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Research Article

MARKERS FOR EARLY DIAGNOSIS AND MONITORING OF THE CLINICAL COURSE OF ONCOLOGICAL DISEASES

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ABSTRACT

In recent decades, oncological diseases have become one of the most important causes of death in people around the world; the annual number of deaths from cancer exceeds half of the number of newly diagnosed cases of the disease. The determination of biomarkers can provide information on the clinical course of the malignant process and predict the chemoresistance of a neoplasm in an individual patient. However, the identification of markers for a more accurate prognosis of the course of the disease and the choice of adequate therapy is still an unsolved problem [1,2]. Therefore, the study of the pathological mechanisms underlying the development of oncological diseases, the elucidation of their molecular markers for early diagnosis and effective treatment, still remain an urgent problem.

KEYWORDS

Oncological diseases, biomarkers, effectiveness of therapy, diagnostics.

INTRODUCTION

Purpose of the study . To analyze the biomarkers of early diagnostics, predicting the effectiveness of therapy and the clinical course of cancer patients.

MATERIAL AND RESEARCH METHODS

When performing the studies , DNA samples obtained from peripheral blood leukocytes of patients suffering from breast cancer (60 patients) and NHL (75 patients) were used. The blood of cancer patients was obtained from the Chemotherapy Department of the Republican Oncological Scientific Center of the Ministry of Health of the Republic of Uzbekistan. DNA of peripheral blood

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leukocytes from healthy donors (35 donors) was used as a control.

Isolation of cfDNA from blood serum/plasma. 1 ml of peripheral blood taken from the cubital vein was transferred into plastic tubes coated with EDTA-Na2 , and blood serum was obtained. The serum was pre-treated with RNase A (100 µg/ml), incubation for 1 hour at 37 0 C, then treated with proteinase K (50 µg/ml), incubation for 1 hour at 37 0 C. Next, blood serum was lysed. . 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 4 0 C for 15 min. The supernatant was treated with 2.5 volumes of chilled 96% ethanol. The cfDNA preparations were stored at -20 0 C.

To determine the concentration of cfDNA, cfDNA preparations were dissolved in 100 µl of DNA binding fluorescent dye (DSFC) 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 cfDNA was used as a control.

Isolation of nuclear DNA. Isolation of DNA from whole blood was carried out using a set of reagents AmpliPrime Ribo Prep and Ribo Sorb AM (InterLabService, Russia) according to the standard protocol. The method has been modified. Lysis of biological samples was carried out for 30-40 minutes at a temperature of 65 0 C. Centrifuged at 11 thousand. rpm DNA extraction was carried out at a speed of 13 thousand. rpm

Results and its discussion. The reasons for low survival and high mortality in cancer patients are the following: asymptomatic disease in the early stages, lack of effective methods for early diagnosis and resistance of patients to chemotherapy. Obviously, to solve the problem of early diagnosis, reliable and simple methods for detecting the tumor process are needed, which would allow identifying the tumor at preclinical stages. Despite the large number of studies on this issue, progress in the widespread use of biomarker analysis for various types of cancer is still insufficient [3,4,5].

The high incidence of this pathology brings to the forefront the problem of early diagnosis and treatment effectiveness. Despite the fact that the prevalence of the tumor process is the most important criterion for prognosis and choice of treatment tactics, its correlation with the effectiveness of treatment and the outcome of the disease is not always revealed. Recently, a number of molecular markers of tumor cells have been included in the list of prognosis criteria [6]. One of the diagnostic perspectives associated with the use of cell-free DNA (cfDNA) as a biomarker, which can be detected in plasma (serum) of human blood. Interest in cfDNA increased immeasurably after it became clear that its amount can increase significantly in a number of diseases, which can be taken into account as an early sign of corresponding pathologies [7]. . This gave a very clear practical significance to the study of circulating extracellular nucleic acids.

Bk DNA can serve as a marker for prognosis of the development of pretumor changes, early diagnosis and prediction of the clinical course of cancer. Much attention is paid to the study of DNA circulating in plasma or serum (circDNA), the concentration and composition of which changes significantly during the development of malignant neoplasms [8]. It is known that the level of circulating DNA increases during the development of a number of cancer diseases ¬. The

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concentration of DNA circulating in the blood can be used for the differentiated diagnosis of benign and malignant tumors of various etiologies. An increase in the concentration of circulating DNA allows one to draw a conclusion about the ineffectiveness of treatment and the progression of the disease, in particular the appearance of metastases.

