ONCOLOGY

Possible Prediction of the Efficiency of Chemotherapy in Patients with Lymphoproliferative Diseases Based on *MDR1* Gene G2677T and C3435T Polymorphisms

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Study of polymorphism in *MDR1* gene exons 21 and 26 revealed that $T^{2677}T$ and $T^{3435}T$ alleles are not a factor predisposing to lymphoproliferative diseases, but they determine the efficiency chemotherapy. Individuals with $T^{2677}T$ and $T^{3435}T$ haplotypes are at highest risk of drug resistance. Association between genotypes $G^{2677}T$ and $C^{3435}T$ was detected in normal subjects and in patients with lymphoproliferative diseases.

Key Words: *MDR1/P glycoprotein; genetic polymorphism; lymphoproliferative diseases; drug resistance*

Human *MDR1* gene encodes integral membrane protein P glycoprotein responsible for ATP-dependent transport of substances from the cell [4,5]. Normally this gene is involved into steroid metabolism and cell protection from toxic compounds. Many drugs, including antitumor agents, serve as the substrates for P glycoprotein. That is why the degree of *MDR1* expression and functional characteristics of P glycoprotein can modulate the therapeutic efficiency of these drugs. This is of particular importance in the therapy of cancer, when high level of *MDR1* expression and high activity of P glycoprotein determine the resistance of tumor cells to many drugs serving as substrates for P glycoprotein [1,5].

More than 28 single nucleotide substitutions in 27 positions of *MDR1* gene were recently described [6, 7,9]. The significance of the discovered variants of *MDR1* polymorphism for drug bioavailability and distribution in the body is not yet clear and clinical significance of many mutations in *MDR1* gene for the formation of predisposition to different forms of cancer and drug resistance of tumors is not yet evaluated. One of the stages in the study of the significance of *MDR1* gene polymorphism for predisposition to tumors and in the development of drug resistance of tumors is the study of distribution of *MDR1* variants in different populations.

We studied the distribution of the most incident single nucleotide substitutions in *MDR1* gene exons 21 ($G^{2677}T$, Ala89Ser) and 26 ($C^{3435}T$, Ilel142Ile) in healthy Europeoids living in West Siberia and patients with hematological tumors and analyzed the relationship between the formation of drug resistance in patients and their *MDR1* genotype.

MATERIALS AND METHODS

The study was carried out on blood samples from 59 normal subjects and 63 patients with lymphoprolife-

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TABLE 1. Primers for PCR Analysis and Restriction Endonucleases

Location/position in *MDR1* gene	Primer sequences for detection of nucleotide replacement	Restriction endonuclease	Restriction site	Size of restriction products, b. p.*
Exon 21/$G^{2677}T$	5' tgc agg cta tag gtt cca gg 3'	AccB1 I	gˆgtgcc	198 26
	5' ttt agt ttg act cac ctt ccc g 3			(224)
Exon 26/$C^{3435}T$	5' tgt ttt cag ctg ctt gat gg 3'	Kzo9 I	ˆgatc	158 39
	5' aag gca tgt atg ttg gcc tc 3'			(197)

Note. *Fragments of wild type allele; fragment of mutant allele in parentheses.

rative diseases (LPD; chronic lympholeukemia and non-Hodgkin's lymphoma) obtained from Novosibirsk Regional Clinical Hospital. The groups were matched for age and sex and included only Europeans. Control group consisted of trauma patients (53% men and 47% women aged 17-81 years, mean age 49.8 years) before discharge from the hospital; the main criterion for selection was the absence of oncopathologies. The group of patients consisted of 68% men and 32% women aged 17-81 years (mean age 53.7 years). The efficiency of drug therapy was evaluated in 53 patients: chemotherapy was considered effective (the tumor sensitive to drugs), if complete or partial remission (decrease in lymphocyte count in the blood and bone marrow, decrease on the lymph node size) was attained. The treatment was considered ineffective (drug-resistant tumor), if clinical picture remained unchanged after drug therapy or if the patient died.

MDR1 polymorphism was detected by analysis of DNA restriction fragment length polymorphism. Genome DNA was isolated from the whole blood using a standard kit (Laboratoriya Medigen): blood cells were lyzed with guanidine isothiocyanate and DNA was adsorbed on a glass carrier. DNA sites containing polymorphic sites were amplified using pairs of *MDR1* gene flanking sequences (exons 21 and 26, Table 1) [3] synthesized on an ASM-800 synthesizer (Biosset Firm). PCR was carried out in 25 μl PCR buffer containing 0.25 mM deoxynucleoside triphosphates, 5 pmol of each primer, 2 Units of DNA Taq polymerase (Biosan), and 5-8 μl DNA sample.

Amplification products (224 b. p.) for exon 21 and 197 b. p. for exon 26) were subjected to endonuclease restriction with the corresponding restrictase (10 U, Sibenzim; Table 1) for 16 h at 37°C. Restriction products were analyzed after electrophoresis in 8.5% polyacrylamide gel and staining with ethidium bromide.

