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Focus on the frequencies of serological HLA antigens in 4,094 Japanese people

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Abstract

Between 1987 and 2005 we performed serological HLA typing in 4,094 Japanese people to evaluate the frequencies of HLA antigens among cancer patients, non-cancer patients and normal subjects. Among these cancer patients compared with non-cancer and normal patients, there were significantly lower frequencies for HLA-24, -A33, -B35, -B44, -DR8, and -DR9, but there was only one significantly lower frequency for HLA-DR8, even after the Bonferroni correction. In cases of gastric cancer, there was a significantly lower frequency for HLA-A33 and -B44 than among non-cancer subjects, even after the Bonferroni correction. In cases of esophageal cancer, significantly lower frequencies for HLA-A33, -B44, -DR6, -DR8, and -DQ4 were found. In cases of hepatoma, the frequency of HLA-DQ3 was significantly lower. In lung cancer, HLA-DR8 and DQ4, and in breast cancer, HLA-Cw3, -DR8, and -DR9 were also significantly lower after the Bonferroni correction. The data demonstrated here shows that there may be an association between different cancers and different antigens. The frequencies of HLA-A33, -B44, -Cw3, -DR6, -DR8, -DR9 and -DQ4 antigens were lower in total; therefore, these antigens may act as defensive factors of carcinogenesis.

Key Words: Serological HLA typing, cancer, HLA-DR8, Frequency of HLA antigens, Japanese people

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Introduction

Human leukocyte antigens (HLA) are glycoproteins that are present on the surface membranes of nearly every cell in the body and are on chromosome number six. Their main function is to help the immune system defend against invaders such as bacteria, viruses, and parasites as well as against cancers. In cases of cancers, however, many reports that evaluated the serological or DNA-typing HLA antigens failed to confirm that the HLA antigens are responsive to cancers. We previously reported that based on a study of 3,219 Japanese individuals that cancer subjects showed a significantly lower frequency of HLA- A33, -B44, and -DR9 than non-cancer subjects[1].

In this study, we re-examined serological HLA typing to clarify the association between cancer risk and HLA antigens in Japanese people. To our knowledge, this is the largest study in terms of numbers that has used serological HLA typing.

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Materials and Methods

Patients

The subjects consisted of 4,094 Japanese individuals (2,897 males and 1,197 females). Among them, 3,651 epithelial cancers, excluding non-epithelial malignant diseases, were confirmed pathologically by means of resected or biopsied specimens. These included gastric cancer (n=2027), esophageal cancer (n=247), colorectal cancer (n=448), hepatoma (n=133), gall bladder and common bile duct cancer (n=23), pancreatic cancer (n=27), lung cancer (n=200), breast cancer (n=135), other cancers (n=21), and double cancers (n=236). It also included 318 who had benign diseases and 125 normal control subjects.

Blood samples were broadly collected throughout Japan from 1987 to 2005 at hospitals of the members of the Japanese Society of Strategies for Cancer Research and Therapy.

HLA typing

HLA antigens were serologically tested using the NIH standard microlymphocytotoxicity method for HLA-A, B, C, DR and DQ antigens. Eight HLA-A antigens, 20 HLA-B antigens, 5 HLA-C antigens, 12 HLA-DR antigens, and 4 HLA-DQ antigens were examined.

Heparinized peripheral blood samples were obtained from a number of research groups, transferred to a single reference laboratory, and centrifuged for 5 min at 2000 RPM. After collecting the buffy coat, B lymphocytes were separated from T lymphocytes using the nylon wool adherence method and HLA class I and II Dynabeads (Dynal AS, Oslo, Norway). Peripheral blood lymphocytes were separated by layering them onto Lymphoprep (Nycomed, Oslo, Norway), followed by centrifugation. For class I typing, cryopreserved peripheral blood lymphocytes were subjected to a complement-dependent microcytotoxicity assay using antisera to HLA-A, -B and -C locus antigens. Class II typing was carried out on cells enriched for class II-positive lymphocytes using magnetic beads coated with a class II monoclonal antibody (Dynal). These cells were subjected to a complement-dependent microcytotoxicity assay using antisera to HLA-DR and -DQ antigens.

Table Frequency of HLA-A antigens in Japanese patients with gastric, esophageal, colorectal, hepatobilial, pancreatic, lung, breast, others, double, and non-epithelial cancer and in normal healthy subjects.