Our task was to develop a method for quantitative assessment of cfDNA in the plasma of cancer patients. The objects for research were samples of biopsy material and blood from newly admitted cancer patients (100 patients) before treatment and after 2 courses of NACT. The age of the patients ranged from 30 to 66 years. The diagnosis in all patients was morphologically verified.

The fluorescence properties of ethidium bromide were used to quantify cfDNA. It is known that ethidium bromide is an artificially synthesized fluorescent nucleotide-specific dye [9]. The advantage of this dye is the simplicity of the staining method and sensitivity to small amounts of DNA in the sample. In Figure 1 Data on the content of cfDNA in the blood plasma of cancer patients are presented. The concentration of cfDNA in the plasma of cancer patients varied from 112.5 ng/ml to 250 ng/ml, and averaged 189.0 ng/ml. This figure shows that the content of cfDNA in the plasma of patients increases by an average of 57% compared to controls.

Of greatest interest is the dynamics of changes in cfDNA content, and not the single value ¬of their concentration in the blood. In order to determine the information content quantitative and qualitative changes in cfDNA to assess the effectiveness of treatment, a comparative analysis of the concentration of cfDNA in the blood plasma of cancer patients before treatment and after a course of chemotherapy was carried out. Figure 2 shows the results of changes in cfDNA concentrations in patients. The table data shows that in patients with signs of disease progression, an increase in cfDNA concentration is observed in 62.5% of cases (out of 16 patients, 10).

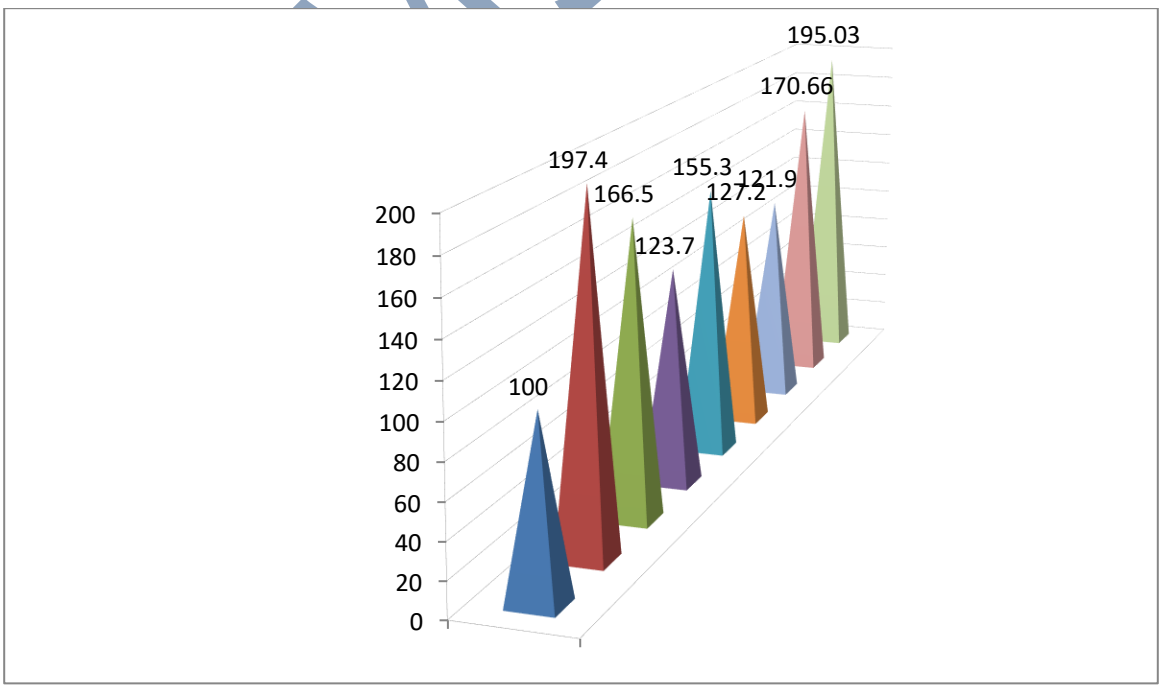

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Fig.1. Content of cfDNA in the blood plasma of cancer patients

Based on the data obtained, we can conclude that an increased content of cfDNA in the blood plasma of cancer patients is associated with an unfavorable outcome, ineffectiveness of treatment and the genotype of the cancer patient.

