Content of 8-Hydroxy-2-Deoxyguanosine in Steroid Receptor-Positive and Receptor-Negative Breast Cancer Cells

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The content of DNA damage marker 8-hydroxy-2-deoxyguanosine in 16 receptor-negative and 18 receptor-positive human breast neoplasms was measured by immunohistochemical methods. Positive staining was revealed in 81.3±9.8 and 50.0±11.7% samples of groups 1 and 2, respectively. The effect of arylhydrocarbon receptor agonist β-naphthoflavone on the content of 8-hydroxy-2-deoxyguanosine and number of estrogen and progesterone receptors was evaluated in MCF-7 breast cancer cells. The degree of genotoxic damage significantly increased 1 h after combined treatment with estradiol and β-naphthoflavone (in contrast to individual treatment) and remained practically unchanged in the follow-up period. According to the estrogen effect-switching phenomenon, genotoxic damage can contribute to the development of R^- breast cancer.

Key Words: *breast cancer; MCF-7 cell line; estrogen and progesterone receptors; 8-hydroxy-2-deoxyguanosine*

Theoretically, all cells in reproductive tissues should carry receptors for steroid hormones, but this is not true for normal and malignant tissues. Moreover, receptors for steroid hormones are absent in 30-40% neoplasms of the mammary gland [3,5,9]. The study of the causes and mechanisms of this phenomenon is an important fundamental and applied problem of tumor growth. Estrogen receptors (ER) are transcription factors. In combination with a considerable number of coactivators and corepressors, they form a specialized apparatus that modifies the access to promoter regions in ER-dependent genes (*e.g.*, gene for progesterone receptors, PR). The function of PR serves as a criterion of normal transduction of the estrogen signal and provides the effect of progestins on target tissues in the reproductive system.

Analysis of the causes and mechanisms of growth of tumors not carrying steroid hormone receptors is based on two different approaches. According to the first approach, the absence of ER and/or PR in the tumor reflects stages of the same disease (tumor progression). The second approach postulates that P^+ and R^- neoplasms are different processes. The development of R^- neoplasms (particularly ER^+PR^-) can result from the estrogen effect-switching phenomenon (ESP) [1]. ESP manifests in weakening the effect of hormones with increasing their genotoxicity, which is related to the formation of free radical products of catechol estrogen metabolism and DNA damage [8]. Several xenoestrogens produce the same effect realized via arylhydrocarbon receptors (AhR) [13].

Here we compared the content of a specific marker of DNA damage 8-hydroxy-2-deoxyguanosine (8-OH-dG) in R^- and R^+ breast tumor tissues. We studied changes in the content of 8-OH-dG and number of ER and PR in MCF-7 breast cancer cells treated with an AhR agonist β-naphthoflavone.

MATERIALS AND METHODS

The concentration of ER and PR in breast tumor tissue and MCF-7 cells was estimated by the radioligand method with tritium-labeled steroids (2,4,6,7-^{3}H Oes-

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tradiol and 1,2,6,7-^{3}H Progesterone, Amersham) [13]. Tumor tissue from 34 postmenopausal patients (stage $T_{1-3}N_{0-1}M_0$) was obtained during surgery and embedded into paraffin. Immunohistochemical study of 8-OH-dG in breast neoplasm sections [7] involved treatment with 1F7 antibodies (Tregiven Inc., dilution 1:100) and biotinylated antibodies (Vecstain kit, Vector Lab., ABC kit), incubation with avidin-biotin-peroxidase complex, and detection of peroxidase by the reaction with diaminobenzidine. Nuclei were stained with methyl green. The results were visually analyzed and expressed as "+" (positive staining), "±" (moderate positive staining), and "-" (negative staining). The data for groups "+" and "±" were pooled. The tumors containing less than 10 fmol ER and PR per 1 mg protein (radioligand study) were considered as R^- neoplasms. MCF-7 cells were defrosted, grown in MEM medium to 70-80% confluence, and reinoculated into 6-well plates (5×10^4). Control samples were maintained in the same medium. MCF-7 cells were incubated with estradiol and β-naphthoflavone in concentrations of 10^{-7} M and 40 μM (40×10^{-6} M), respectively. Combined treatment involved estradiol and β-naphthoflavone in the same doses. Samples were taken 1, 24, and 48 h after the start of incubation. For immunocytochemical analysis of 8-OH-dG the cell smears were fixed with 70% ethanol for 10 min, dried in air, and kept in foil at -20°C. The data were subjected to independent visual evaluation with a scale that varied from 0 (no staining) to 1.5 points (maximum staining) at an increment of 0.25 points. The results were analyzed by Statistica 6 software.