		Gastric cancer				Esophageal cancer				Colorectal cancer				Hepatoma				Gall bladder and common bile duct cacner				Pacreatic cancer			
	a	2238				283				532				149				34				63			
	b	2027				247				448				133				23				57			
	c	4476				566				1064				298				68				126			
	d	4054				494				896				266				46				114			
Antigen		N0.	%	p Value	correct p value	N0.	%	p Value	correct p value	N0.	%	p Value	correct p value	N0.	%	p Value	correct p value	N0.	%	p Value	correct p value	N0.	%	p Value	correct p value
A1	c	19	0.42%			2	0.35%			3	0.28%			1	0.34%			1	1.47%			1	0.79%		
	d	18	0.44%			2	0.40%			3	0.33%			1	0.38%			1	2.17%			1	0.88%		
A2	c	903	20.17%			114	20.14%			214	20.11%			60	20.13%			15	22.06%			22	17.46%		
	d	832	20.52%			104	21.05%			186	20.76%			54	20.30%			11	23.91%			22	19.30%		
A3	c	15	0.34%			0	0.00%			4	0.38%			2	0.67%			0	0.00%			1	0.79%		
	d	15	0.37%			0	0.00%			4	0.45%			2	0.75%			0	0.00%			1	0.88%		
A11	c	411	9.18%			65	11.48%			68	6.39%			22	7.38%			6	8.82%			9	7.14%		
	d	365	9.00%			50	10.12%			50	5.58%			18	6.77%			3	6.52%			9	7.89%		
A24	c	1362	30.43%	B:p=0.015, C:p=0.014		165	29.15%			310	29.14%			80	26.85%			16	23.53%			38	30.16%		
	d	1228	30.29%	B:p=0.021		143	28.95%			264	29.46%			71	26.69%			10	21.74%			33	28.95%		
A26	c	472	10.55%			61	10.78%			123	11.56%			36	12.08%			11	16.18%			15	11.90%		
	d	432	10.66%			58	11.74%			110	12.28%			33	12.41%			4	8.70%			13	11.40%		
A31	c	373	8.33%			48	8.48%			95	8.93%			37	12.42%	A:p=0.01, B:p=0.027, C:P=0.009		3	4.41%			10	7.94%		
	d	338	8.34%			43	8.70%			79	8.82%			33	12.41%	A:p=0.011, B:p=0.034		3	6.52%			10	8.77%		
A33	c	312	6.97%	B:p=0.001, C:p=0.008	B:p<0.05	33	5.83%	B:p=0.002, C:p=0.013	B:p<0.05	83	7.80%			22	7.38%			7	10.29%			11	8.73%		
	d	281	6.93%	B:p=0.001	B:p<0.05	29	5.87%	B:p=0.004	B:p<0.05	71	7.92%			19	7.14%			6	13.04%			10	8.77%		
A blank	c	609	13.61%			78	13.78%			164	15.41%			38	12.75%			9	13.24%			19	15.08%		
	d	545	13.44%			65	13.16%			129	14.40%			35	13.16%			8	17.39%			15	13.16%		
B7	c	229	5.12%			31	5.48%			59	5.55%			13	4.36%			4	5.88%			3	2.38%		
	d	202	4.98%			26	5.26%			49	5.47%			11	4.14%			1	2.17%			3	2.63%		
B13	c	46	1.03%	A:p=0.022		3	0.53%	A:p=0.018		9	0.85%	A:p=0.022		5	1.68%			1	1.47%			4	3.17%	B:p=0.029	
	d	43	1.06%	A:p=0.021		3	0.61%	A:p=0.024		8	0.89%			4	1.50%			1	2.17%			4	3.51%	B:p=0.021	
B17	c	22	0.49%			1	0.18%			5	0.47%			1	0.34%			1	1.47%			1	0.79%		
	d	20	0.49%			1	0.20%			5	0.56%			1	0.38%			0	0.00%			1	0.88%		
B27	c	12	0.27%				0.00%			2	0.19%			1	0.34%			1	1.47%			1	0.79%		
	d	11	0.27%				0.00%			1	0.11%			1	0.38%			1	2.17%			1	0.88%		
B35	c	328	7.33%	C:P=0.023		37	6.54%	C:p=0.045		79	7.42%			23	7.72%			4	5.88%			12	9.52%		
