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Mechanism of Estrogen-Induced Apoptosis in Breast Cancer Cells: Role of the NF-κB Signaling Pathway

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Abstract—The ability of sex steroid hormones to up-regulate the apoptotic signaling proteins is well documented; however, the apoptotic potential of sex hormones is not remarkable and fully compensated by their growth stimulatory action to target cells. In the present study using the long-term cultivation of estrogen-dependent MCF-7 breast cancer cells in steroid-free medium, we have established a cell subline, designed as MCF-7/LS, which was characterized by the resistance to growth stimulatory estradiol action and hypersensitivity to estrogen-induced apoptosis. We have demonstrated that estrogen treatment of the cells does not influence on the level of TNF-R1 or Fas, but dramatically decreases the transcriptional activity of NF-κB. Importantly, the MCF-7/LS cells, which are insensitive to growth stimulatory estrogen action, retain the ability to decrease in the NF-κB activity in response to estrogen stimulus. Furthermore, the significant increase in the basal (in the absence of ligand) estrogen receptor (ER)-dependent transcriptional activity in the MCF-7/LS cells was revealed and reciprocal transcriptional antagonism between ER and NF-κB was demonstrated. Finally, we proved the possible involvement of phosphatidylinositol-3 kinase (PI3K) in the ligand-independent ER activation. In general, the results presented suggest that long-term growth of MCF-7 breast cancer cells in steroid-free medium is accompanied with the increase in the basal ER-dependent transcriptional activity as well as the maintenance of the negative regulatory loop ER–NF-κB. The latter may be considered as one of the factors resulting in a disbalance between pro- and anti-apoptotic pathways and enhancement in estrogen apoptotic action in the cells.

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The efficiency of endocrine therapy of tumors, including breast cancer, is limited by development of hormone-independent tumors which are resistant to antiestrogens initially or acquire resistance *de novo* during the therapy with antiestrogens (tamoxifen, raloxifene) [1-5]. The classic scheme of hormonal signal transmission in cells includes the following stages [1, 4, 6-8]: hormone binding to specific receptor, dimerization and phosphorylation of the receptor, translocation of the complex into the cell nucleus, and receptor binding with specific DNA sequences. The activated hormone–receptor complex functions not only as a transcriptional factor, but also as a signaling protein that interacts with other signaling molecules located in the cytoplasm (growth factor receptors, regulatory proteins of the phosphatidylinositol-3 kinase (PI3K) cascade, etc.) and, thus, influences the efficiency of major mitogenic and apoptotic signaling pathways of the cell [1, 3, 4, 9-12]. The development of hormonal resistance of tumor cells can be associated with disorders or changes in any stage of the hormone action. It has been shown that constitutive activation of hormone-independent mitogen-transducing pathways (including the hyperexpression of receptor tyrosine kinases, the cell cycle proteins) is among the possible mechanisms controlling growth of antiestrogen-resistant breast cancer cells [1-5].

Abbreviations: ER) estrogen receptor; ERE) estrogen responsive element; MTT) 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; PI) propidium iodide; PI3K) phosphatidylinositol-3 kinase; PTEN) phosphatase dephosphorylating 3-OH-phosphoinositides; TNF-α) tumor necrosis factor α; TNF-R1) type I tumor necrosis factor receptor.

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Recently it was described the phenomenon of the paradoxical sensitization of the antiestrogen-resistant breast cancer cells to estrogen-induced apoptosis [13-18]. Paradoxical induction of apoptosis by estrogens has been reported for estrogen-deprived breast cancer cells and/or breast tumors after tamoxifen or raloxifene long-term treatment. Several observations revealed that long-term estrogen deprivation as well as the retained high level of estrogen receptors (ER) may play a causal role in the acquisition of the cell response to estradiol-induced apoptosis [13, 14, 16, 19]. These findings correlate with the clinical and experimental data demonstrating that estrogen treatment of antiestrogen-resistant breast tumors can cause tumor regression in vivo [13, 14, 16, 17, 19-24]. Taken together, these facts demonstrate that cell sensitization to estrogen apoptotic action may be considered as one of the possible ways of breast tumor progression under conditions of estrogen withdrawal and/or long-term treatment with antiestrogens.

