RESEARCH ARTICLE

Association of an LMP2 Polymorphism with Acute Myeloid Leukemia and Multiple Myeloma

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Abstract

Hematological malignancies (HM) are a group of neoplasms derived from the cells of the bone marrow and lymphatic system. Genetic factors leading to susceptibility to HM have been investigated for years but little is known yet. Low molecular weight polypeptide (LMP) 2 and LMP7 genes are important subunits of the immunoproteasome and play significant role in antigen presentation. The polymorphisms of LMP genes have been reported to be risk factors for various types of diseases. The aim of this study was to investigate the association of LMP2 and LMP7 polymorphisms with the occurrence of particular types of HM. A total of 132 patients with HM and 130 control subjects were investigated. No significant difference was obtained in the distribution of genotype and allele frequencies of LMP7 gene in HM patients and the control group. On the other hand, the prevalence of LMP2-AA genotype was found to be higher in acute myeolid leukemia (AML) patients while it was significantly lower in multiple myeloma (MM) cases than in the control subjects. Our results suggested that LMP7 could not be a risk factor for susceptibility to HM, whereas LMP2 polymorphisms could play a role in the development of AML and MM.

Keywords: LMP2 - LMP7 - hematological malignancy - polymorphism - Turkish

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Introduction

Hematological malignancies (HM) include leukemias, lymphomas, myeloma, myelodysplastic syndromes and myeloproliferative diseases all originating from cells of the bone marrow and the lymphatic system. Having high level of heterogeneity in clinical features and acquired genetic alterations, hematological malignancies are complex to classify. Generally, the morphology of the tumor cells, immunophenotype, genetic abnormalities and clinical features are used in classification (Weng et al., 2012). Although the etiology is largely unknown, several exogenous toxicants including cytotoxic drugs, benzene, ionizing radiation and tobacco smoking were described as causative factors (Descatha et al., 2005; Irigaray et al., 2007). Targeted disease association studies aiming to identify genetic factors leading to susceptibility to HM resulted in identification of several candidate genes but positive results generally have not been replicated in subsequent studies. Findings from genome wide association study of CLL provided evidence that the variation in SP140, IRF4, PRKD2 (Di Bernardo et al., 2008) and IRF8 genes (Crowther-Swanepoel et al., 2010) influence the risk of disease development. Similarly, IKZF1, ARIDB5, CEBPE genes were determined to be associated with ALL (Papaemmanuis et al., 2009). It was argued that these low-suscebtibility alleles contribute to the risk of developing CLL and ALL and genes involved in transcriptional regulation and differentiation of B-cell progenitors as the biological basis of predisposition (Houlston, 2010).

Antigen processing machinery (APM) components play key roles in the human leukocyte antigen (HLA) class I-linked endogenous antigen presentation (Van Kaer, 2002). The production and processing of the peptide influence the efficiency of peptide-HLA complex presentation on the cell surface. Intracellular proteins are degraded by the proteasome and resulting peptides selected and transported to endoplasmic reticulum by the transporter associated with antigen processing (TAP) (Gromme and Neefjes, 2002). The low molecular weight polypeptide 2 (LMP2; PSMB9) and low molecular weight polypeptide 7 (LMP7; PSMB8) proteins are catalytic subunits of the immunoproteasome which can be induced with interferon γ resulting in distinct subunit composition and altered catalytic characteristics (Liu et al., 2011). LMP2 and LMP7 genes, localized in MHC class II, are less polymorphic than other MHC genes, but the importance of these polymorphisms has been indicated by the analysis of the spectrum of peptides generated, transported and presented; disease association studies and population genetic studies (Rueda Faucz et al., 2000). It is generally assumed that the function of antigen processing and transport pathway might be influenced by

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structural differences encoded by TAP and LMP alleles and immunoproteasome improves quality and quantity of generated class-I ligands.

