Original paper

Association of *ABCB1* T-129C polymorphism and multiple myeloma risk in Polish population

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The possible interaction between gene polymorphism and cancer risk development is a very interesting issue. The genetic variants of the ATP-binding cassette superfamily B member 1 (*ABCB1*) are known to be involved in developing cancer risk and individual differences in chemotherapeutic response. Polymorphisms may affect the reduction of the activity and/or expression of important protective cellular proteins. The increased exposure to toxic compounds, including carcinogens is associated with an increased risk of developing cancers. The present study was aimed to evaluate the possible effect of *ABCB1* T-129C single nucleotide polymorphism in risk of cancer development in Polish patients diagnosed with multiple myeloma. 91 multiple myeloma patients and 94 healthy controls were enrolled in this case-control study. The *ABCB1* T-129C genotypes were determined by polymerase chain reaction-restriction fragment length polymorphism method (PCR-RFLP). The distribution of particular genotypes between multiple myeloma patients and controls group was not significantly different for T-129C SNP ($p = 0.4297$). The studied polymorphism does not seem to affect the increased risk of multiple myeloma development.

Key words: *MDR1*, *ABCB1*, P-gp, single-nucleotide polymorphism, plasma cell myeloma.

Introduction

Plasma cell myeloma (multiple myeloma – MM) is a cancer of blood cells derived from bone marrow. It belongs to the group of malicious monoclonal gammapathy. Memory B cells are considered to be responsible for clonal proliferation. The expansion of neoplastic cells in bone marrow and the production of monoclonal immunoglobulin are responsible for disease symptoms manifestation [1]. As a result of disseminated marrow infiltrated by tumor cells, its function is disturbed – anemia, coagulation disorders, immunity disorders. Other characteristic symptoms of myeloma are osteolytic lesions, hypercalcemia, and renal failure [2]. Multiple myeloma is the second cause of neoplasms of the hematopoietic system in terms of the frequency of disease, it accounts for 1% of all cancers and approximately 10% of all hematologic malignancies. In Europe, 4.6/100 000 males and 3.2/100 000 females develop MM every year. The median age of patients at the time of diagnosis is about 65 years [3, 4]. The molecular mechanism of disease development has not yet been fully understood, attention is paid to the simultaneous participation of many environmental and genetic factors. Genetic variants like SNPs may be a protective or risk factor in the cancer development and be correlated with response to chemotherapy [5, 6].

ATP-binding cassette transporters play an important role in outflowing of xenobiotics and toxic compounds from cells [7]. ATP-binding cassette sub-family B member 1 – *ABCB1* gene, also named

as *MDR1* gene - Multidrug Resistance Gene 1 is located on the chromosome 7q21.1 *ABCB1* consists of 28 introns and 28 exons and encodes transmembrane P-gp – P-glycoprotein with molecular weight 170 kDa or 1280 amino acids [7]. Firstly this protein was described in drug-resistant cells [8]. P-gp occurs in both normal tissues and cancer cells. The *ABCB1* gene is widely expressed in human organs and tissues, significantly in the apical membranes of excretory tissues: brain microvessel endothelium cells, intestinal epithelial cells, renal proximal tubular epithelial cells, placenta and testes [5, 9]. This protein is responsible for efflux endogenous metabolites and toxic xenobiotics, including carcinogens from cells, what is a protective mechanism against carcinogenesis. On the other hand, P-gp plays an important role in drug response trough the reduction the effect of the drug through modulating absorption, metabolism and promoting elimination from cells [10, 11]. P-gp also plays a role in the development of immune response by activating lymphocytes. Among the substrates for P-gp are: lipids, sterols, analgesics, antidepressants, anticancer drugs and immunomodulators such as doxorubicin, vinblastine, vincristine, epirubicin, etoposide and imatinib, are widely used in chemotherapy, including multiple myeloma therapy [11, 12, 13].

Genetic changes that affect the activity or expression of P-gp may contribute to the risk of developing cancer as well as potential response to chemotherapy [14, 15, 16, 17]. Recent studies suggest that genetic components may play an important role in the ethiopahology of MM [17]. SNPs in *ABCB1* gene are highly diverse in different ethnic populations. So far, more than 50 SNPs have been identified in *ABCB1* gene [11, 18]. Some of them have been studied more widely, others not enough. The aim of this study was to determine the potential significance of SNP T-129C of *ABCB1* gene (rs3213619) in the development of multiple myeloma. According to the state of our knowledge, the role of this polymorphism in promoter region at *ABCB1* gene in multiple myeloma has not been studied in the Polish population.