It should be noted that the ability of sex steroid hormones to stimulate apoptotic signaling proteins (p53, Fas/FasL) has been known for a long time [17, 25-27], but in the hormone-dependent cells this effect of steroids is poorly expressed and fully compensated by the proliferative action of the hormones. Estrogen-induced apoptosis is shown to develop with involvement of proapoptotic proteins Fas, Bax, and Bim, with a subsequent activation of caspases 7 and 9 [13, 16, 28], but the molecular mechanism of sensitization of breast cancer resistant cells to the apoptotic action of estrogens remains unclear.

We found earlier that development of resistance of breast cancer cells MCF-7 to the antiestrogen tamoxifen was associated with cell sensitization to estrogen-induced apoptosis [18]. It was also shown that the activation of mitogenic signaling pathways specific for the tamoxifen-resistant cells was not sufficient for cell sensitization to estrogen-induced apoptosis. We revealed that cell sensitization to estrogen-induced apoptosis emerged later, possibly independently of the cell ability to estrogen-independent growth, and required a long-term cultivation in a steroid-free medium. Estradiol stimulated the expression of p53 and suppressed the DNA-binding activity of NF-κB, suggesting that these signalings might be involved in estrogen apoptotic action.

The purpose of the present work was to study the sensitization of the MCF-7 cells to estrogen-induced apoptosis during cultivation in steroid-free medium and elucidate the role of NF-κB and estrogen receptor signaling in the regulation of cell sensitivity to the apoptotic action of estrogens.

MATERIALS AND METHODS

Cell culture. The human breast cancer cell line MCF-7 was cultured in standard DMEM medium supplemented with 5% fetal calf serum (FCS) (PAA, Austria) and gentamicin (50 U/ml) (Paneco, Russia) at 37°C and 5% CO_2. The subline MCF-7/LS was developed by long-term (60 days) cultivation of the parental cell line MCF-7 in phenol red-free DMEM medium supplemented with 5% steroid-free fetal serum. The steroid-free serum was prepared by treatment of fetal serum with dextran-coated charcoal (Sigma-Aldrich, USA), according to the routine method described in [29]. The cell growth was evaluated by the MTT-test based on the accumulation by living cells of a fluorescent MTT reagent (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) [30].

Cell extract preparation and immunoblotting. To perform immunoblotting, the cells at the stage of 80% monolayer were removed from the dishes with 1 ml of phosphate buffer. Then the samples were lysed and placed onto nitrocellulose membranes (Amersham, Great Britain), as described in [1]. Antibodies to ER-α (E-1396; Sigma-Aldrich) and TNF-R1 (type I tumor necrosis factor receptor) (#AF225; R&D Systems, USA) were used for immunoblotting. To prevent nonspecific protein binding, the filters were treated with 5% fat-free milk (Nestle, France) solution and then incubated with primary antibodies for 3 h at room temperature. The filters were washed and incubated for 1.5 h with secondary antibodies conjugated with peroxidase, and the signals were detected using chemiluminescent substrate (Amersham).

ER binding assay. The binding ability of estrogen receptors was determined by a modified radioligand method described in [31]. The specific binding was determined at the saturating concentration of ^{3}H-labeled 17β-estradiol (specific radioactivity 85-95 Ci/mmol; GE Healthcare, Great Britain) in the presence or absence of 200-excess unlabeled hormone (diethylstilbestrol; Sigma-Aldrich). Cells were incubated with the hormone for 1.5 h at 37°C and 5% CO_2, washed twice in phosphate buffer and treated with ethanol; after that ethanol extracts were transferred to scintillation vials for analysis in a LS 6500 β-counter (Beckman, Germany).