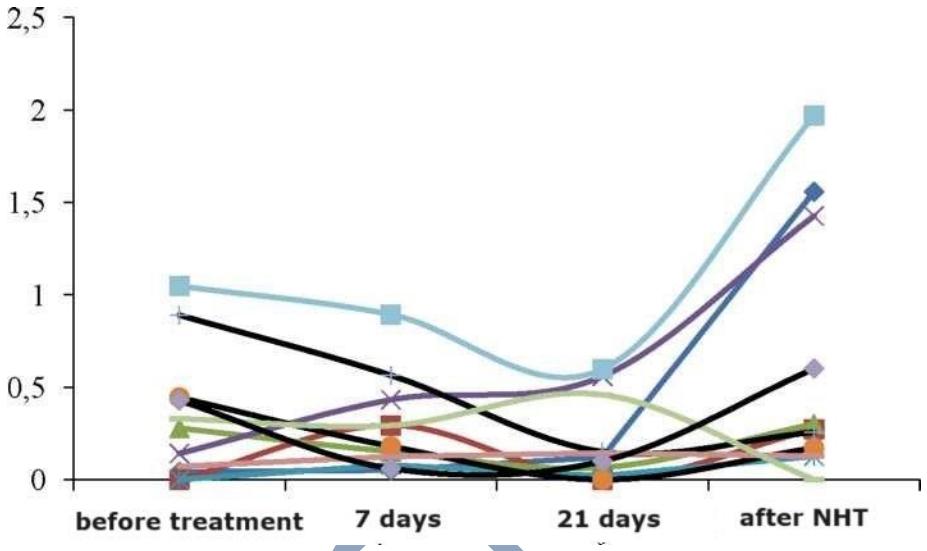

Fig.2. Changes in cfDNA concentration before treatment and after chemotherapy in cancer patients.

In cancer, there is not only a quantitative change in plasma cfDNA, but also qualitative disturbances, i.e. DNA fragmentation occurs, and low molecular weight nucleosomal DNA appears [10]. A qualitative assessment of cfDNA in the plasma of cancer patients suffering from breast cancer was carried out. Molecular analysis cfDNA masses were performed by electrophoresis in a 2% agarose gel. Figure 3 shows an electropherogram of cfDNA from healthy donors and cancer patients. From the data in the figure it can be seen that in cancer, extracellular DNA fragments and low molecular weight fragments appear, measuring 2.0; 0.6; 0.4 and 0.2 kb. A comparative quantitative analysis of cfDNA in the blood plasma of cancer patients was carried out using real-time PCR, specific to the polymorphic marker C3435T of the MDR 1 gene. Real-time PCR revealed significant differences in the amount of cfDNA of patients. Figure 4 shows data on cfDNA content in the plasma of cancer patients

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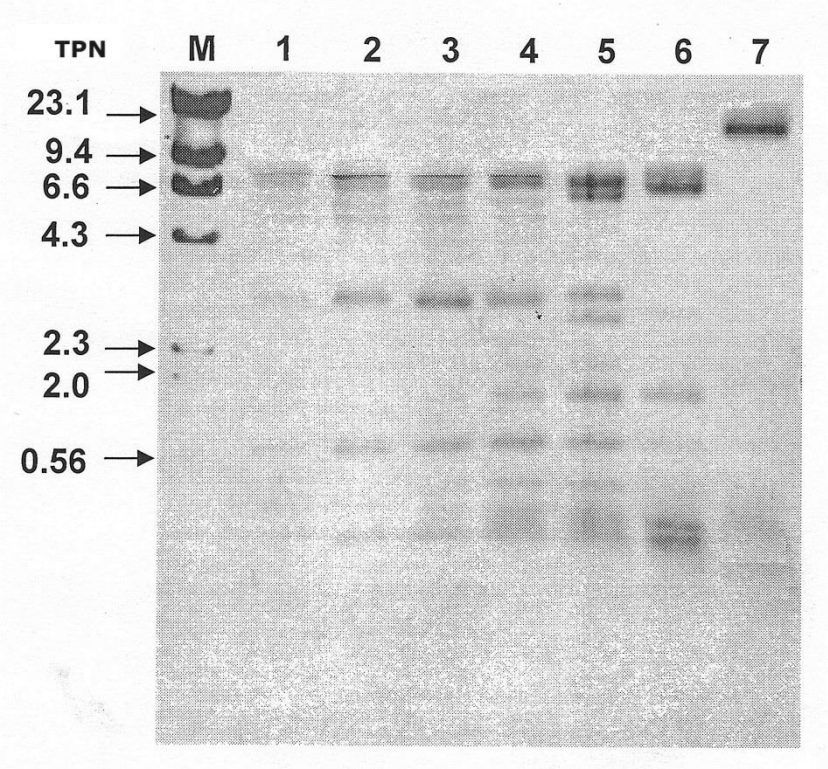

