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Predicting the Effectiveness of Therapy for Cancer Diseases

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Abstract: Revealing of genetic features at patients on polymorphic marker C3435T MDR1 gene allows to predict character of the pharmacological answer that gives the chance to raise efficiency and safety of application of medical products - a dose, frequency rate of introduction, an introduction way, replacement of medical products and real possibility of an individualization of pharmacotherapy at various oncological diseases. Definition of frequency of occurrence of genotypes among healthy donors and oncological patients with the diagnosis of breast cancer and non-Hodgkin's lymphoma is spent. It is shown, that frequency of occurrence of homozygous TT genotype at breast cancer and non-Hodgkin's lymphoma above, than occurrence of CC and CT genotypes.

Keywords: C3435t Marker Of The Mdr1 Gene, Breast Cancer, Non-Hodgin Lymphoma, Homozygous Tt Genotype, Drug Resistance, Atp-Binding Cassette (Abc) Transporters, Atp-Dependent Transporters, Transmembrane Protein P-Glycoprotein.

1. Introduction

Malignant neoplasm's lead to a significant loss of the labor and life potential of society and are one of the main causes of mortality in the population. Despite numerous studies of the cancer problem, cancer therapy faces a number of difficulties, one of which is multidrug resistance (MDR) of tumor cells to various anticancer drugs. Drug resistance of ontological diseases is one of the main reasons for their clinical progression (1). It is this property of cancer cells that makes it difficult to treat cancer patients: the tumor is insensitive to chemotherapy, regardless of the combination of drugs used. To date, the clinical significance of various mechanisms of multidrug resistance has been proven, one of which is the mechanism based on the transport of drugs from the cell through transmembrane proteins ABC transporters (ATP Binding Cassette (ABC) transporters, ATP-dependent transporters). One of the most studied and characterized ABC transporters is P-glycoprotein (Pgp), the overexpression of which is associated with the phenomenon of MDR (2). Resistance of tumor cells to chemotherapy can be a consequence of various processes: from a decrease in intracellular the concentration of the antitumor drug, caused by the active release of the substance into the intercellular environment by the ATP-dependent transmembrane protein P-glycoprotein, which is a product of the ABCB1 or MDR1 gene, until the mechanisms of apoptosis in the tumor cells themselves are disrupted (mutation or decreased expression of the p53 gene, disrupting its proapoptotic function). In addition to the multiplicity of mechanisms the drug resistance status of a tumor may vary depending on the genotype of the individual. Genetic determinants make a significant contribution to the development of cancer: the identified genetic markers make it possible, on the one hand, to identify pathogenetic and clinical phenotypes, to predict a "high-risk group" for the development of severe disease and complications; on the other hand, they allow you to select therapy individually, for a specific patient (3). The study of individual genetic differences leading to differences in the body's response to a particular drug, the development of the MDR phenomenon in the treatment of cancer in modern medicine is an urgent problem and is of paramount importance.

The purpose of this work is to determine the personal genotype of drug resistance of cancer patients with breast cancer (BC) and non-Hodgin lymphoma (NHL) using the C3435T polymorphic marker of the MDR1 gene to predict an individual chemotherapy regimen

2. Materials and Methods

Research methods: When performing the research, DNA samples were used obtained from peripheral blood leukocytes of patients suffering from breast cancer (30 patients) and NHL (25 patients). The blood of cancer patients was obtained from the chemotherapy department of the Republican Oncology Research Center of the

Ministry of Health of the Republic of Uzbekistan (head D.A. Pulatov). DNA of peripheral blood leukocytes from healthy donors (25 donors) was used as a control.