Endogenous antigen presentation is a crucial mechanism for recognition of virally infected cells, maintenance of self-tolerance and the surveillance of newly arising tumors by the immune system (Atkins et al., 2004). For cytotoxic T lymphocytes to detect and subsequently kill antigen-specific malignant cells, tumor antigens should be properly presented on MHC class I surface molecules (Seliger et al., 2002). Defects in APM components have been correlated with progression and survival in various human tumors (Meissner et al., 2005; Seliger et al., 2006; Mehta et al., 2007). A tissue microarray analysis indicated significant downregulation of APM components in acute myeloid leukaemic (AML) blasts and it was concluded that multiple deficiencies in APM expression play role in the failure of immunosurveillance and may therefore contribute to relapse in the disease (Hoves et al., 2009).

In addition to other types of diseases, LMP2 and/or LMP7 genes have been associated with the risk of occurrence of several malignant tumors such as esophageal carcinoma (Cao et al., 2005), cervical carcimoma (Mehta et al., 2007), oral squamous cell carcinoma (Tang et al., 2009), prostate cancer (Seliger et al., 2010), colon cancer (Fellerhoff et al., 2011), esophageal squamous cell carcinoma (Zheng et al., 2013). However, little is known about the role of LMP2 and LMP7 genes in development of hematopoietic malignancies. In this study, we have investigated for the first time to the best of our knowledge, the association of LMP2 and LMP7 gene polymorphisms with hematological malignancies.

Materials and Methods

Subjects

A total of 132 patients with a hematological malignancy (chronic myeloblastic leukemia-CML, chronic lymphoid leukemia-CLL, acute myeloid leukemia-AML, multiple myeloma-MM) were included in this study. Patients were diagnosed and followed in the Department of Internal Medicine Section of Hematology at Gaziantep University. Of 132 subjects; 48 were CML (15 males and 33 females aged 19-78), 26 were CLL (9 males and 17 females aged 40-80), 33 were AML (21 males and 12 females aged 16-67) and 25 were MM patients (15 males and 10 females aged 36-74).

The control group consist of 130 unrelated healthy subjects (62 males and 68 females; aged between 19-80) with similar ethnic background and from the same geographic area of the patients. Subjects with no evidence of any personal or family history of cancer or other serious illness were included in control group. Informed consent was obtained from each participant before blood sampling and the study was approved by the local Ethical Committee of Gaziantep University.

DNA extraction and genotyping

Blood samples (10 cc) were collected into ethylenediaminetetraacetic acid (EDTA) tubes. Genomic deoxyribonucleic acid (DNA) was isolated using a standard salting-out procedure (Miller et al., 1988) and stored in Tris EDTA buffer at -20ºC until use.

PCR-RFLP method was used to analyze LMP2 (rs17587) and LMP7 (rs2071543) polymorphisms. PCR primers and restriction enzymes used for these experiments are as previously described (Sugimoto et al., 2002). Briefly, genomic DNA samples (1 μg) were amplified in 25 μl reaction mixtures containing 1 μmol/l of each primer, 200 μM dNTPs, 1xTaq DNA polymerase buffer, 1.5 mmol/l $MgCl_2$ and 0.5 units of Taq DNA polymerase (Fermentas, Lithuania) using a thermal cycler (Takara PCR Thermal Cycler Dice, Otsu, Shiga, Japan). Reaction conditions were as follows: 95ºC for 5 min, 35 cycles of 95ºC for 30 seconds, 53ºC for 30 seconds, 72ºC for 30 seconds and a final extension at 72ºC for 5 min. Reaction products were electrophoresed on a 2% agarose gel containing ethidium bromide for visualization.

The amplified fragments were digested with appropriate restriction enzymes (4-4.5 h) and digestion products were separated on a 3% agarose gel. Re-genotyping of randomly selected samples was performed to confirm the results, and all the results were in agreement with the previous ones.

Statistical analysis

Genotype and allele frequencies of LMP2 and LMP7 polymorphisms were determined by direct counting and compared between control and patient groups using the chi-square test, and odds ratio (OR) with 95% confidence interval (CI) was calculated to assess the relative risk conferred by a particular allele or genotype. Yates correction (<25) and Fisher exact test (<5) were performed where necessary. Hardy-Weinberg equilibrium was also tested by chi-square analysis. Statistical significance was assumed at the $p<0.05$ level. The SPSS statistical software package version 13.0 was used for all of the statistical analyses.