Material and methods

Study subject

91 blood samples collected from patients (50 females; 41 males, median age of the group: 63 years) with multiple myeloma diagnosed at the Cathedral of Hematology Medical University of Lodz, Poland were recruited to the study. Various treatment regimens have been used in the therapy of patients, such as: MP (melphalan; cisplatin), VAD (vincristine; doxorubicin; dexamethasone). In almost half of the study group, the clinical stage was assessed to III, according to Durie-Salomon classification.

The healthy control group consisted of 94 blood samples obtained from healthy individuals (56 females, 38 males, median age of group: 33 years) from the local blood bank were ethnic and geographically matched with the group of multiple myeloma patients. The investigation was in accordance with the Declaration of Helsinki, the Good Laboratory Practice rules and was approved by the Ethical Committee of the Medical University of Lodz No: RNN/88/16/KE. All patients provided a written informed consent before their inclusion in the study.

Genotyping

ABCB1 T-129C (rs3213619) polymorphism was evaluated applying the PCR-RFLP technique. DNA was isolated from peripheral blood according to "Blood Mini" protocol (*A&A Biotechnology, Poland*). DNA samples, until analysis, were stored at –20°C. PCR reaction for both investigated and control groups was performed according to 2xPCR Super Master Mix (*Biotool.com, USA*) protocol in the volume 20 μl PCR mixture. The PCR mixture composed of 5 μl of 2xPCR Super Master Mix, 0.5 mM of each primer, 50 ng of DNA template and distilled water up to 20 μl. Negative control (without DNA) was included in every experiment. PCR products were evaluated during electrophoresis in 2% agarose gel with ethidium bromide. Products of PCR reaction for SNP T-129C in *ABCB1* gene had the size of 258 bp.

PCR products were digested by restriction enzyme, specific to the studied polymorphism: *MspII*. The digestion mixture consist of: 16 μl of PCR product, 2 μl of 10× PCR buffer, 0.2 μl of specified enzyme 10 U/μl and 1.8 μl of distilled water up to 20 μl. Digestion by restriction enzyme was performed for SNP T-129C: at 37°C for 16 h. Amplified DNA fragments after digestion by restriction enzyme were underwent electrophoresis on 2% agarose with ethidium bromide. The details of PCR-RLFP method are given in Table I.

Statistical analysis

STATISTICA 10 statistical software (StatSoft Inc. 2011) was used for data analysis. The χ^2 Pearson test with the Yates correction was applied to evaluate the conformity between the observed and expected genotype frequencies in the investigated and control groups according to Hardy-Weinberg equilibrium. Differences in genotype frequencies among MM patients and control group and association of the various genotypes with clinical date were determined using the χ^2 Pearson test with the Yates correction test. All p-values were two-sides and $p < 0.05$ was considered as statistically significant.

Table I. Primers sequences and basic PCR-RLFP reaction conditions

SNP T-129 C in *ABCB1* gene		
Primers:	**Genotype:**	**Length after digestion: [bp]**
Forward primer:		
5' TTTCACTACTTGCCCTTTCTAGAG 3'	TT	258
Reverse primer:	CT	32, 226, 258
5' CGGCCTCTGCTTCTTTGAG 3'	CC	32, 226

Results

In this study all blood samples from investigated and control groups were successfully analyzed for T-129C of *ABCB1* gene. The frequency of particular genotypes in both groups was consistent with the Hardy-Wineberg equilibrium (p = 1.000).

First, genotype and allele frequencies for studied SNP between group of patients with multiple myeloma and healthy individuals were compared. In both groups the dominant genotype was homozygote TT (98% – multiple myeloma patients; 96% – healthy individuals). There was no significant statistical difference between multiple myeloma cohort and healthy individuals (p = 0.4297, p = 0.4296). All results are shown in Table II.

Secondly, the group of patients with multiple myeloma was divided, according to gender, into two subgroups (Table III), than the frequencies of genotypes for SNP T-129C were compared. Similarly, there was no statistically significant differences between the presence of a specific genotype and gender (p = 0.8870).

In the next step of the analysis the multiple myeloma cohort was divided according to age. Frequencies of particular genotypes of studied SNP were compared between the subgroup of patients with age 63 years and under this and subgroup of patients with age over 63 years. No statistically significant differences were found (p = 0.1572).

In the last part of the research, the group of patients with multiple myeloma were divided according to the type of the produced by myeloma cells immunoglobulin into four subgroups. Results are summarized in Table III. Then the analysis of genotype frequencies of SNP T-129C and these subgroups of patients were performed, no statistically significant differences were found (p = 0.6901).