Isolation of ecDNA from blood serum/plasma. 1 ml of peripheral blood taken from the cubital vein was transferred into plastic tubes coated with EDTA-Na2. The blood was centrifuged at 40C sequentially at 1500 rpm for 10 minutes, at 3000 rpm for 15 minutes, at 5000 rpm for 15 minutes. After centrifugation, 400 µl were taken from the tubes blood serum and transferred to new sterile tubes. The serum was pretreated with RNase A (100 µg/ml), incubated for 1 hour at 370C, then treated with proteinase K (50 µg/ml), incubated for 1 hour at 370C. After enzymatic treatment, 200 µl of lysis buffer (100 mM Tris HCl, pH 8.0; 25 mM EDTA, pH 8.0; 0.15 M NaCl; 0.7 M β-mercaptoethanol), SDS was added to the blood serum to a final concentration of 2%. Lysis was performed in the cold for 3 minutes (over ice). Aliquots were deproteinized for 15 min in 1.5 ml of a phenol/chloroform mixture (1:2), followed by centrifugation at 5000 rpm, at a temperature of 40°C for 15 min. The supernatant was transferred to new test tubes, 1/10 was added volume of 3M sodium acetate pH 5.2, as well as 2.5 volumes of chilled 96% ethanol, were left overnight at -200C. The cfDNA preparations were centrifuged at 5000 rpm, 30 min, at a temperature of 40C. The cfDNA sediment was washed in 1 ml of chilled 70% ethanol, followed by centrifugation at 13,000 rpm, 15 min, at 40°C. The cfDNA sediment was dried in a vacuum desiccator for 15 min. The dried sediment was dissolved in 300 µl of TE buffer, pH 8.0 and stored at -200C. Aliquots of cfDNA were analyzed on a 2% agarose gel containing 0.5 µg/ml ethidium bromide. Electrophoresis was carried out for 1 hour at 100 V, and the gel was photographed under transmitted UV rays.
To determine the concentration of ecDNA, ecDNA preparations were dissolved in 100 µl of DNA-binding fluorescent dye (DBFK) containing 10 mM Tris HCl, pH 8.0; 1mM EDTA, pH 8.0; ethidium bromide 0.5 µg/ml. The cfDNA solutions were measured on a spectrophotometer at a wavelength of 260 nm. Fluorescence intensity increased linearly with cfDNA concentration. DSPC without ecDNA was used as a control.
Synthesis of complementary DNA for the MDR1 gene. Complementary DNA for the MDR1 gene was synthesized by PCR using primers specific for this gene: forward 5'- GATGGCAAAGAAATAAAGCGACTG -3' reverse 5'- ACCAGCCCCTTATAAATCAAACTA -3' PCR was carried out in an incubation medium: 50 µl contained 60 mM Tris-HC1 (pH 8, 6), 6 mM EDTA, 10 mM β-mercaptoethanol, 10 µg/ml BCA, 1 mM each of the 4 nucleotides, 2 units. reverse transcriptase. The PCR had only 55 cycles. The synthesis was carried out at 720C for 4 minutes. Subsequent cycles included denaturation (1 min, 940C), primer annealing (1 min, 550C), and cDNA synthesis (2 min, 720C). After 55 amplification cycles, the samples were kept for 10 min. at 720C and then cooled. After amplification and restriction, the amplification products were analyzed by electrophoresis in a 2% agarose gel with the addition of ethidium bromide. Restriction fragments were visualized under an ultraviolet transilluminator.

3. Results and Discussion

The MDR1 gene is highly polymorphic. Currently, the clinical significance of 4 allelic variants, which represent the replacement of one nucleotide in the DNA nucleotide sequence with another, is being actively studied. Polymorphisms (in exon 12) and C3435T (in exon 26) are localized in the promoter zone of the MDRI gene and lead to changes in its expression The C3435T polymorphic marker of the MDR1 gene plays a key role in oncology in identifying predisposition to various diseases and response to the effects of drugs. Identification of genetic characteristics in patients based on the C3435T polymorphic marker of the MDR1 gene will make it possible to predict the nature of the pharmacological response, which makes it possible to increase the effectiveness and safety of drug use (4,5).
Complementary DNA (cDNA) was synthesized by PCR using specific primers for the MDR1 gene. The synthesized cDNA was treated with Sau3A restriction enzyme. After amplification and restriction, electrophoresis was performed in a 2% agarose gel with the addition of ethidium bromide. Restriction fragments were visualized under an ultraviolet transilluminator. Genotyping was performed based on restriction fragment length polymorphism analysis. In Figure 1 an electropherogram of genotypes for the polymorphic marker C3435T of the MDR1 gene is presented.
In order to identify the frequency of occurrence of genotypes among healthy donors and cancer patients, we carried out genotyping using the C3435T polymorphic marker of the MDR1 gene. The C3435T polymorphic marker of the MDR1 gene, which is a substitution in the nucleotide sequence at position 3435 of cytosine to thymine, is the most clinically informative (3,4). Therefore, we conducted studies using only this marker. Genotypes were identified: TT - resistant, CT - moderately resistant and CC - sensitive to the effects of drugs.