Results

Patients with hematological malignancies (CML, AML, CLL, MM) (n=132) and healthy control subjects (n=130) were investigated for LMP2 and LMP7 polymorphisms. The result of the Hardy-Weinberg (HW) equilibrium test indicated that the genotypes in control and all patient groups were distributed as expected under HW equilibrium ($p>0.05$) (data not shown).

Table 1 shows the distribution of the genotype and allele frequencies for LMP2 and LMP7 gene polymorphisms in HM patients (all, not stratified) and the control group. No significant difference between HM patiens (all) and the control group was found when the allele and genotype frequencies of two genes were compared ($p>0.05$).

Allele frequencies and genotype distributions of LMP7 gene were determined after stratification of the HM subjects into different sub-groups namely CML, AML, CLL and MM (Table 2). No statistically significant difference was found between each patient group and the control group ($p>0.05$). This result suggested that LMP7 gene could not have any role in the development of hematological malignancies.

Allele and genotype frequencies of LMP2 polymorphism were compared between patient groups and the control group seperately. LMP2 polymorphism was not found to be associated with CML and also CLL (p>0.05). However, as shown in Table 2 the genotype distribution of LMP2 gene was significantly different from the control group in AML (p=0.034) and MM patients (p=0.028). Compared to the LMP2-GG genotype, the frequency of AA genotype was found to be higher in AML cases than in the control group (OR=3.97, 95%CI 1.42-11.08, p=0.008). The A allele was observed 2.48-fold more in AML cases than in the control group (95%CI 1.41-4.38, p=0.0017).

On the other hand, when compared to the GG and GA genotypes the prevelance of the AA genotype was significantly lower in MM cases (OR=25.5, 95%CI 3.08-211.11, p=0.003). The frequency of A allele was also found to be lower in MM patients than in the control group (OR=2.29, 95%CI 1.16-4.51, p=0.016).

Discussion

Proteasomes, distributed throughout eukaryotic cells at a high concentration, cleave foreign peptides in a non-lysosomal pathway. As being important subunits of the immunoproteasome, the LMP2 and LMP7 proteins have a significant role in antigen presentation and therefore they have been suggested as susceptibility factors for a large variety of autoimmune, infectous and inflammatory diseases as well as for different types of neoplasms. As the genes that code for these proteins are polymorphic, it is possible that particular genotype/allele in the LMP genes might confer susceptibility or protection in patients with hematological malignancies. Here, we report for the first time the allele and genotype distibution of LMP2 and LMP7 polymorphisms in healthy Southeastern Anatolian population and patients with hematological malignancies diagnosed as CML, CLL, AML and MM.

We have not found any evidence of association between polymorphism of LMP7 gene and CML, CLL, AML and MM. Our results did not suggest LMP7 gene as a susceptibility factor in HM but the possibility of modulating disease severity still remains to be investigated. The lack of association of LMP7 polymorphism with hematological malignancy risk is interesting, as it was previously shown to be associated with increased risk of HPV-associated esophageal carcinoma (Cao et al, 2005), cervical carcinoma (Mehta et al., 2007) and colorectal carcinoma (Fellerhoff et al., 2011). Low expression of LMP7 has been reported in malignant melanoma (Kageshita et al., 1999) and carcinomas of colorectum (Atkins et al., 2004a), cervix (Ritz et al., 2001; Mehta et al., 2008) and kidney (Atkins et al., 2004b). LMP7 gene promoter methylation and protein downregulation were also correlated at high extent in Kazakh's esophageal squamous cell carcinomas (Zheng et al., 2013). It could be argued that the clinical significance of LMP7 polymorphism may likely vary depending on the disease context as well as on the allele distribution in a particular population.

The allele and genotype distribution of LMP2 polymorphism were not found to be significantly different in CML, CLL cases and the control group. On the other hand, significant associations of LMP2 polymorphism with AML and MM were found in this study. The prevalence of AA genotype (Arg/Arg) was significantly higher in AML patients than in the control group. Carrying AA genotype for LMP2 polymorphism could be a risk factor for developing AML. On the contrary, the prevelance of AA genotype and A allele in MM patients were significantly lower than in the control group. The high frequency of G allele in cases could suggest a possible role of this allele as a risk factor in MM. These results clearly indicated that mechanisms of immune modulation vary with histology.