RESULTS

The concentration of ER and PR in R^+ breast neoplasms (18 tumors) was 20-250 and 20-470 fmol/mg protein, respectively. The content of these receptors in R^- breast neoplasms (16 tumors) corresponded to 0-4 and 0-8 fmol/mg protein, respectively. Immunohistochemical study revealed specific positive staining for 8-OH-dG in 9 of 18 P^+ tumors (50.0±11.7%) and in 13 of 16 R^- tumors (81.3±9.8%, t=2.05, p=0.05). It should be emphasized that 8-OH-dG was more often detected in R^- tumors than in R^+ tumors. These results indicate that the genotoxic factor is of considerable importance for the growth and progression of R^- breast cancer (Fig. 1).

Estradiol (10^{-7} M) and β-naphthoflavone (40×10^{-6} M) and their combination reduced the concentration of ER in MCF-7 cells as soon as 1 h after the start of incubation. As differentiated from cells treated with estradiol, in samples treated with β-naphthoflavone alone or in combination with estradiol the concentration of ER continued to decrease after 24 and 48 h. As expected PR concentration increased by the 48th hour under the influence of estradiol and, to a lesser extent, after treatment with estradiol and β-naphthoflavone (clinical tumor ER^-PR^-).

Immunocytochemical analysis showed that the content of 8-OH-dG progressively increased by the 48th hour of incubation with estradiol or β-naphthoflavone alone. However, the degree of genotoxic damage significantly increased 1 h after combined treatment with these substances (the period when the concentration of ER decreases, while the content of PR is low) and then remained practically unchanged (Fig. 2). It cannot be excluded that the hormonal effect of estradiol is modified by an additional factor, which contributes to accumulation of genotoxic damages. These changes are accompanied by cell transition from the R^+ to R^- state.

The absence of steroid hormone receptors in breast carcinomas not only determines the therapeutic strategy [4,6], but also serves as an important biological characteristic of the neoplasm. The concentration of ER in breast tumor tissue is often higher than in normal mammary epithelium. However, transition from normal to malignant tissue is not necessarily accompanied by the loss of sensitivity to hormones [9]. The ratio of ER^+PR^+ tumors increases during menopause (relative to other receptor phenotypes). It should be emphasized that the incidence of PR^- neoplasms often increases in this period [14], which is consistent with the results of our study [2]. These changes correspond to the mechanism for ESP. In breast cancer patients above 50 years, the increase in the percentage of ER^+PR^- neoplasms is accompanied by decreased binding of tumor DNA with Sp1 cofactor and increased concentration of oxidative stress marker P-Erk5 in tumor tissue [11]. 8-OH-dG content in breast tumor tissue is 10-fold higher than in normal epithelium (2.44±0.49 and 0.25±0.03 pmol/mg DNA, respectively). Published data show that 8-OH-dG content in ER^+ neoplasms is 3.5 -fold higher than in ER^- neoplasms [10]. However, the presence or absence of PR in examined samples was not taken into account in these researches. Other authors reported that estradiol probably promotes accumulation of 8-OH-dG in MCF-7 cells. However, the same phenomenon was observed in receptor-negative MDA-MB-231 cells. This cell line does not proliferate under the influence of estradiol. These data show that DNA damage is not associated with the effect of classic estradiols, but develops due to the influence of their catechol derivatives [7]. The results of our study indicate that genotoxic damage can contribute to the development of R^- breast cancer.