Association of *MDR1* genotypes with the studied sign (disease or treatment effect) was evaluated by the ratio of chances (RC), showing the probability of falling ill (or being drug resistant) for an individual with a certain genotype in comparison with the chance to remain healthy (or sensitive to drug therapy). The ratio of chances was calculated using EpiInfo 6 software. The significance of differences between the groups was evaluated by χ^2 test with Yates corrections and by two-tail Fisher's test, when necessary ($n<5$).

RESULTS

Genotyping showed that the incidence of T allele in exons 21 and 26 in controls was slightly higher than in patients (Table 2). Analysis of distribution of *MDR1* gene polymorphic variants in exons 21 and 26 in normal subjects and patients with LPD showed that, first, the incidence of the studied genotypes in exons 21 and 26 in West Siberian Europeans was closer to the genotype incidence in the population of German Europeans [3] and exon 26 was characterized by special simi-

TABLE 2. Incidence of *MDR1* Gene Alleles and Genotypes in exon 21 ($G^{2677}T$) and exon 26 ($C^{3435}T$) in Normal Subjects and Patients with CLD

Exon; allele, genotype	Group	
	healthy	patients
Exon 21		
allele G	0.47	0.56
T	0.53	0.44
genotype G/G	0.22	0.35
G/T	0.51	0.43
T/T	0.27	0.22
Ratio of chances*, significance of differences	0.52 $p<0.29$	
Exon 26		
allele C	0.41	0.55
T	0.59	0.45
genotype C/C	0.20	0.35
C/T	0.41	0.40
T/T	0.39	0.25
Ratio of chances*, significance of differences	0.38 $p<0.07$	

Note. *The ratio of chances was calculated with consideration for the number of mutant and wild type homozygotes in patients with LPD and normal subjects.

TABLE 3. Coupling of *MDR1* Gene Polymorphisms in Positions 3435 and 2677 in Normal Subjects and Patients with Lymphoproliferative Diseases

Genotype	Normal subjects			Patients		
	G/G^{2677}	G/T^{2677}	T/T^{2677}	G/G^{2677}	G/T^{2677}	T/T^{2677}
C/C^{3435}	11	1	0	22	0	0
C/T^{3435}	0	23	1	0	21	4
T/T^{3435}	2	6	15	0	6	10

TABLE 4. Association of *MDR1* Gene Genotypes and Resistance of CLD Patients to Drug Therapy (n=53)

Genotype	Combinations of genotypes for which ratio of chances was calculated	Ratio of chances	Confidence interval	Significance of differences
T/T^{2677}	G/G^{2677}	4.5	0.80-27.11	$p<0.05$
	$G/G^{2677}+G/T^{2677}$	6.8	1.35-35.87	$p<0.0084$
T/T^{3435}	C/C^{3435}	6.3	1.03-40.99	$p<0.023$
	$C/C^{3435}+C/T^{3435}$	10.08	1.88-53.79	$p<0.024$
$T/T^{2677}+T/T^{3435}$	$G/G^{2677}+C/C^{3435}$	10.50	1.44-85.8	$p<0.007$
	All except $T/T^{2677}+T/T^{3435}$	17.73	2.71-131.34	$p<0.00045$

larity with the distribution in Portuguese Europeans (C/C, C/T, T/T - 0.22, 0.42, 0.36, respectively) [2]. The second important result is that the incidence of the genotypes in exon 21 was virtually the same in normal subjects and patients (RC=0.52; $p<0.29$). This suggests that mutation in position 2677 is not a factor predisposing to LPD development. On the other hand, mutation in position 3435 in exon 26 can be a factor of LPD resistance, although the data did not reach statistical significance (RC=0.38; $p<0.07$).

Analysis of haplotypes revealed an association between mutant homozygotes T/T^{3435} and T/T^{2677} in normal subjects (65.2%) and patients with LPD (62.5%), which indicates the significance of these *MDR1* haplotypes (Table 3). Similar data on cosegregation of mutations in positions 3435 and 2677 (62%) in American Europeans were reported [8].

Analysis of associations between genetic variants in exons 21 and 26 and treatment efficiency (Table 4) showed that the probability of resistance to chemotherapy was 4.5 higher in patients with T/T^{2677} genotype in comparison with individuals with G/G^{2677} genotype and 6.8 times higher in comparison with the patients possessing one or both G alleles in position 2677. A similar relationship was observed in patients with T/T^{3435} genotype: the probability of drug resistance was 6.3 times higher than in patients with C/C^{3435} genotype and even higher than in patients with one or two C alleles. Moreover, the predicted resistance to chemotherapy in a patient with the T/T^{2677} T/T^{3435} haplotype was 10-fold higher than in a patient with the G/G^{2677} C/C^{3435} haplotype and 17-fold higher than in patients with other haplotypes. All differences were statistically significant.

Hence, analysis of genetic polymorphism of *MDR1* in comparison with drug resistance of patients with lymphoid tumors showed that $G^{2677}T$ and $C^{3435}T$ polymorphisms of *MDR1* gene are essential for the results of therapy in patients with chronic lymphoproliferative diseases, and therefore determination of *MDR1* genotype in these patients is important for predicting the course of the disease and choice of therapy.

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