	d	302	7.45%			34	6.88%			71	7.92%			21	7.89%			3	6.52%			10	8.77%		
B37	c	18	0.40%			4	0.71%			3	0.28%			2	0.67%			1	1.47%			1	0.79%		
	d	17	0.42%			4	0.81%			3	0.33%			2	0.75%			1	2.17%			1	0.88%		
B39	c	173	3.87%			26	4.59%			38	3.57%			6	2.01%	C:p=0.046		3	4.41%			3	2.38%		
	d	151	3.72%			23	4.66%			31	3.46%			5	1.88%			3	6.52%			3	2.63%		
B44	c	287	6.41%	B:p=0.001, C:p=0.011	B:p<0.05	26	4.59%	B:p<0.001, C:p=0.002	B:p<0.05	95	8.93%	A:p=0.042		26	8.72%			5	7.35%			14	11.11%	A:p=0.03	
	d	261	6.44%	B:p=0.001, C:p=0.011	B:p<0.05	21	4.25%	B:p<0.001	B:p<0.05	81	9.04%	A:p=0.04		24	9.02%			5	10.87%			12	10.53%		
B46	c	212	4.74%			29	5.12%			40	3.76%	A:p=0.045		7	2.35%	A:p=0.014		3	4.41%			2	1.59%	A:p=0.04	
	d	199	4.91%	B:p=0.035		26	5.26%			32	3.57%	A:p=0.034		6	2.26%	A:p=0.015		3	6.52%			2	1.75%		
B48	c	115	2.57%			13	2.30%			21	1.97%			2	0.67%	A:p=0.031, B:p=0.024, C:p=0.019		1	1.47%			7	5.56%		
	d	107	2.64%			12	2.43%			17	1.90%			2	0.75%	B:p=0.038		0	0.00%			6	5.26%		
B51	c	417	9.32%	A:p=0.018		44	7.77%			91	8.55%			33	11.07%	A:p=0.009		6	8.82%			8	6.35%		
	d	374	9.23%	A:p=0.021		41	8.30%			72	8.04%			28	10.53%	A:p=0.019		4	8.70%			8	7.02%		
B52	c	537	12.00%	B:p=0.018, C:p=0.04		54	9.54%			115	10.81%			31	10.40%			8	11.76%			16	12.70%		
	d	478	11.79%	B:p=0.03		45	9.11%			95	10.60%			27	10.15%			6	13.04%			14	12.28%		
B54	c	342	7.64%			46	8.13%			66	6.20%			34	11.41%	A:p=0.028, C:p=0.035		3	4.41%			10	7.94%		
	d	317	7.82%			41	8.30%			59	6.58%			31	11.65%	A:p=0.025		1	2.17%			10	8.77%		
B55	c	94	2.10%			19	3.36%			29	2.73%			5	1.68%			2	2.94%			1	0.79%		
	d	85	2.10%			16	3.24%			26	2.90%			4	1.50%			1	2.17%			1	0.88%		
B56	c	35	0.78%			4	0.71%			15	1.41%			3	1.01%			0	0.00%			0	0.00%		
	d	31	0.76%			3	0.61%			12	1.34%			3	1.13%			0	0.00%			0	0.00%		
B59	c	57	1.27%			4	0.71%			11	1.03%			1	0.34%			0	0.00%			1	0.79%		
	d	55	1.36%			3	0.61%			9	1.00%			0	0.00%	B:p=0.045		0	0.00%			0	0.00%		
B60	c	260	5.81%			45	7.95%	B:p=0.021, C:p=0.011		61	5.73%			18	6.04%			3	4.41%			11	8.73%		
	d	234	5.77%			39	7.89%	B:p=0.029		49	5.47%			16	6.02%			3	6.52%			9	7.89%		
B61	c	421	9.41%			61	10.78%			97	9.12%			41	13.76%			7	10.29%			6	4.76%	A:p=0.014, B:p=0.04, C:p=0.027	
	d	379	9.35%			54	10.93%			81	9.04%			37	13.91%			2	4.35%			6	5.26%	A:p=0.005	
B62	c	369	8.24%			51	9.01%			84	7.89%			17	5.70%			7	10.29%			12	9.52%		
	d	337	8.31%			44	8.91%			73	8.15%			17	6.39%			4	8.70%			11	9.65%		
B67	c	41	0.92%			5	0.88%			3	0.28%			3	1.01%			0	0.00%			1	0.79%		
	d	37	0.91%			4	0.81%			1	0.11%	A:p=0.044, B:p=0.034		0	0.00%			0	0.00%			1	0.88%		
B blank	c	461	10.30%			63	11.13%			141	13.25%			26	8.72%			8	11.76%			12	9.52%		
	d	414	10.21%			54	10.93%			121	13.50%			26	9.77%			7	15.22%			11	9.65%		