Fig.3. Qualitative assessment of cfDNA in blood plasma of patients with breast cancer

m – DNA marker 100 kb , M – λ/ EcoR I ; 1 – Chakalova (9.4; 6.6 kb), 2 – (9.4; 3.5 kb); 3- Holierova (9.4; 3.2; 2.0; 0.6; 0.4 kb); 4- Nigmatulina (9.4; 6.8; 1.8; 0.6; 0.2 kb); 7 – normal (22.1 kb).

The data in the figure shows that the ratio of the amount of cfDNA in the blood plasma of cancer patients differs from the amount of cfDNA from healthy donors.

Changes in the amount of cfDNA according to real-time PCR data specific to the polymorphic marker C3435T of the MDR 1 gene can serve as an additional criterion for detecting breast cancer and can be used to assess the stage of the disease and monitor treatment. Figure 4 shows a PCR analysis of the concentration of cfDNA in the blood plasma of cancer patients using the polymorphic marker C3435T/ MDR 1.

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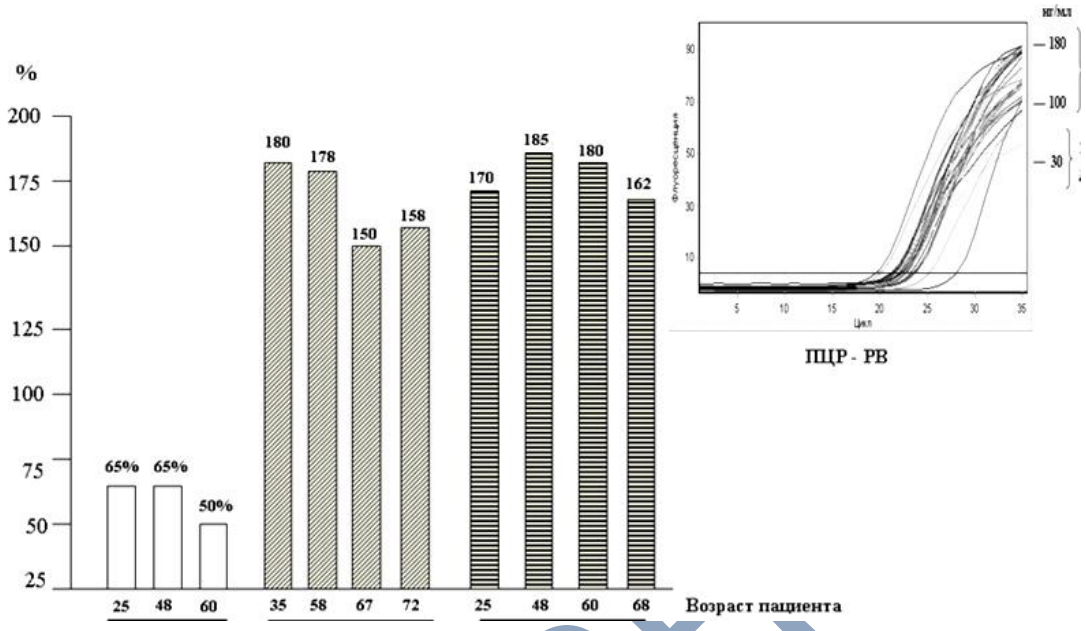

Fig. 4. PCR analysis of cfDNA concentration in the blood plasma of cancer patients using the polymorphic marker C3435T/ MDR 1

HL - healthy donors, NHL - non-Hodgkin's lymphoma, BC - breast cancer; C3435T – polymorphic marker of the MDR 1 gene; Malignant neoplasms – malignant neoplasms.

Tumors are characterized by an imbalance of epigenetic regulation: against the background of total hypomethylation, local hypermethylation of the promoter regions of suppressor genes is observed. Abnormal methylation is considered one of the early signs of carcinogenesis, and can also be a marker for predicting the effectiveness of cancer treatment and can be used in practical oncology. First, identifying the methylation pattern of the promoter region of the suppressor gene blood plasma can be used to detect a tumor process [11]. Secondly, abnormal methylation of a number of CpG islands can serve as a marker of a particular tumor phenotype, prognosis of the disease, response to therapy and/or side effects of treatment. Third, abnormal methylation in morphologically intact cells is associated with an increased risk of malignancy and may serve as a marker for early cancer diagnosis [12]. .