Table II. Frequencies of genotypes alleles of *ABCB1* gene SNP T-129C in the group of patients with multiple myeloma and healthy individuals

ABCB1 T-129C	Multiple myeloma patients n = 91	Healthy Individuals n = 94	p
CC	0 (0%)	0 (0%)	
CT	2 (2%)	4 (4%)	0.4297
TT	89 (98%)	90 (96%)	
C	2 (1%)	4 (2%)	0.4296
T	180 (99%)	184 (98%)	

Discussion

The P-gp protein encoded by the *ABCB1* gene is an active exporter responsible for the transport of substances from the cytoplasm outside the cell or to specific intracellular compartments. The physiological function of P-gp is protection cells against harmful substances – metabolites or toxins of both endogenous and exogenous origin. The presence of membrane P-gp contributes to the removal of xenobiotics, ensuring the proper functioning of the intestines, kidneys, liver barrier or blood-brain barrier [12]. P-gp is involved in the regulation of the immune

Table III. The frequency of particular genotypes in the investigated group

Prevalence of the investigated SNP T-129C in multiple myeloma patients (n = 91)						
Genotype	**Gender**		**Type of the immunoglobulin produced by myeloma cells**			
	Male	**Female**	**IgG**	**IgA**	**IgD**	**LCD**
TT	40	49	51	19	18	1
CT	1	1	2	0	0	0
CC	0	0	0	0	0	0
Total	41	50	53	19	18	1

response and the process of proliferation and differentiation of hematopoietic stem cells [19].

Previous knowledge about P-gp protein suggests that a wild variance of T-129C *ABCB1* can protect cells from carcinogen accumulation [20]. Therefore, it is likely mutant form of this SNP in *ABCB1* may reduce ability to eliminate carcinogens, what could promotes carcinogenesiss. On the other hand, SNPs in the *ABCB1* gene might influence the efficiency of chemotherapy by inducing multidrug resistance and could become a prognostic factor [12, 21].

So far, genetic variants in *ABCB1* have been studied in various diseases such as Parkinson's disease, mood disorders, breast cancer and colorectal cancer [22, 23]. *ABCB1* SNPs (C1236T, G2677T/A, C3435T) have also been studied in hematological diseases such as chronic myeloid leukemia (CML), chronic lymphocytic leukemia (CLL), acute lymphocytic leukemia (ALL), diffuse large B lymphoma (DLBCL) and multiple myeloma (MM) [5, 18, 20, 24]. From the previously published data, it can be assumed that polymorphisms in the gene regulatory region can also affect the expression and function of P-gp e.g. T-2410C, T-1910C, T-692C and T-129C and could be associated with hematological tumors [8].

The T-129C SNP in *ABCB1* gene is located in the promoter region. The role of this polymorphism in cancer development and progression has not been sufficiently recognized. It was confirmed that this SNP increases the risk of DNA damage in response to carcinogens such as pesticides [24]. Also some data about neuropathic pain induced by chemotherapy in multiple myeloma have been reported [25] what confirmed that investigation of genes from the group of ABC transporters could help to solve that problem. Especially, that some data from the literature indicate that some genes [26] may also be involved in sensitivity to the use of treatment. However, in case of our study we did not get information about neuropathic pain in the group of investigated patients during chemotherapy. Therefore, we could not evaluate it and this could become the next step in the future analysis.

Distribution of individual genetic variants of the SNP T-129C varies within the populations. The results obtained in our study were consistent with the distribution of the frequency of genotypes for the healthy members of the Polish population in the study conducted by Tan *et al*. in which the distribution of individual genotypes was as follows: TT 93.5%, CT 6.5%, CC 0% [27]. In another research on the French population, the following distribution was obtained: TT 92%, CT 8%, CC 0% [28]. The presence of a CC homozygous has not been demonstrated for the Caucasian ethnic group. In contrast to the Japanese population, where the presence of CC homozygotes has been observed with frequencies CC 1.3% [29].

Our results indicate a lack of association between the studied T-129C polymorphism and the increased risk of multiple myeloma development ($p = 0.4297$). Contrary to our results, Hu *et al.* showed that polymorphisms of *ABCB1* T-129C is connected with the risk of DLBCL [30]. In turn, results obtained in our study are consistent with the data published by Komoto *et al.* where there were no statistically significant differences between the group of healthy people and the group of patients with oesophageal cancer or with a group of patients with colorectal cancer. Similar results were obtained by Liu *et al.* in the study of the role of SNP in *ABCB1* gene in susceptibility to primary open-angle glaucoma. In this study there were no significant differences in *ABCB1* T-129C frequencies in investigated and control groups [31]. Studies on the role of T-129C polymorphism in the *ABCB1* gene were also conducted in ovarian cancer. Similarly, there were no differences in the incidence of alleles between patients with ovarian cancer and the control group for SNP T-129C [32]. In the next step it could be important to compare of studied SNPs of *ABCB1* gene with others recently investigated polymorphisms, including promising *CD4* gene, tested on an animal model [33].

We are aware of the limitations of the study, however, we hope that our research on the role of genetic variants in this incurable cancer will contribute to the increasing of knowledge about multiple myeloma.

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The authors declare no conflict of interests.

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