Transient transfection and measurement of reporter gene activity. Cells were transfected with a plasmid containing cDNA of PTEN (phosphatase dephosphorylating 3-OH-phosphoinositides) for 4 h at 37°C using lipofectamine (Life Technologies-BRL, USA). The mean efficiency of gene transfection with this reagent was 25-30%. To determine the transcriptional activity of ER and NF-κB, the cells were transfected with the plasmids containing luciferase reporter gene controlled by the promoter with estrogen-responsive and NF-κB-responsive elements, respectively. The plasmids used in this work were kindly provided by Reid [32], Gasparian [33], and Chan [34]. To control the efficiency and potential toxicity of the transfection, the cells were transfected with the β-galactosidase plasmid. All subsequent experiments were performed during 48 h after the transfection. The

luciferase activity was measured according to a standard protocol (Promega, USA) using a Turner BioSystems 20/20^n luminometer (USA). The luciferase activity was calculated in arbitrary units evaluated as the ratio of the luciferase activity to the galactosidase activity.

Assessment of Fas expression. The percentage of Fas-positive cells was evaluated by indirect immunofluorescence flow cytometry. The cells were incubated for 30 min at 20°C with primary antibodies to CD95/Fas (ICO160) (MedBioSpektr, Russia), washed twice in phosphate buffer, and then incubated for 30 min at 4°C with secondary antibodies (MedBioSpektr) conjugated with fluorescein isothiocyanate (FITC). Then the cells were washed twice in phosphate buffer and stored in 1% formalin until the analysis by flow cytofluorimetry. The samples were analyzed in a FACSCalibur cytometer (Becton Dickinson, USA).

Assessment of apoptosis. The apoptosis was determined by flow cytometry using staining with propidium iodide (PI) (Sigma-Aldrich). Cells were fixed in 70% cold ethanol, centrifuged and resuspended in 1 ml of solution containing PI (5 μg/ml), 0.1% sodium citrate, 0.1% Triton X-100, and then incubated for 15 min in the dark. Then the samples were analyzed in the FACSCalibur cytometer. The further processing of the data was performed with the WinMDI 2.9 software program (Joseph Trotter, La Jolla). The percent of apoptotic cells was determined as a pre-G1 peak in the DNA histogram [35].

Statistical analysis was performed using the Statistica 5.0 and Origin 5.1 software programs. The criterion for statistical significance was $p < 0.05$.

RESULTS

The establishment and properties of MCF-7/LS subline of breast cancer cells. The content and activity of ER in MCF-7 and MCF-7/LS cells. The MCF-7/LS cell subline was developed from the parental MCF-7 cells cultured for 60 days in phenol red-free DMEM supplemented with 5% steroid-free FCS. The comparison of growth rates of the MCF-7 and MCF-7/LS cell lines showed that the MCF-7/LS cells were less sensitive to the growth-stimulating action of estradiol and revealed relatively higher growth rate in the steroid-free medium than the parental line cells (Fig. 1a).

Determination of ER expression by immunoblotting (Fig. 1b) and of the binding ability of ERs by the standard radioligand approach (Fig. 1c) revealed a slight (25-30%) decrease in the ER level and estradiol binding in the hormone-resistant subline MCF-7/LS as compared to the MCF-7 cells. To analyze the ER-dependent promoter activity, the cells were transfected with plasmid ERE-TK-LUC containing the luciferase reporter gene controlled by the estrogen-responsive element (ERE) [32]. As revealed, despite the reduced total level of ERs in the MCF-7/LS cells, the basal (in the absence of estradiol) activity of the reporter gene in these cells was significantly higher than in the parental strain (Fig. 1d).

Sensitivity of MCF-7 and MCF-7/LS cells to estrogen-induced apoptosis. The effect of a long-term estradiol treatment on cell growth and survival was studied. As shown, 8-day treatment of the parent MCF-7 cells with 10^{-9} M estradiol markedly decreased the cell number, when tamoxifen addition partially restored the cell growth (Fig. 2a). Flow cytometry analysis revealed that the estradiol-induced suppression of the MCF-7/LS cell growth was associated with enhanced apoptosis of these cells, whereas the parental MCF-7 cells were insensitive to the apoptotic action of estradiol (Fig. 2b).