Statistical analysis

All of the statistical analyses that follows was carried out using SPSS software, version 13 (SPSS Inc., Chicago, USA.). The chi-square test with the Bonferroni correction was used to compare the prevalence of the incidence of HLA antigens; Fisher's exact test was used if the number was less than 5. The odds ratio (relative risk (RR)) and 95% confidence intervals (CI) were calculated. Results were considered significant when the value was less than 0.05.

Results

The most frequent specificities were HLA-DQ1 (phenotype frequency = 33.3%) followed by HLA-DQ3 (29.7%), HLA-A24 (29.6%), HLA-Cw3 (23.1%), HLA-A2 (20.5%), HLA-DR4 (19.3%) and HLA-DR2 (17.4%). Among non-cancer, normal subjects and cancer patients, there were significantly lower frequencies for HLA-24, -A33, -B35, -B44, -DR8, and -DR9, but there was only one significantly lower frequency for HLA-DR8 even after the Bonferroni correction.

In patients with gastric cancer, there was a significantly lower frequency for HLA-A33 and -B44 than for non-cancer subjects even after the Bonferroni correction. In the case of esophageal cancer, significantly lower frequencies for HLA-A33, -B44, -DR6, -DR8, and -DQ4 were found. In cases of hepatoma, the frequency

Table continued

		Lung cancer				Breast cancer				Others				Double cancer				Non-cancer				Normal healthy	
	a	229				150				93								427				140	
	b	200				135				21				236									
	c	458				300				186								854				280	
	d	400				270				42				472									
Antigen		N0.	%	p Value	correct p value	N0.	%	p Value	correct p value	N0.	%	p Value	correct p value	N0.	%	p Value	correct p value	N0.	%	p Value	correct p value	N0.	%
A1	c	2	0.44%			3	1.00%			0	0.00%												
	d	2	0.50%			3	1.11%			0	0.00%			1	0.21%			5	0.59%			0	0.00%
A2	c	92	20.09%			64	21.33%			36	19.35%												
	d	81	20.25%			57	21.11%			11	26.19%			80	16.95%	B:p=0.037		180	21.08%			60	21.43%
A3	c	0	0.00%			2	0.67%			0	0.00%												
	d	0	0.00%			2	0.74%			0	0.00%			0	0.00%			5	0.59%			1	0.36%
A11	c	38	8.30%			28	9.33%			18	9.68%												
	d	32	8.00%			26	9.63%			1	2.38%			54	11.44%			76	8.90%			30	10.71%
A24	c	132	28.82%			96	32.00%	B:p=0.045		59	31.72%												
	d	114	28.50%			85	31.48%			12	28.57%			148	31.36%	B:p=0.042		233	27.28%			80	28.57%
A26	c	53	11.57%			22	7.33%	A:p=0.013, B:p=0.032, C:p=0.016		17	9.14%												
	d	47	11.75%			18	6.67%	A:p=0.007, B:p=0.016		4	9.52%			45	9.53%			98	11.48%			37	13.21%
A31	c	36	7.86%			14	4.67%	B:p=0.03, C:p=0.48		19	10.22%												
	d	32	8.00%			12	4.44%	B:p=0.027		4	9.52%			39	8.26%			71	8.31%			18	6.43%
A33	c	32	6.99%	B:p=0.043		24	8.00%			11	5.91%												
	d	25	6.25%			21	7.78%			2	4.76%			35	7.42%			87	10.19%	A:p=0.029		17	6.07%
