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EPIGENETIC INACTIVATION OF TUMOR SUPPRESSOR GENES- MARKER FOR MONITORING THE EFFECTIVENESS OF THERAPY

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ABSTRACT

Epigenetic inactivation of tumor suppressor genes caused by hypermethylation of the promoter area is as characteristic of the tumor process as genetic disorders, serving as an alternative mechanism of the function loss by the tumor suppressor genes. An analysis of the promoter area of RARв2 gene-suppressors has been performed in the normal condition and in breast cancer by methyl-sensitive polymerase chain reaction. Methylation of CpG-dinucleotides in the RARв2 gene promoter area was demonstrated. Abnormal methylation of the RARв2 gene promoter area may be used as a marker for early diagnosis and monitoring of the treatment efficacy in patients with the breast cancer.

KEY WORDS

breast cancer, tumor suppressor, marker, early diagnosis, monitoring of treatment efficacy.

INTRODUCTION

It is known that the breast cancer (BC) has a multifactor nature. In pathogenesis of this disease the oncogenes and genes of tumor suppression play important roles. The reason of inactivation of tumors suppressor genes (here termed as gene-suppressors) and growth of malignantly transformed cells are genetic and epigenetic violations. It is thought that epigenetic violation in malignancy is the earliest sign and appears before clinical manifestations of the disease [1]. At present, it is established that gene methylation is associated with aggressive forms of tumor and disease progression [2] Therefore, determination of methylation degree of gene-suppressors of the tumor may serves as an early marker of aggressiveness of the tumor process and can be applied for prognosis.

Tumor cells are characterized by global changes in the pattern of DNA methylation which has a fairly complex character: within the same tumors, happen both total demethylation of DNA and activation of transcription of appropriate genes on the one hand, and local hypermethylation and suppression of transcription of genes associated with them on the other hand [3]. Mechanisms activated in tumor cells for such a multidirectional process as hyper- and hypo-methylation of DNA, as well as mechanisms determining the specificity of markers of methylation for different tumor types, remain unknown [4].

Methylation of tumor-suppressor gene promoters in breast cancer. The breast cancer is the most common oncological pathology of women. It was studied in detail, what is the range and rate of molecular-genetic

violations in BC. In BC, the methylation of cytosine within so-called "CpG-islands" as well as the process of deacetylation of histones, take place, which lead to the changes is configuration of chromatin and local suppression of transcription [5]. The «CpG-islands» are short sites in the promoter areas of genes containing higher number of the CG-dinucleotides in comparison with the rest of the genome. These areas are almost always not methylated in normal cells. The transition of CpG islands in hypermethylation condition sharply decreases expression of tumor suppressors, which leads to an activation of the tumor process [6]. According to this notion, determination of the hypermethylated CpG islands of tumor-suppressors gene in the DNA of tumors growing in humans is very important and allows performing an early diagnostic of the diseases.