Effect of estradiol on the level of TNF-R1, Fas, and the activity of NF-κB in MCF-7 and MCF-7/LS cells. The phenomenon of reciprocal negative regulation of ER and NF-κB. Determination of the basal level of TNF-R1 by immunoblotting and of Fas expression by immunofluorescence did not reveal considerable differences between the contents of these proteins in the MCF-7/LS and MCF-7 cells. Cell cultivation in the presence of estradiol also did not markedly change the expression of TNF-R1 and Fas (Fig. 3). Importantly, the low Fas expression in the MCF-7 cells (Fig. 3b) was also noted by other investigators, indicating an insignificant activity of the FasL/Fas signaling pathway in this cell line [36].

We found earlier that MCF-7 cell sensitization to estrogen-induced apoptosis was not accompanied by changes in basal activity of NF-κB [18]. To study the effect of estradiol on NF-κB-dependent transcriptional activity, we transfected MCF-7 and MCF-7/LS cells with a plasmid containing the luciferase reporter gene controlled by the NF-κB-sensitive promoter, with subsequent treatment of the cells with estradiol for 24 h and determination of the luciferase activity according to the standard protocol [33]. Estradiol significantly decreased the reporter gene activity, and, despite the resistance to the mitogenic action of estradiol, the MCF-7/LS cells fully retained the ability to suppress the NF-κB-dependent transcriptional activity in the response to estradiol treatment (Fig. 4a).

Parallel study of the influence of estradiol on the plasmid ERE-TK-LUC transcription revealed an acute increase in the ER-dependent transcriptional activity in the presence of estradiol in both the MCF-7 and MCF-7/LS cells (Fig. 4b). Taking into account the well-known data on the reciprocal negative regulation between NF-κB and ERs [37-40], we studied the effect of tumor necrosis factor α (TNF-α), which is a specific inducer of NF-κB, on expression of the ERE-containing reporter plasmid. As revealed, treatment of the cells with TNF-α suppressed the ERE-dependent luciferase gene activity (Fig. 4c), demonstrating the existence of a negative regu-