A blank	c	73	15.94%			47	15.67%			26	13.98%												
	d	67	16.75%			46	17.04%			8	19.05%			70	14.83%			99	11.59%			37	13.21%
B7	c	15	3.28%	C:p=0.05		13	4.33%			14	7.53%												
	d	13	3.25%			11	4.07%			4	9.52%			30	6.36%			46	5.39%			17	6.07%
B13	c	5	1.09%			3	1.00%			0	0.00%	A:p=0.044											
	d	4	1.00%			3	1.11%			0	0.00%			3	0.64%	A:p=0.044		6	0.70%	A:p=0.014		7	2.50%
B17	c	3	0.66%			2	0.67%			1	0.54%												
	d	3	0.75%			2	0.74%			0	0.00%			0	0.00%			2	0.23%			7	2.50%
B27	c	3	0.66%	B:p=0.018, C:p=0.006		0	0.00%			0	0.00%												
	d	3	0.75%	B:p=0.032		0	0.00%			0	0.00%			0	0.00%			1	0.12%			0	0.00%
B35	c	30	6.55%			22	7.33%			13	6.99%												
	d	28	7.00%			17	6.30%			7	16.67%			27	5.72%	A:p=0.023 B:p=0.025		77	9.02%			28	10.00%
B37	c	2	0.44%			4	1.33%			1	0.54%												
	d	2	0.50%			4	1.48%			0	0.00%			1	0.21%			5	0.59%				0.00%
B39	c	23	5.02%			8	2.67%			12	6.45%												
	d	20	5.00%			6	2.22%			2	4.76%			24	5.08%			38	4.45%			13	4.64%
B44	c	32	6.99%			23	7.67%			9	4.84%	B:p=0.032											
	d	26	6.50%			19	7.04%			1	2.38%			33	6.99%			81	9.48%	A:p=0.024		15	5.36%
B46	c	18	3.93%			8	2.67%	A:p=0.025		8	4.30%												
	d	15	3.75%			8	2.96%	A:p=0.05		2	4.76%			17	3.60%			28	3.28%	A:p=0.018		18	6.43%
B48	c	11	2.40%			6	2.00%			1	0.54%	C:p=0.047											
	d	9	2.25%			6	2.22%			0	0.00%			9	1.91%			25	2.93%			9	3.21%
B51	c	32	6.99%			25	8.33%	A:p=0.041		18	9.68%												
	d	25	6.25%			25	9.26%			4	9.52%			48	10.17%			75	8.78%			15	5.36%
B52	c	59	12.88%	B:p=0.036		41	13.67%	B:p=0.026, C:p=0.05		21	11.29%												
	d	51	12.75%			36	13.33%	B:p=0.047		3	7.14%			62	13.14%	B:p=0.024		80	9.37%			33	11.79%
B54	c	26	5.68%			22	7.33%			10	5.38%												
	d	23	5.75%			19	7.04%			3	7.14%			27	5.72%			70	8.20%			18	6.43%
B55	c	12	2.62%			6	2.00%			4	2.15%												
	d	9	2.25%			6	2.22%			1	2.38%			11	2.33%			15	1.76%			7	2.50%
B56	c	8	1.75%			4	1.33%			5	2.69%	B:p=0.046											
	d	7	1.75%			4	1.48%			1	2.38%			6	1.27%			7	0.82%			4	1.43%
B59	c	5	1.09%			4	1.33%			1	0.54%												
	d	4	1.00%			4	1.48%			0	0.00%			4	0.85%			13	1.52%			4	1.43%
B60	c	35	7.64%	B:p=0.049, C:p=0.03		14	4.67%			8	4.30%												
	d	33	8.25%	B:p=0.021		13	4.81%			1	2.38%			28	5.93%			43	5.04%			13	4.64%
B61	c	46	10.04%			32	10.67%			18	9.68%												
	d	43	10.75%			31	11.48%			2	4.76%			46	9.75%	A:p=0.016		87	10.19%			34	12.14%
B62	c	49	10.70%			24	8.00%			17	9.14%												
	d	43	10.75%			23	8.52%			3	7.14%			37	7.84%			68	7.96%			23	8.21%
B67	c	3	0.66%			3	1.00%			2	1.08%												
	d	3	0.75%			3	1.11%			1	2.38%			4	0.85%			7	0.82%			3	1.07%
B blank	c	41	8.95%			36	12.00%			23	12.37%												
	d	36	9.00%			30	11.11%			7	16.67%			55	11.65%			80	9.37%			12	4.29%

