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A PRIMARY STUDY OF THE CORRELASTIONS BETWEEN HUMAN LEUKOCYTE ANTIGEN (HLA) AND OSTEOSARCOMA*

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Objective: To study the correlations between human leukocyte antigen (HLA) and osteosarcoma in Chinese Han nationality. Methods: The frequencies of HLA-A, B, DR, DQ locus antigens were tested in a group of 25 osteosarcoma patients in comparison with 250 healthy controls by using complement-dependent microlymphocytotoxity technique. Both of them are Chinese Han nationality. The results were compared statistically. Results: The frequency of HLA-B35 was 0.400 in patient group, and comparing with 0.048 in controls. The relative risk of suffering from osteosarcoma in persons carrying HLA-B35 was 13.220 times as high as that in those without this antigen ($P<0.01$). Patients with HLA-B13 had increased in the relative risk of poor prognosis with 12.048 fold comparing with those without this antigen ($P<0.05$). A tendency of the worst prognosis was presented in the patients who carry both HLA-B13 and HLA-B35. For those patients with HLA-B40, the relative safety was 7.057 times higher than the negative persons ($P<0.05$). Conclusion: HLA-B35 is in close linkage to osteosarcoma susceptibility genes in Chinese Han nationality. HLA-B13 and HLA-B40 may be associated to the malignant and resistant genes of osteosarcoma respectively.

Key words: Osteosarcoma, HLA.

Up to now human leukocyte antigen (HLA) system is the most complex polymorphism system in human heredity. It modulates the susceptibility, resistance, immune response and immunosuppression genes of many disorders and also seems to be responsible for the occurrence of many kinds of tumors. HLA phenotypes of twenty-five Chinese Han nationality osteosarcoma patients were studied in this research for the evidence of association between HLA and osteosarcoma.

MATERIALS AND METHODS

Patients

The osteosarcoma group included 25 patients of Chinese Han nationality, aged from 13 to 31 years old, 17 males and 8 females, presented the pathological identification of typical osteosarcoma. 250 homogeneous healthy young persons without any history of bone tumor were established as the controls. Of them 250 and 100 controls were tested in comparison with osteosarcoma patients for the identification of HLA-A, B and HLA-DR, DQ respectively.

Typing Antigens

10 ml of peripheral blood from patient or control was run into a glass tube containing heparin 100 U. Lymphocytes were isolated by the Ficoll-hypaque gradient centrifugation technique and T, B cells were

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separated using nylon wool method. The cell concentration was adjusted to 2000/μl. Antigens of HLA class I A, B and HLA class II DR, DQ were typed by using NIH two-step complement-dependent microlymphocytotoxity technique. The antisera prepared by Shanghai Institute of Immunology were used to type the class of HLA and to identify 7, 17, 10 and 3 antigens that located in Locus A, B, DR and DQ respectively.

Statistics

The frequencies of individual phenotype on each HLA locus were studied statistically in the osteosarcoma group in comparison with its controls. Relative risk and safety (percentage in expression) were calculated by four-fold table. P value was determined by Woolf's formula, and P value times the number of antigens of each locus was equal to modified P value.

RESULTS

Two types of HLA antigens (paternal and maternal origin) could be tested on account of the distribution of coupled allele on each HLA locus. Only one phenotype of HLA presents while the allele couple is homozygote. The frequency of phenotype actually is the ratio between positive phenotypes to total members. The frequencies of 37 phenotyped antigens on HLA-A, B, DR and DQ locus were studied in the osteosarcoma group in comparison with its controls (Table 1 and 2). The frequency of HLA-B35 presented

Table 1. Frequencies of antigens distributed in HLA-A, B locus in osteosarcoma patients and its controls

HLA	HLA	Osteosarcoma group				Controls			
locus	antigens	n	Phenotypable Ag	Phenotyped Ag	Frequency	n	Phenotypable Ag	Phenotyped Ag	Frequency
A	A1	25	50	0	0.000	250	500	19	0.076
	A2			11	0.440			119	0.476
	A3			1	0.040			8	0.032
	A9			9	0.360			104	0.416
	A10			0	0.000			19	0.076
	A11			9	0.360			98	0.392
	A19			5	0.200			81	0.324
B	B5	25	50	3	0.120	250	500	43	0.172
	B7			0	0.000			10	0.040
	B8			0	0.000			7	0.028
	B12			0	0.000			14	0.056
	B13			6	0.024			42	0.168
	B14			0	0.000			1	0.004
	B15			3	0.120			73	0.292
	B16			0	0.000			22	0.088
	B17			0	0.000			39	0.156
	B18			0	0.000			1	0.004
	B21			0	0.000			1	0.004
	B22			1	0.040			42	0.168
	B27			3	0.120			36	0.148
	B35			10	0.400			12	0.048
	B37			0	0.000			2	0.008
	B40			12	0.480			84	0.336
	B46			2	0.080			33	0.132