Vasuri et al. (2010) have reported strong expression of LMP2 and LMP7 in fetal hematopoietic elements and suggested a possible role of immunoproteasome during the hepatic phase of human hematopoiesis. Hoves et al. (2009) investigated the expression of APM components including LMP2 and LMP7 in AML blasts and observed no detectable or only partially detectable expression in

Table 1. Genotype and Allele Frequencies of LMP2 and LMP7 Polymorphisms in Hematological Malignancies and Controls

SNP	Genotype/Allele	Control n=130 (%)	HM Cases n=132 (%)	p value
LMP2 rs17587	GG	49 (37.7)	46 (34.8)	
	GA	46 (35.4)	52 (39.4)	0.794
CGC--CAC	AA	35 (26.9)	34 (25.8)	
Arg (R)--His (H)	G	144 (55.4)	144 (54.5)	0.847
	A	116 (44.6)	120 (45.5)	
LMP7 rs2071543	CC	112 (86.1)	111 (84.1)	
	CA	17 (13.1)	18 (13.6)	0.878
CAG--AAG	AA	1 (0.8)	3 (2.3)	
Gln (Q)--Lys (K)	C	241 (92.7)	240 (90.9)	0.558
	A	19 (7.3)	24 (9.1)	

*p>0.05, statistically non-significant

Table 2. Genotype and Allele Frequencies of LMP2 and LMP7 Polymorphisms in CML, CLL, AML and MM Cases and Controls

Genotype/Allele		Control n=130 (%)	CML n=48 (%)	p	CLL n=26 (%)	p	AML n=33 (%)	p	MM n=25 (%)	p
LMP2	GG	49 (37.7)	20 (41.7)	0.919	7 (26.9)	0.342	6 (18.2)	**0.034**	13 (52.0)	**0.028**
	GA	46 (35.4)	17 (35.4)		14 (53.9)		10 (30.3)		11 (44.0)	
	AA	35 (26.9)	11 (22.9)		5 (19.2)		17 (51.5)		1 (4.0)	
	G	144 (55.4)	57 (59.4)	0.500	28 (53.9)	0.959	22 (33.3)	**0.002**	37 (74.0)	**0.022**
	A	116 (44.6)	39 (40.6)		24 (46.1)		44 (66.7)		13 (26.0)	
LMP7	CC	112 (86.1)	42 (87.5)	1.000	20 (76.9)	0.170	26 (78.8)	0.279	23 (92.0)	0.166
	CA	17 (13.1)	6 (12.5)		5 (19.2)		6 (18.2)		1 (4.0)	
	AA	1 (0.8)	0 (0)		1 (3.9)		1 (3.0)		1 (4.0)	
	C	241 (92.7)	90 (93.7)	0.910	45 (86.5)	0.234	58 (87.9)	0.309	47 (94.0)	1.000
	A	19 (7.3)	6 (6.3)		7 (13.5)		8 (12.1)		3 (6.0)	

*Significant values (p<0.05) are in bold

the majority of AML blasts (70-90%) with the exception of LMP7 which was positive in the majority (66%) of all AML samples. It can be concluded that downregulation of APM components may play a role in the failure of immuno-surveillance and may therefore contribute to relapse in acute leukemia.

How the polymorphism of LMP2 gene could act to determine the susceptibility to AML and MM is largely unknown. However, polymorphism of this gene could change the substrate specificity and proteolytic activity of the proteasome and influence both the generation of peptides and their transport to the endoplasmic reticulum and consequently resulting in different sets of peptides derived from the same antigen being presented to T cells in different individuals.

The impact of environmental factors in addition to the genetic factors in cancer development should not be ignored. Regulation of immune response is a complicated process involving numerous genes, so individual genes might have only limited effect on disease susceptibility. Our current report is a preliminary one, further research with a larger population in different regions of Turkey is suggested to confirm the results.

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