Our main task is the analysis of the possibility of using of the anomalous methylation as a biomarker of early diagnostics for oncological patients. At present, the analysis of methylation markers is considered as an optimal instrument for molecular-genetic diagnostics and monitoring in oncology. By specificity, it is comparable to the analysis of genes expression, and significantly exceeds the latter in simplicity and accessibility. While the analysis of structural abnormalities remains to be an expensive enough procedure and is applied, particularly, for diagnostics of the hereditary oncological syndromes, for detecting the standard mutations, and for determining the sensitivity of tumors to chemical preparations (drugs). At present, the anomalous methylation of DNA is one of the most technically available markers. The tumors are characterized by an imbalance of epigenetic regulation: on the background of total hypermethylation, the local hypermethylation of promoter areas of tumor suppressors is also observed. Anomalous methylation is one of the early signs of carcinogenesis. For the determination of CpG-islands methylation of promoter areas of investigated genes, the method of methyl-sensitive PCR (MS-PCR) is employed. This method is based on the ability of methyl-sensitive restriction enzymes to hydrolyse DNA not containing modified bases (5-methylcytosine) and to abandon unhydrolyzed areas containing methyl cytosine [7]. As matrix for polymerase chain reaction, the DNA preliminarily hydrolysed by the methyl-sensitive restriction enzyme, HpaII (CCGG) is used. The methyl-sensitive PCR of DNA sequences of RARв2 gene-suppressors treated by the restriction enzyme HpaII (CCGG) is then performed, which is sensitive to methylation of cytosine residues. The presence of the areas containing methyl cytosine in the promoter areas in breast cancer and unhydrolyzed by the restriction enzyme HpaII has been demonstrated [8,9]. In Fig. 1, an example of the result of the methyl-sensitive PCR of RARв2 gene is presented. From the data of Fig. 1, it can be seen that in BC, there is a hypermethylation of CpG -islands in the promoter region of this gene-suppressor. If in tumor DNA, there is an anomalous methylation of the promoters of RARв2 gene, the cytosines in CpG-dinucleotides will be replaced with 5-methylcytosines, including the sites of HpaII recognition. In these cases, the restriction enzyme HpaII cannot hydrolyse DNA in sites of recognition, and matrix for MS-PCR remains intact. As a result, during electrophoresis it is seen as a fragment in the agarose gel which corresponds to the promoter region of RARв2B gene (Fig. 1, lanes 6-11). This figure shows that the 2nd lane of in agarose gel has fragments of unhydrolyzed DNA [10]. On the basis of this, one may come to conclusion that this donor may have tumor cells.

It was of interest for us to check how hypermethylation of promoter area of RARв2 gene of tumor suppression influences the treatment efficacy. For this purpose, we investigated the patients suffering from breast cancer six months after the treatment. All patients received 2 courses of chemotherapy in standard scheme. The material of study was peripheral blood of women after 6 months of treatment. In Fig. 2, the data of methyl-sensitive PCR of RARв2 gene of patients with breast cancer after treatment is presented. The data show that in some oncology patients during BC (lanes 7,9,11) the methylation of RARв2 gene has not been revealed, while in other patients (lanes 6,8,10,12) electropherogram, unhydrolyzed, containing methyl cytosine, fragments of promoter area of RARв2 gene can be seen that indicates ineffectiveness of the performed therapy [11].

In those samples, where there is not methylation of genes, the hydrolysis of DNA in sites of HpaII recognition takes place. It is destroyed, and the PCR product, which corresponds to the gene promoter, is absent (Fig. 1, lanes 1-3). This figure shows that 2nd lane of the donor in agarose gel has fragments of unhydrolyzed DNA. On the basis of this, one may come to a conclusion that this donor may have tumor cells.

Thus, in this study we analysed the opportunity of the use of methyl-sensitive polymerase chain reaction for determination of pathologic methylation of tumor suppressors gene on an example of methylation of 5′-promoter area of RARв2 gene in normal subjects and in BC. On the basis of anomalous methylation of promoter

areas of RARв2 gene, one may conclude about high probability of malignant process in breast, and in diagnosed cancer cases the diagnostics is confirmatory. On the other hand, hypermethylation may be used also as a predictive marker, which provides information about efficacy of chemotherapy in the treatment of oncological patients.