In the next series of experiments, the role of methylation of suppressor genes in early diagnosis and assessment of the effectiveness of treatment of cancer patients was studied . Tumor cells are distinguished by a global change in the DNA methylation pattern, which is complex: in the same tumors, both total DNA demethylation and activation of transcription of the corresponding genes, as well as local hypermethylation and suppression of transcription of genes associated with them, occur. Recent studies have shown that tumors of different locations, including breast cancer (BC), may differ in the set of genes that undergo aberrant methylation. The mechanisms that activate such multidirectional processes in tumor cells as hyper- and hypomethylation of DNA, as well as the mechanisms that determine the specificity of methylation markers for different types of tumors, are still unknown.

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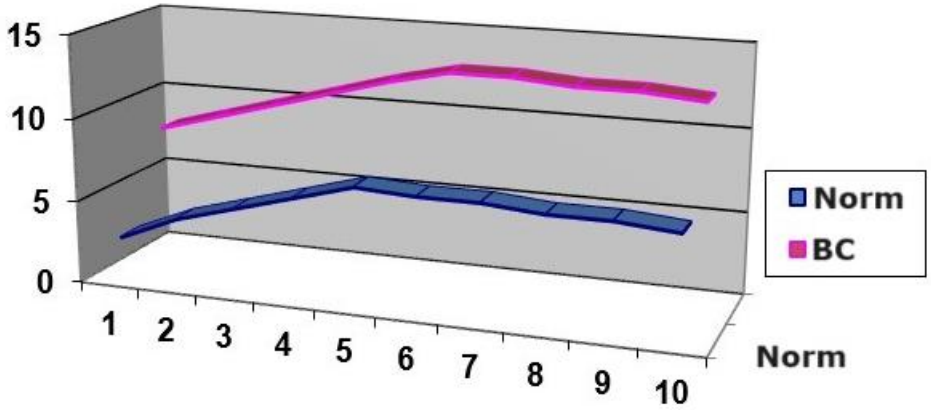
In oncological diseases, cytosine methylation occurs in the so-called "CpG islands", the process of histone deacetylation is activated, which leads to a change in chromatin configuration and local suppression of transcription. "CpG islands" are short regions in the promoter regions of genes that contain an increased number of CG dinucleotides compared to the rest of the genome; these regions are almost always unmethylated in normal cells. The transition of CpG islands to a hypermethylated state sharply reduces the expression of suppressor genes, which leads to activation of the tumor process [11,12]. In this regard, the identification of hypermethylated CpG islands of tumor suppressor genes in human DNA is extremely important and allows for early diagnosis diseases.

The methylation pattern in neoplastic cells changes significantly compared to normal cells; total demethylation is accompanied by an increase in DNA methyltransferase activity and local hypermethylation of CpG islands. The mechanism of local hypermethylation is not entirely clear. Apparently, an increase in methyltransferase activity plays an important role in this process. In order to understand the reason for simultaneous hypermethylation and hypomethylation of DNA in cancer, we studied the activity of DNA methyltransferase in the DNA of normal and tumor cells. To study methyltransferase activity, DNA molecules were treated with the DNA enzyme methyltransferase. Changes in methyltransferase activity have been shown in breast cancer. Figure 5 shows the results reflecting changes in the activity of this enzyme in normal conditions and in breast cancer. From the data in the figure it can be seen that in breast cancer the activity of methyltransferase increases. The molecular mechanisms of increased DNA methyltransferase expression in tumor cells have not been elucidated. Apparently, this may be a compensatory reaction of the cell to general demethylation. Increased methyltransferase activity significantly affects the DNA methylation profile. High activity of methyltransferase characterizes an imbalance of epigenetic regulation in cancer: against the background of total hypomethylation, local hypermethylation of the promoter regions of suppressor genes is observed.



Rice. 5. DNA methyltransferase activity in normal conditions and in breast cancer

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Abnormal methylation can be considered a marker of a high probability of a malignant process and ineffectiveness of the therapy.

Thus, the identified biochemical markers can identify a tumor at an early preclinical stage. Biomarkers can also predict and monitor the adequacy of therapy for cancer patients who are forced to endure single-course chemotherapy regimens, which leads to the development of adverse reactions and multidrug resistance. The biological complexity of cancer continues to confound efforts to eradicate the disease entirely. To increase life expectancy and improve its quality, it is necessary to have markers of early diagnosis, prognosis and response to treatment of cancer patients.

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