15	92.9	9.452	0.024	91.7	11.105	0.011	90.3	12.843	0.005	96.3	10.202	0.017
	N1	40	86.3			87.8			82.9			88.4		

Clinical stage	N2	51	79.8			74.7			71.2			79.7		
	N3	11	76.6			66.3			61.0			68.5		
	I	2	100.0	2.805	0.423	100.0	5.119	0.163	100.0	8.472	0.037	100.0	2.928	0.403
	II	15	92.9			100.0			0.917			91.7		
	III	50	88.4			89.2			0.809			79.0		
	IV	50	78.3			72.8			0.482			70.2		
HLA-A*24	Positive	50	0.749	0.635	0.426	0.799	0.620	0.430	0.749	0.635	0.426	0.645	2.932	0.087
	Negative	67	0.868			0.872			0.868			0.822		
HLA-B*46	Positive	36	0.860	0.241	0.624	0.716	3.004	0.083	0.681	0.157	0.692	0.787	1.291	0.256
	Negative	81	0.823			0.897			0.699			0.830		
HLA-A*2-B*46	Positive	31	90.0	2.810	0.094	0.664	8,362	0.003	0.635	0.014	0.907	0.798	0.730	0.393
	Negative	86	79.9			0.903			0.715			0.845		

OS, overall survival; LRFS, local recurrence-free survival; DMFS, distant metastasis-free survival; DFS, disease-free survival

Table 2 Cox multivariate analysis of the effect of multiple factors on nasopharyngeal carcinoma prognosis in the 117 patients

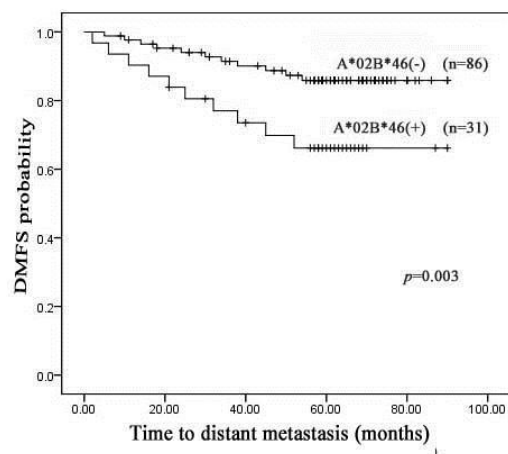
Factors	β	<i>SE</i>	χ^2	<i>P</i>	<i>RR</i> (95% CI)
LRFS					
Age	0.761	0.463	4.613	0.032	2.039 (1.112–10.094)
DMFS					
HLA-A*02-B*46	1.016	0.206	5.027	0.024	2.762 (1.024–7.450)
N stage	0.563	0.356	4.258	0.039	1.756 (0.942–9.540)
DFS					
Age	0.363	0.321	4.231	0.040	1.438 (0.789–5.449)
T stage	0.520	0.219	5.734	0.017	1.682 (0.799–7.577)
N stage	0.558	0.318	5.943	0.014	1.800 (0.989–7.096)
Clinical stage	0.751	0.211	5.362	0.021	2.119 (1.012–8.936)
OS					
Age	0.588	0.393	6.210	0.013	1.800 (0.799–8.232)
N stage	0.786	0.202	5.634	0.018	2.195 (0.896–7.138)

OS, overall survival; LRFS, local recurrence-free survival; DMFS, distant metastasis-free survival; DFS, disease-free survival; CI, confidence interval

Fig. 1 Role of HLA-A*02-B*46 haploid type in the prognosis of nasopharyngeal carcinoma patients without distant metastasis

Comparison of total (A) and clinical stage III–IV (B) HLA-A*02-B*46 haplotype carriers ($n = 31$ and 26 , respectively) and non-carriers ($n = 86$ and 74 respectively)

A



B