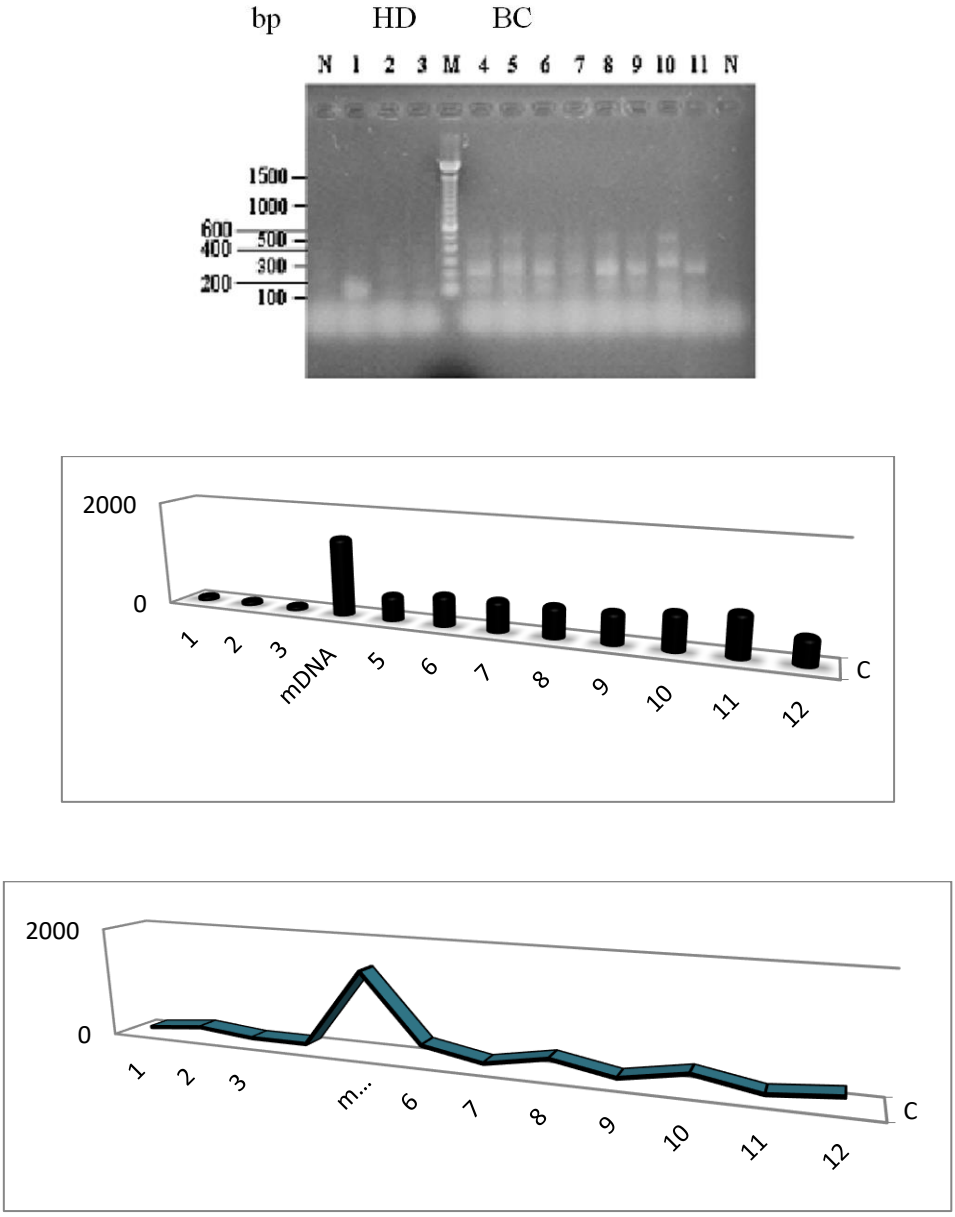

Figure 1. (Methyl-sensitive PCR of RARв2 gene.)

HD – healthy donors (1,2,3). BC – breast cancer (5-11), N – control (primers + water), M – DNA markers 1500kb (Modified from Ref. 9).