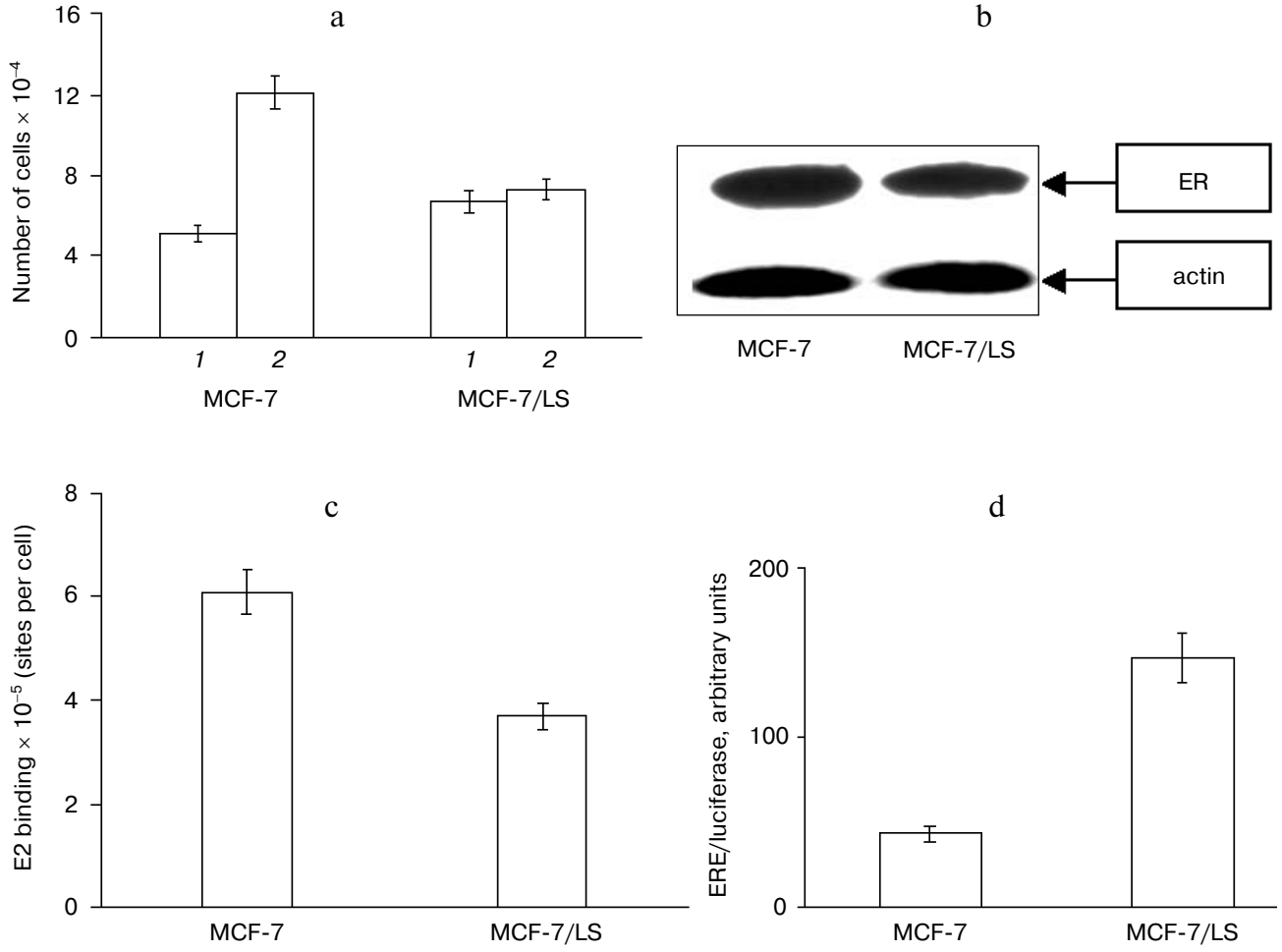

Fig. 1. Basic properties of MCF-7 and MCF-7/LS breast cancer cells. a) Effect of estradiol on growth of MCF-7 and MCF-7/LS cells. The cells were cultured in the absence (*1*) and in the presence of 10^{-9} M 17β-estradiol (*2*) for 4 days, and then the cell number was determined by the standard MTT test. b) Expression of estrogen receptors (ER) in the MCF-7 and MCF-7/LS cells. The ER contents in the cells were determined by immunoblotting. Protein loading was controlled by membrane hybridization with anti-actin antibodies. c) Binding ability of ERs in MCF-7 and MCF-7/LS cells. Specific binding was determined routinely with radioligands, using ^{3}H-labeled 17β-estradiol (E2). d) Expression of ERE-containing reporter plasmid ERE-TK-LUC in MCF-7 and MCF-7/LS cells. The cells were transfected with the ERE-TK-LUC plasmid containing the luciferase reporter gene under the control of the estrogen-responsive element. The transfection efficiency was controlled by co-transfection of the cells with plasmid containing the β-galactosidase gene. The cells were taken from the plates 24 h later, and the luciferase and galactosidase activities were determined as described in "Materials and Methods". The mean values ± standard deviations are given for three independent experiments.

latory loop with involvement of ERs and NF-κB that functions in the MCF-7 cells and retains its activity in the MCF-7/LS cells. Probably, during the long-term cultivation in the steroid-free medium (MCF-7/LS cells), the activity of this regulatory loop can be maintained, in particular, via the constitutive activation of ER.

Effect of tamoxifen on ER-dependent transcriptional activity of the cells. Addition of the antiestrogen tamoxifen to the MCF-7 and MCF-7/LS cells transfected with the ERE-TK-LUC plasmid induced a significant, more than sixfold, decrease in the estrogen-induced activity of the reporter gene and only a slight suppression of its basal activity (Fig. 5a). A weak influence of tamoxifen on the basal activity of the reporter gene was more likely to be caused by a ligand-independent activation of ERs, the process based on formation of active complexes of ER with some signaling proteins and responsible for maintaining of the minimal receptor activity in the cells in the absence of estradiol. Importantly, a relative increase in the ER-dependent transcriptional activity in the MCF-7/LS cells compared to the parental cells was also retained in the presence of tamoxifen, and this could be associated with the increased ligand-independent activation of ER in the MCF-7/LS cells.