of HLA-DQ3 was significantly lower. In cases of lung cancer, the frequencies of HLA-DR8 and DQ4 were also significantly lower after the Bonferroni correction. The same was true in cases of breast cancer for frequencies of HLA-Cw3, -DR8, and -DR9.

Discussion

It has been proposed that disease-associated imbalances in HLA allele frequencies between diseased and healthy populations may signify efficient immune responses[2]. It has also been well known that HLA antigen expression loss in malignant cells has been demonstrated by immunohistochemistry. Abnormally decreased HLA antigen expression may not permit the immune system to avoid or survive an attack, and consequently the clinical course of a disease would be not so good.

Wank et al. reported an association between squamous cell carcinoma of the cervix for women and HLA-DQw3[3]. Cancer diseases whose carcinogenesis may be induced by viral infections such as squamous cell carcinoma of the cervix for women or head and neck carcinoma have been associated with HLA antigen[4, 5], but others failed to have been significantly associated with HLA antigens.

In the case of gastric cancer, Lee et al. reported that the frequency of HLA-DQB1*0301 in patients with gastric adenocarcinoma was higher than in controls but were not associated with colorectal or pancreatic adenocarcinoma[6]. Ohmori et al. reported that after the Bonferroni correction the difference in frequencies of HLA alleles and haplotypes between gastric cancer patients and controls was of no statistical significance[7].

