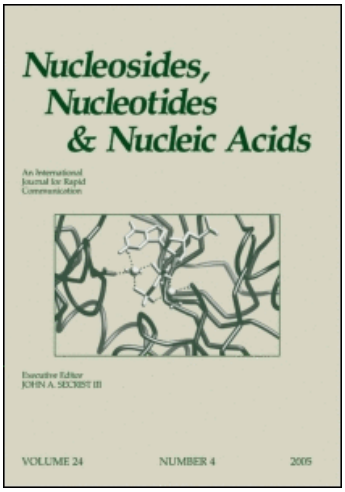

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Studies on the Methylation Status of CpG Sequences Located in Promoters of Selected Tumour Suppressor Genes in Breast Cancer Cells

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STUDIES ON THE METHYLATION STATUS OF CpG SEQUENCES LOCATED IN PROMOTERS OF SELECTED TUMOR SUPPRESSOR GENES IN BREAST CANCER CELLS

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□ *In the tested samples of sporadic breast cancer (100 cases), hypermethylation of CpG sequences located in ERα promoter was observed in 62 cases. It correlated with: (i) deficiency of* ERα *protein in 45%, (ii) hypermethylation of BRCA1 promoter in 95%, and (iii) nonmethylated E-cadherin promoter in 90%. Fifty-eight percent of the patients with nonmethylated E-cadherin promoter (56 cases) did not show metastasis to lymphatic nodes. The analysis of the methylation level of the promoter of ERα, BRCA1, and E-cadherin, frequently connected with their activity, shows that it can be an important parameter in the diagnosis and therapeutic strategies in sporadic breast cancer.*

Keywords Hypermethylation of gene promoter; Breast cancer

INTRODUCTION

One-third of breast cancer, mainly resistant to antiestrogenic therapy,[1] is probably associated with epigenetic alteration, which can include both decreased methylation level (hypomethylation) of genomic DNA and increased methylation (hypermethylation) of CpG islands located mainly in regulatory regions of tumour suppressor genes.[2–5] The methylation of cytosine in CpG sequences located in promoters of the genes is often involved in the mechanism which regulates gene activity.[4,5] The present studies

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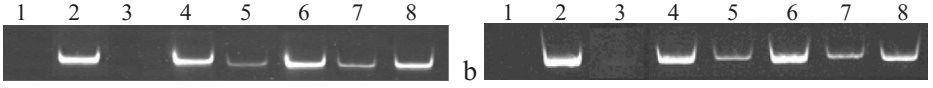
were designed to estimate the methylation status of CpG sequences located in promoters of three tumour suppressor genes (i.e., *ERα*, *BRCA1*, and *E-cadherin*) in comparison with the expression of ERα protein in sporadic breast cancer cells and in comparison with clinical and pathological data such as tumour size or nodal metastasis. To achieve this goal, we used methylation-sensitive restriction analysis, MSRA.[6]

MATERIALS AND METHOD

The experiments were performed on neoplastic (100 cases) and noncancerous lesion (used as control) tissues which were received from cancer tissue bank (Department of Pathology). The methylation status of the promoters of *ERα*, *BRCA1*, and *E-cadherin* was estimated using methylation-sensitive restriction analysis, MSRA,[6] in which isolated and purified DNA was digested with a restriction enzyme recognizing non-methylated CpG sequences. Then, digested DNA was amplified in PCR reaction, and amplified DNA fragments were electrophoretically analyzed in 6% polyacrylamide gel. The following restriction enzymes were used: HpaII (recognizing C$^{\downarrow}$CGG), BstU1 (CG$^{\downarrow}$CG), and AatII (GACGT$^{\downarrow}$C, the sequence is recognized by CREB protein). The endonucleases were products of New England Bio Labs. The description of clinical and pathological parameters (nodal metastasis and immunohistochemical examination of ERα receptor) was received from the Department of Pathology.