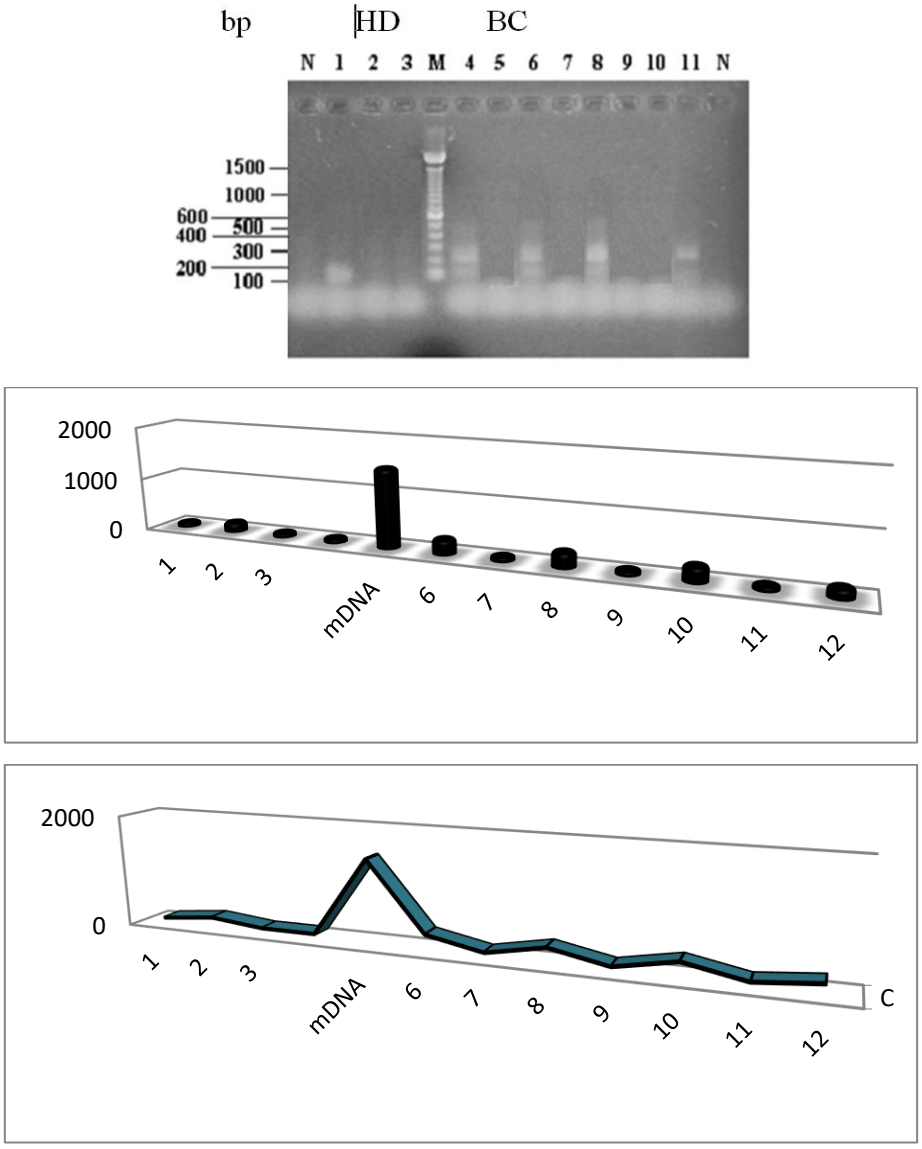

Figure2. (Methyl-sensitive PCR of RARв2 gene after treatment.)

HD – healthy donors (1,2,3,4). BC – breast cancer (6-11). N – Control (primers + water), M – DNA markers 1500kb (Modified from Ref. 9).

Therefore, studying the methylation status of RARв2 gene has showed that their epigenetic change may be one of the consecutive molecular changes in BC and correlates with the trend to the development of tumor in breast. It may be considered that methylation of DNA is one of the most significant reasons of appearing of tumor in patients [12]. Revealed features of epigenetic changes of RARв2 gene can be used not only for early diagnostics

of BC, but also as new prognostic tests, allowing to reveal the risk groups of patients with BC development. On the basis of the obtained results one may come to conclusion that anomalous methylation of DNA can be used in practical oncology as marker of early diagnostics and treatment efficacy of oncological diseases.

Epigenetic alterations, such as DNA methylation, are widely accepted as a potential source of early biomarkers for better diagnosis/prognosis [13]. Many tumors exhibit excessive methylation of CpG islands that are found near the transcriptional promoters of genes [14]. Indeed, hypermethylation of CpG islands in specific gene promoters

is thought to contribute to carcinogenesis through transcriptional silencing of tumor suppressor gene expression, leading to the initiation and progression of cancer. Hypermethylation of CpG islands located in the promoter regions of tumor suppressor genes is now firmly established as an important mechanism for gene inactivation. CpG island hypermethylation has been described in almost every tumor type.