Our findings should be taken into account in the development of new methods for induction of steroid

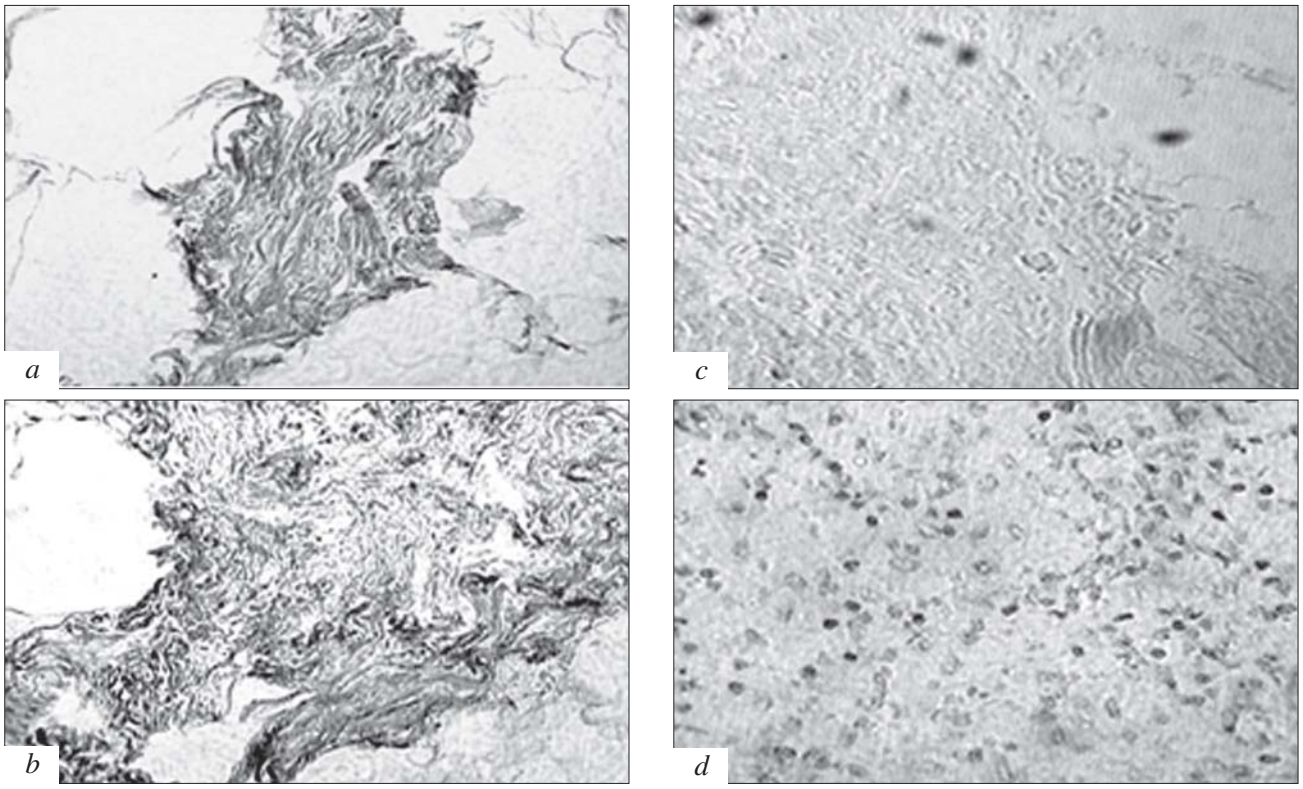

Fig. 1. Sections of breast cancer (BC) tissue, immunohistochemical detection of 8-hydroxy-2-deoxyguanosine: positive staining, BC^- (*a*); positive staining, BC^+ (*b*); negative staining, BC^+ (*c*); control (without primary antibodies). ×200.