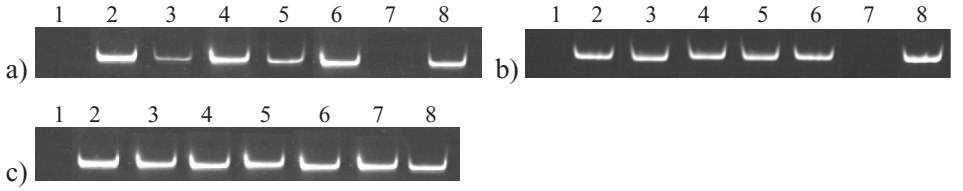
RESULTS AND DISCUSSION

In the sporadic cancer samples hypermethylation of *ERα* was detected in 62% of cases (62/100). An example of electrophoretic separation of amplified *ERα* from breast cancer tissue is shown in Figure 1 (lack of a band indicates non-methylated CpG sequences located in the tested fragments of promoters, but lower intensity of a band means that methylation level



Channels:
1 – control of digestion; non-methylated DNA from human placenta
8 – control of amplification; undigested DNA from K562 cells
2, 4, and 6 – undigested DNA from three different breast cancer tissues
3, 5, and 7 – digested DNA from breast cancer tissues corresponding to 2, 4, and 6 channels

FIGURE 1 Amplified fragments of *ERα* promoter of 3 selected breast cancer tissues previously digested with HpaII (a) and BstU1 (b).



Channels:
1 – control of digestion; non-methylated DNA from human placenta
8 – control of amplification; undigested DNA from K562 cells
2, 4, and 6 – undigested DNA from three different breast cancer tissues
3, 5, and 7 – digested DNA from breast cancer tissues corresponding to 2, 4, and 6 channels

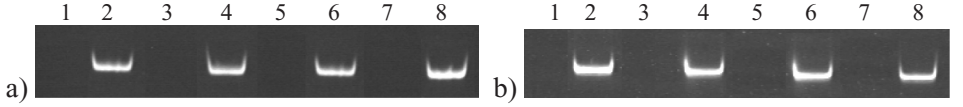
FIGURE 2 Amplified fragments of *BRCA1* promoter of 3 selected breast cancer tissues previously digested with HpaII (a), AatII (b), and BstU1 (c).

of specific sequence was partially reduced). Hypermethylation of *ERα* promoter was related to the deficiency of its protein in 45% of cases (45/62). The results confirm previous observations of other authors.[1]

ERα hypermethylation correlated with hypermethylation of *BRCA1* promoter ($p = 0.0079$; 59/62). Figure 2 presents an example of electrophoretic separation of amplified *BRCA1* from selected breast cancer tissues.

Hypermethylation of *ERα* (62 cases) was associated with nonmethylated promoter of *E-cadherin* (56 cases). Metastasis to lymphatic nodes was not detected in 33 patients (58%) of the group with nonmethylated *E-cadherin*. Examples of electrophoretic analysis of amplified *E-cadherin* promoter from neoplasic tissues are shown in Figure 3.

Noncancerous tissue of breast did not show methylation of promoters of corresponding genes (data are not shown). The highest increase of *ERα* promoter methylation in the group of ERα (-) breast cancer cases is noted



Channels:
1 – control of digestion; non-methylated DNA from human placenta
8 – control of amplification; undigested DNA from K562 cells
2, 4, and 6 – undigested DNA from three different breast cancer tissues
3, 5, and 7 – digested DNA from breast cancer tissues corresponding to 2, 4, and 6 channels

FIGURE 3 Amplified fragments of *E-cadherin* promoter of 3 selected breast cancer tissues previously digested with HpaII (a) and BstU1 (b).

at perimenopausal age (in the early forties), which may result from the aberration of hormonal balance in the body, and also in the seventies, probably due to ageing.

Referring to *ERα* and its expression, the results confirmed that the hypermethylation of gene promoters plays an important role in the silencing of gene activity.[1] In breast cancer cells hypermethylation concerned usually several genes (in our assays *ERα* and *BRCA1*) encoding proteins whose actions are interdependent.[7]

The analysis of the methylation level of the promoter of *ERα*, *BRCA1*, and *E-cadherin*, frequently connected with their activity, shows that it can be an important parameter for diagnosis and monitoring of carcinogenesis and metastasis processes in sporadic breast cancer.

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