We reported that among cancer patients the frequencies of HLA-A33, -B44, and -DR9 were lower, but the hypothesis of overdominant selection ("heterozygote advantage") at MHC has more correlation with carcinogenesis[2]. It has been suggested that the hypothetical advantage of heterozygosity at HLA with respect to increased resistance to cancer may be responsible, at least

Table continued

		Gastric cancer				Esophageal cancer				Colorectal cancer				Hepatoma				Gall bladder and common bile duct cacner				Pacreatic cancer			
	a	2238				283				532				149				34				63			
	b	2027				247				448				133				23				57			
	c	4476				566				1064				298				68				126			
	d	4054				494				896				266				46				114			
Antigen		N0.	%	p Value	correct p value	N0.	%	p Value	correct p value	N0.	%	p Value	correct p value	N0.	%	p Value	correct p value	N0.	%	p Value	correct p value	N0.	%	p Value	correct p value
Cw1	c	740	16.53%			98	17.31%			164	15.41%			54	18.12%			11	16.18%			16	12.70%		
	d	686	16.92%			88	17.81%			141	15.74%			49	18.42%			9	19.57%			16	14.04%		
Cw3	c	1016	22.70%			138	24.38%			238	22.37%			75	25.17%			18	26.47%			29	23.02%		
	d	913	22.52%			124	25.10%			201	22.43%			65	24.44%			8	17.39%			26	22.81%		
Cw4	c	186	4.16%			30	5.30%			41	3.85%			14	4.70%			1	1.47%			9	7.14%	B:p=0.038, C:p=0.049	
	d	172	4.24%			24	4.86%			36	4.02%			14	5.26%			1	2.17%			6	5.26%		
Cw6	c	19	0.42%	A:p=0.19		3	0.53%			4	0.38%			4	1.34%			1	1.47%			2	1.59%		
	d	18	0.44%	A:p=0.025		3	0.61%			4	0.45%			4	1.50%			1	2.17%			2	1.75%		
Cw7	c	523	11.68%			76	13.43%			125	11.75%			22	7.38%	A:p=0.02, B:p=0.017, C:p=0.011		10	14.71%			11	8.73%		
	d	465	11.47%			65	13.16%			107	11.94%			19	7.14%	A:p=0.019, B:p=0.016		6	13.04%			11	9.65%		
Cw blank	c	1992	44.50%			221	39.05%			492	46.24%			129	43.29%			27	39.71%			59	46.83%		
	d	1800	44.40%			190	38.46%			407	45.42%			115	43.23%			21	45.65%			53	46.49%		
DR1	c	208	4.65%			26	4.59%			52	4.89%			10	3.36%			6	8.82%			3	2.38%		
	d	190	4.69%			23	4.66%			45	5.02%			8	3.01%			3	6.52%			3	2.63%		
DR2	c	801	17.90%	B:p=0.042		105	18.55%			165	15.51%			47	15.77%			17	25.00%	B:p=0.02, C:p=0.029		25	19.84%		
	d	714	17.61%			93	18.83%			132	14.73%			40	15.04%			11	23.91%			24	21.05%		
DR3	c	4	0.09%			0	0.00%			0	0.00%			0	0.00%			0	0.00%			0	0.00%		
	d	4	0.10%			0	0.00%			0	0.00%			0	0.00%			0	0.00%			0	0.00%		
DR4	c	889	19.86%			102	18.02%			202	18.98%			70	23.49%	C:p=0.044		8	11.76%			22	17.46%		
	d	808	19.93%			84	17.00%			172	19.20%			63	23.68%			4	8.70%	B:p=0.048		22	19.30%		
DR5	c	311	6.95%			44	7.77%			69	6.48%			11	3.69%			3	4.41%			8	6.35%		
	d	282	6.96%			39	7.89%			58	6.47%			10	3.76%			3	6.52%			7	6.14%		
DR6	c	581	12.98%	B:p=0.034		53	9.36%	B:p<0.001, C:p=0.001	B:p<0.05, C:p<0.05	167	15.70%			46	15.44%			11	16.18%			18	14.29%		
	d	533	13.15%			49	9.92%	B:p=0.002	B:p<0.05	143	15.96%			42	15.79%			5	10.87%			15	13.16%		
DR7	c	10	0.22%			1	0.18%			1	0.09%			2	0.67%			0	0.00%			2	1.59%		
	d	10	0.25%			1	0.20%			1	0.11%			2	0.75%			0	0.00%			2	1.75%		
DR8	c	385	8.60%			28	4.95%	A:p=0.001, B:p=0.001, C:p<0.001	A:p<0.05, B:p<0.05, C:p<0.05	72	6.77%	A:p=0.021, B:p=0.028, C:p=0.009		20	6.71%			4	5.88%			5	3.97%	A:p=0.026, B:p=0.033, C:p=0.025	
	d	345	8.51%			24	4.86%	A:p=0.001, B:p=0.002	A:p<0.05	60	6.70%	A:p=0.021, B:p=0.031		15	5.64%	A:p=0.024B: p=0.045		2	4.35%			3	2.63%	A:p=0.006, B:p=0.008	
DR9	c	583	13.03%	B:p=0.01, C:P=0.006		87	15.37%			155	14.57%			34	11.41%	B:p=0.033, C:p=0.034		8	11.76%			12	9.52%	B:p=0.036, C:p=0.038	
	d	529	13.05%	B:p=0.011		75	15.18%			130	14.51%			32	12.03%			7	15.22%			8	7.02%	A:p=0.015, B:p=0.005	
DR blank	c	704	15.73%			120	21.20%			181	17.01%			58	19.46%			11	16.18%			31	24.60%		
	d	639	15.76%			106	21.46%			155	17.30%			54	20.30%			11	23.91%			30	26.32%		
DQ1	c	1489	33.27%			183	32.33%			344	32.33%			106	35.57%			25	36.76%			43	34.13%		
	d	1349	33.28%			162	32.79%			287	32.03%			94	35.34%			15	32.61%			39	34.21%		
DQ2	c	12	0.27%			1	0.18%			1	0.09%	A:p=0.03		2	0.67%			0	0.00%			2	1.59%		
	d	12	0.30%			1	0.20%			1	0.11%	A:p=0.044		2	0.75%			0	0.00%			2	1.75%		
DQ3	c	1337	29.87%	A:p=0.016		166	29.33%	A:p=0.023		319	29.98%	A:p=0.029		79	26.51%	A:p=0.003	A:p<0.05	17	25.00%	A:p=0.027		36	28.57%		
	d	1211	29.87%	A:p=0.016		145	29.35%	A:p=0.027		270	30.13%	A:p=0.038		69	25.94%	A:p=0.002	A:p<0.05	12	26.09%			31	27.19%	A:p=0.037	
DQ4	c	539	12.04%	B:p=0.049		59	10.42%	B:p=0.021		131	12.31%			41	13.76%	A:p=0.035, C:p=0.04		6	8.82%			10	7.94%	B:p=0.034	
	d	488	12.04%			48	9.72%	B:p=0.008	B:p<0.05	111	12.39%			39	14.66%	A:p=0.017		3	6.52%			10	8.77%		
DQ blank	c	1099	24.55%			157	27.74%			269	25.28%			70	23.49%			20	29.41%			35	27.78%		
	d	994	24.52%			138	27.94%			227	25.33%			62	23.31%			16	34.78%			32	28.07%		