Nevertheless, we still distinguish little about the mechanisms of abnormal methylation and why convinced genes are nominated over others. DNA methyltransferases and histone deacetylase are supposed to be elaborate, but our sympathetic of the grade of specificity of these epigenetic pathways in the quieting of exact growth suppressor genes leftovers imperfect. The detonation of comprehensible technologies has given increase to a fast-growing list of hypermethylated genes.

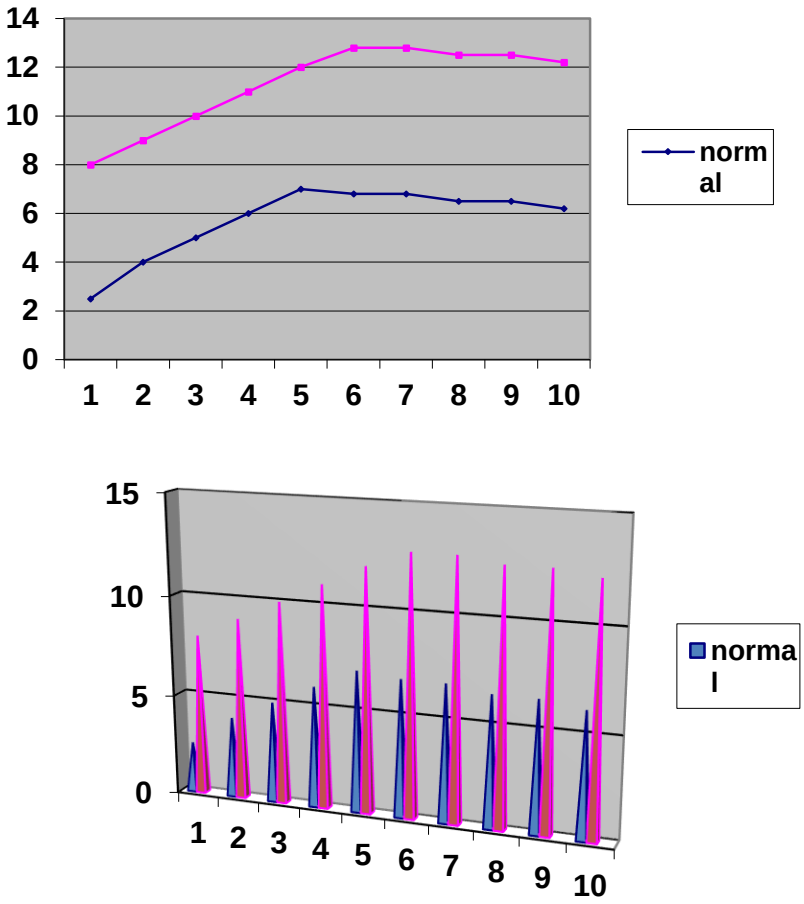

Figure 3. (The activity of DNA methyltransferase in normal and breast cancer.)

(1 - healthy donors; 2 - breast cancer.)

From the figure, it can be seen that, with BC, methyltransferase activity increases by 58%, compared with the norm. Molecular mechanisms of enhanced expression of DNA methyltransferase in tumor cells have not been elucidated. Apparently, this can be a compensatory reaction of the cell to general demethylation. The increase in methyltransferase activity significantly affects both the DNA methylation profile and local hypermethylation.

Finally, CpG island hypermethylation has demonstrated its great versatility for the molecular monitoring of cancer patients, and is a likely target for future and smarter therapeutic approaches. Careful functional and

genetic studies are necessary to determine which hypermethylation events are truly relevant for human tumor genesis. The development of CpG island hypermethylation profiles for every form of human tumors has yielded valuable pilot clinical data in monitoring and treating cancer patients based on our knowledge of DNA methylation. An epigenetic defect in cancer cells is a new area of carcinogenesis studies. We presume that epigenetics cancer diagnostics and therapy will open new perspectives for early diagnostics of BC.

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