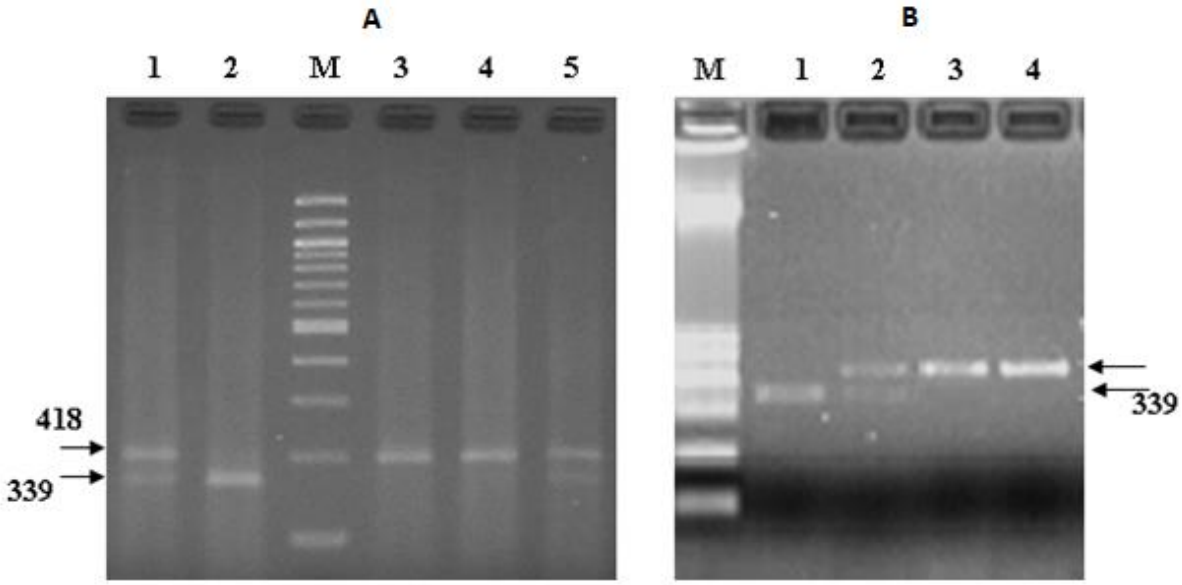

Fig 1. Electropherogram of genotypes for the polymorphic marker C3435T of the MDR1 gene. A) lanes: 3.4 – TT genotype, 2 – CC genotype, 1.5 – ST genotype. B) tracks: 1 – CC genotype, 2 – ST genotype, 3,4 – TT genotype. PCR products were digested with restriction enzyme Sau3AI. Electrophoretic separation of cDNA genotypes was carried out in 2.0% agarose gel (Fig. A) and 2.5% agarose (Fig. B).

The genotyping results showed different genotype variants in each group of donors. The TT genotype accounts for 30% of healthy people; 33% - breast cancer; 50% -Non-Hodgkin's lymphoma. The CT genotype is 40% healthy, 16.6% breast cancer, 16.6% non-Hodgkin lymphoma. The CC genotype is 30% - healthy, 25% - breast cancer and 28.3% - Non-Hodgkin's lymphoma. Table 1 shows data on the frequency of genotypes of patients sensitive to chemotherapy for the polymorphic marker C3435T of the MDR1 gene.

Table 1 Frequency of occurrence of genotypes of cancer patients sensitive to chemotherapy

Diagnosis	**CC - genotype**	**TT- genotype**	**CT - genotype**
Healthy donors	30±0,8	30±1,6	40±1,8
Breast cancer	25±1,4	33±1,1	16,6±0,4
Non-Hodgkin lymphoma	28,3±0,9	50±0,8	16,6±0,6

From the data presented in the table, it can be seen that the frequency of occurrence of the homozygous TT genotype in patients with breast cancer and Non-Hodgin lymphoma is higher than the frequency of occurrence of the CC and CT genotypes. In carriers of the TT genotype, there is a violation of the expression of the MDR1 gene at the transcription level, which leads to a decrease in the amount of glycoprotein-P and a slow elimination of drugs from the body. As a result, carriers of the TT genotype are likely to have a significant increase in the concentration of drugs in the blood, which, in turn, leads to the development of undesirable drug reactions, side effects and a decrease in treatment effect. Increasing the dosage of drugs impairs the functioning of the kidneys and liver and can ultimately lead to death (5). Identification of the TT genotype using the C3435T polymorphic marker of the MDR1 gene allows predicting disease relapse and the presence of distant metastases. Thus, genetic polymorphism caused by the C3435T marker may be an important factor determining both susceptibility to cancer and resistance to cancer drug therapy. Based on the data obtained, we can come to the conclusion that identifying genetic characteristics in patients using the C3435T polymorphic marker of the MDR1 gene makes it possible to predict the nature of the pharmacological response, which makes it possible to increase the effectiveness and safety of drug use - dose, frequency of administration, route of administration, replacement of drugs and the real possibility individualization of pharmacotherapy for various ontological diseases.

4. Conclusions

1. The frequency of occurrence of genotypes was determined among healthy donors and cancer patients diagnosed with breast cancer and Non-Khodgin lymphoma.

2. It has been shown that the frequency of occurrence of the homozygous TT genotype in breast cancer and Non-Hodgin lymphoma is higher than the frequency of occurrence of the CC and CT genotypes.

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