in part, for maintaining the tremendous variety of HLA alleles that exists in the human population[8]. On the other hand, we also reported that the HLA-Cw3 antigen resulted from subsequent malignancies after gastrectomy in cases of gastric cancer[9]. The data demonstrated here shows that there may be an association between different cancers and different antigens. The frequencies of HLA-A33, -B44, -Cw3, -DR6, -DR8, -DR9 and -DQ4 antigens were lower in total. Therefore, they may be act as defensive factors of carcinogenesis.

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Table continued

		Lung cancer				Breast cancer				Others				Double cancer				Non-cancer				Normal healthy	
	a	229				150				93								427				140	
	b	200				135				21				236									
	c	458				300				186								854				280	
	d	400				270				42				472									
Antigen		N0.	%	p Value	correct p value	N0.	%	p Value	correct p value	N0.	%	p Value	correct p value	N0.	%	p Value	correct p value	N0.	%	p Value	correct p value	N0.	%
Cw1	c	72	15.72%			48	16.00%			29	15.59%												
	d	62	15.50%			44	16.30%			8	19.05%			63	13.35%			132	15.46%			47	16.79%
Cw3	c	111	24.24%			52	17.33%	A:p=0.008, B:p=0.003, C:p=0.001	A:p<0.05, B:p<0.05, C:p<0.05	48	25.81%												
	d	99	24.75%			49	18.15%	A:p=0.022, B:p=0.01		11	26.19%			112	23.73%			209	24.47%			70	25.00%
Cw4	c	23	5.02%			14	4.67%			9	4.84%												
	d	19	4.75%			13	4.81%			2	4.76%			20	4.24%			29	3.40%			12	4.29%
Cw6	c	1	0.22%			4	1.33%			1	0.54%												
	d	1	0.25%			4	1.48%				0.00%			1	0.21%			5	0.59%			4	1.43%
Cw7	c	54	11.79%			31	10.33%			31	16.67%												
	d	49	12.25%			26	9.63%			6	14.29%			64	13.56%			103	12.06%			36	12.86%
Cw blank	c	197	43.01%			151	50.33%			68	36.56%												
	d	170	42.50%			134	49.63%			15	35.71%			212	44.92%			376	44.03%			111	39.64%
DR1	c	16	3.49%			16	5.33%			11	5.91%												
	d	14	3.50%			15	5.56%			3	7.14%			21	4.45%			39	4.57%			16	5.71%
DR2	c	82	17.90%			58	19.33%			36	19.35%												
	d	72	18.00%			52	19.26%			8	19.05%			94	19.92%	B:p=0.017		131	15.34%			50	17.86%
DR3	c	0	0.00%			0	0.00%			0	0.00%												
	d	0	0.00%			0	0.00%			0	0.00%				0.00%			3	0.35%			0	0.00%
DR4	c	81	17.69%			51	17.00%			34	18.28%												
	d	72	18.00%			45	16.67%			7	16.67%			89	18.86%			162	18.97%			53	18.93%
DR5	c	30	6.55%			29	9.67%	B:p=0.024, C:p=0.023		14	7.53%												
	d	24	6.00%			26	9.63%	B:p=0.031		3	7.14%			33	6.99%			51	5.97%			18	6.43%
DR6	c	69	15.07%			40	13.33%			19	10.22%	B:p=0.044											
	d	59	14.75%			35	12.96%			4	9.52%			58	12.29%			132	15.46%			37	13.21%
DR7	c	1	0.22%			1	0.33%			0	0.00%												
	d	1	0.25%			1	0.37%			0	0.00%			0	0.00%			3	0.35%			3	1.07%
DR8	c	26	5.68%	A:p=0.009, B:p=0.014, C:p=0.006		12	4.00%	A:p=0.001, B:p=0.002, C:p=0.001	A:p<0.05, C:p<0.05	14	7.53%												
	d	19	4.75%	A:p=0.002, B:p=0.003	A:p<0.05, B:p<0.05	10	3.70%	A:p=0.001, B:p=0.002	A:p<0.05	3	7.14%			42	8.90%			80	9.37%			30	10.71%
DR9	c	63	13.76%			27	9.00%	A:p=0.011, B:p=0.001, C:p=0.001	B:p<0.05, C:p<0.05	26	13.98%												
	d	55	13.75%			25	9.26%	A:p=0.019, B:p=0.002		4	9.52%			64	13.56%			137	16.04%			43	15.36%
DR blank	c	90	19.65%			66	22.00%			32	17.20%												
	d	84	21.00%			61	22.59%			10	23.81%			71	15.04%			116	13.58%			12	4.29%
DQ1	c	152	33.19%			101	33.67%			57	30.65%												
	d	132	33.00%			90	33.33%			13	30.95%			157	33.26%			296	34.66%			96	34.29%
DQ2	c	0	0.00%			1	0.33%			0	0.00%												
	d	0	0.00%			1	0.37%			0	0.00%			0	0.00%			3	0.35%			3	1.07%
DQ3	c	133	29.04%	A:p=0.022		82	27.33%	A:p=0.007		56	30.11%												
	d	115	28.75%	A:p=0.019		74	27.41%	A:p=0.009		10	23.81%	B:p=0.042		143	30.30%			255	29.86%	A:p=0.029		98	35.00%
DQ4	c	37	8.08%	B:p<0.001, C:p=0.004	B:p<0.05	31	10.33%			21	11.29%												
	d	33	8.25%	B:p<0.001	B:p<0.05	27	10.00%	B:p=0.049		5	11.90%			53	11.23%			122	14.29%			24	8.57%
DQ blank	c	136	29.69%			85	28.33%			52	27.96%												
	d	120	30.00%			78	28.89%			14	33.33%			119	25.21%			178	20.84%			59	21.07%

A: vs normal healthy control
B: vs patients with benign diseases
C: vs non cancer

a: No. of cases included double cancers
b: No. of cases excluded double cancers
c: No. of examined samples included double cancers
d: No. of examined samples excluded double cancers