Table 2. Frequencies of antigens distributed in HLA-DR, DQ locus in osteosarcoma patients and its controls

HLA locus	HLA antigens	Osteosarcoma group				Controls			
		n	Phenotypable Ag	Phenotyped Ag	Frequency	n	Phenotypable Ag	Phenotyped Ag	Frequency
DR	DR1	25	50	0	0.000	100	200	1	0.010
	DR2			7	0.028			31	0.310
	DR3			0	0.000			6	0.060
	DR4			11	0.440			23	0.230
	DR5			9	0.360			52	0.520
	DR6			7	0.280			22	0.220
	DR7			4	0.160			9	0.090
	DR8			0	0.000			21	0.210
	DR9			5	0.200			21	0.210
	DR10			0	0.000			3	0.030
DQ	DQ1	25	50	11	0.440	100	200	59	0.590
	DQ2			0	0.000			17	0.170
	DQ3			19	0.760			77	0.770

N=number Ag=antigen

in 10 patients (10/25) was 0.400 obviously higher than that 0.048 (12/250) in controls. The relative risk of suffering from osteosarcoma in Chinese Han nationality persons who carry HLA-B35 was 13.220 times as high as that in those without this antigen. P value was 1.6×10^{-6}. Modified P value was 2.77×10^{-5} ($P<0.01$) with statistical significance.

Twenty patients were followed-up ranging from 19 to 210 months with an average of 48.3 months. Among them, eight patients with poor prognosis died or occurred pulmonary metastasis within 3 years while the others (12 cases) had a good prognosis (3/12 cases keep alive 14 years after initial surgery). Five of six patients carrying HLA-B13 antigen (3/5 also with HLA-B35) showed poor prognostic results when the comparison was studied in the two categories. It was statistically significant that the relative risk of poor prognosis in these patients who carry HLA-B13 was 12.048 times as high as that in those without it. P=0.017 ($P<0.05$). It was no statistical difference that only 3 of total 8 patients with HLA-B35 had a poor prognosis. Statistical significance was also obtained that 9 cases including 2 keeping alive patients showed good prognosis among 11 patients with HLA-B40. The relative safety was 7.057 times higher in patients with this antigen than in those without it, $P=0.036$ ($P<0.05$).

DISCUSSION

HLA, the major human histocomptibility complex located in the petit of chromosome 6 (6p), is a life-constant inheritant segment which consists of very complex genetic locus and plays a very important role in immunological recognition, immunological response and immunoregulation. Its extreme polymorphism is also an excellent mark for the evidence of inheritant background in studying the occurrence of mankind disorders.

Osteosarcoma is the most common representative malignant bone tumor whose predilection is teenagers with active cellular proliferation. That simultaneous incidence in monozygotic twins,[1] multiple familial incidence,[2,3] different regional and ethnical occurrence[4] indicate that the occurrence of osteosarcoma is associated with certain population and individual inheritant background. Tabacchi et al.[5] reported that HLA-A3 may develop poor prognosis in Japanese osteosarcoma patients. Shimizu et al.[6] suggested that the susceptibility genes of osteosarcoma distribute along HLA and its adjacent locus such as HLA-A11 which may be responsible for the occurrence of osteosarcoma in Japanese.

HLA antigens related to individual disorder may alter in different population on account of its different

distribution in different nations. Our results showed that HLA-B-35 and HLA-13 may be associated to the occurrence and poor prognosis of osteosarcoma respectively in Chinese Han nationality. A prognostic tendency was observed that the worst in patients who carry both HLA-B13 and HLA-B35 antigens in contrast to those with HLA-B40, which suggest that HLA-B35 antigen links up closely with susceptibility genes while HLA-B13 may attach itself to genes controlling the malignant behavior of osteosarcoma. These genes, just like recessive genes which normally present in the state of "inactivation", could be activated by gene translocation, crossing-over and recombination of chromosome during the cellular proliferation so as to induce the occurrence and poor prognosis of osteosarcoma. Zhao Wushu et al.[7] suggested that the nature of this malignant gene which is responsible for the poor prognosis may be the immunosuppression gene which is related to the HLA. HLA-B40, correlated osteosarcoma resistant gene, performs the protective action for the osteosarcoma patients. Our conclusion is only the primary report on the correlations between HLA and the occurrence as well as the prognosis of osteosarcoma owing to the limitation of case account. But this study provides a new way to explore the differences of molecular structure and function of HLA between osteosarcoma patients and healthy person as well as the etiology and pathogenesis of osteosarcoma.

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