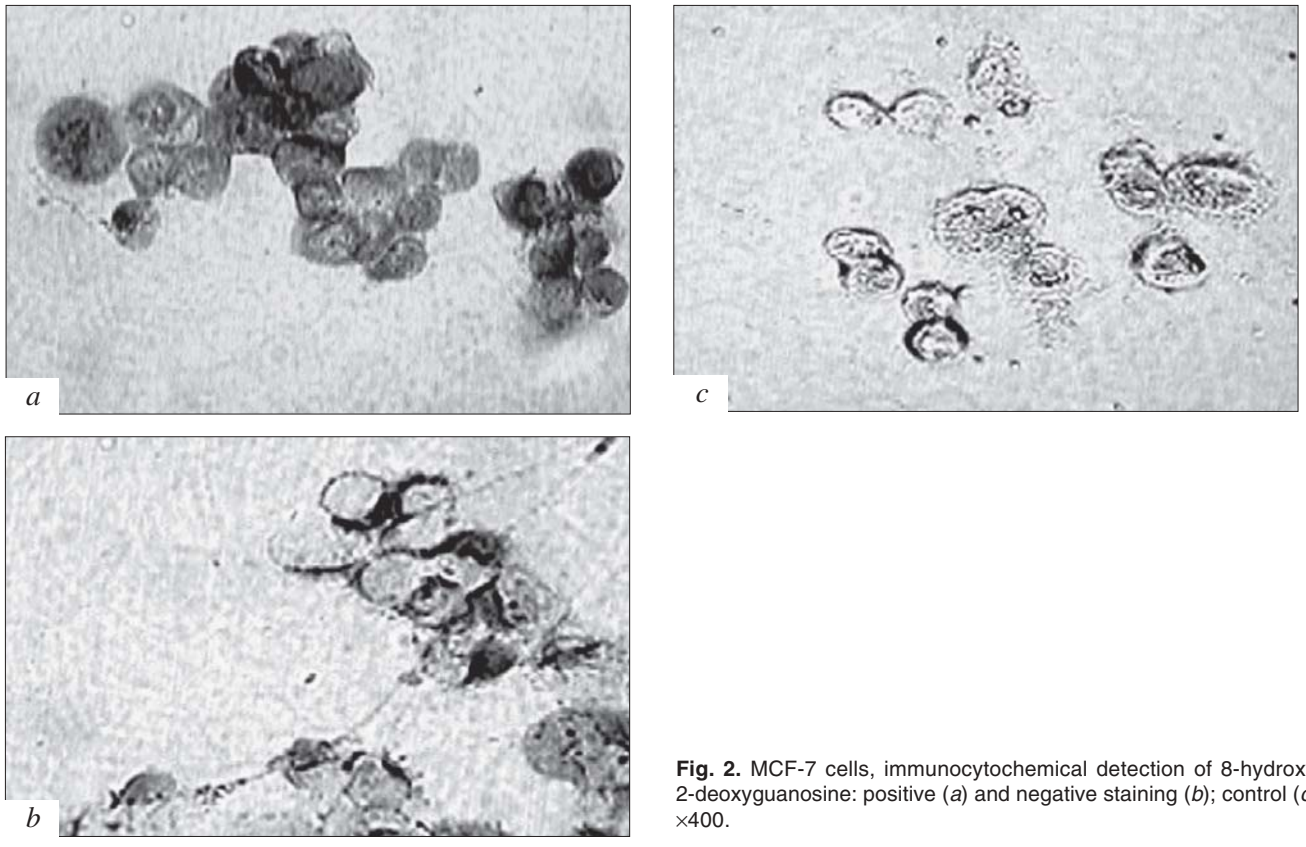

Fig. 2. MCF-7 cells, immunocytochemical detection of 8-hydroxy-2-deoxyguanosine: positive (*a*) and negative staining (*b*); control (*c*). ×400.

receptors in tumor tissue (*e.g.*, prevention of DNA damage by treatment with estradiol and estradiol-containing preparations). This approach will increase the sensitivity of neoplasms to hormones and improve the results of therapy.

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