Mechanism of ligand-independent ER activation: role of the PI3K-signaling pathway. Phosphatidylinositol-3 kinase (PI3K) and one of its main effectors, Akt/PKB, are known to directly interact with ERs with production

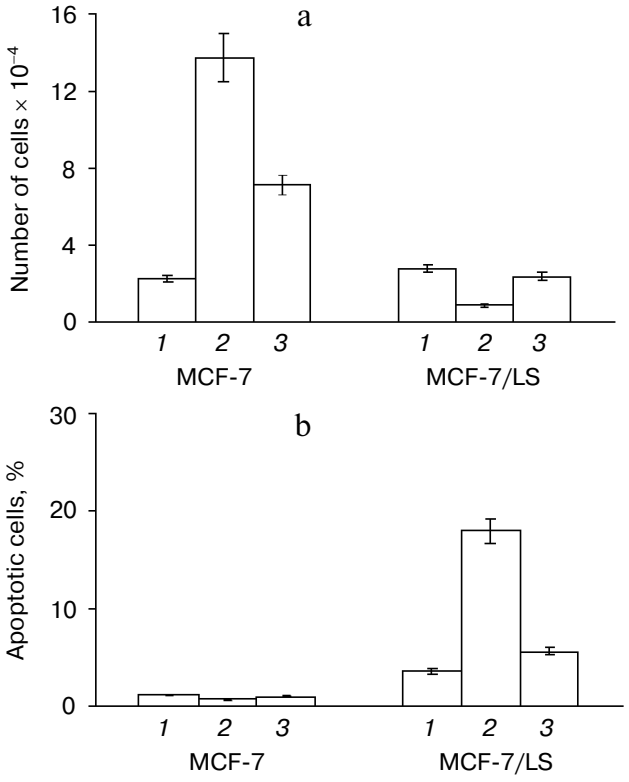

Fig. 2. Effect of estradiol on growth rate (a) and level of apoptosis (b) in MCF-7 and MCF-7/LS cells. The cells were cultured without additions (*1*), in the presence of 10^{-9} M 17β-estradiol (*2*), and 10^{-9} M 17β-estradiol + 10^{-6} M tamoxifen (*3*) for eight days, and then the number of cells was determined by the standard MTT-test (a), or the apoptosis level was analyzed by flow cytometry using staining with propidium iodide (b). The mean values ± standard deviations are given for three independent experiments.

of active complexes, and, according to some data, can be involved in the ligand-independent activation of ERs [9, 41-43]. To study the role of the PI3K-signaling pathway in the regulation of the ERE-dependent promoter expression, we co-transfected MCF-7/LS cells with the plasmid ERE-TK-LUC and the plasmid containing cDNA of PTEN, phosphatase dephosphorylating 3-OH-phosphoinositides. Transfection of the cells with cDNA of PTEN considerably decreased the reporter gene activity (Fig. 5b), and this suggested a possible involvement of the PI3K-signaling pathway in the regulation and maintaining of the ligand-independent activation of ERs.

DISCUSSION

As known, in many cases progression of breast cancer tumors to estrogen-independent phenotype is not associated with the loss of estrogen receptors [4]. The tumor resistance to antiestrogens and cell ability to estrogen-independent growth may be formed as a result of changes in the intracellular signaling pathways, including the activation of receptor tyrosine kinases, disorders in the regulation of cell cycle proteins, changes in the functional activity of ERs, etc. [1-5].

The problem of sensitization of breast cancer hormone-resistant cells to the apoptotic action of estradiol has currently attracted the attention of researchers. The long-term *in vitro* cultivation of breast cancer cells in the presence of antiestrogen or in the steroid-free medium in some cases resulted in the development of a paradoxical sensitivity of the cells to the apoptotic action of estradiol [15, 16]. The cell sensitization to the estrogen-induced apoptosis requires the long-term exposure in the absence of steroids, and occurs concurrently with acquired ability to estrogen-independent growth [13]. The retention of the high level of ERs in the cells is necessary for the cell sensitization to the apoptotic action of estradiol, and ER-α has been directly proven to be involved in the transmission of estrogen apoptotic signal [13]. The proapoptotic proteins Fas, Bax, and Bim and caspases 7 and 9 activated by estrogens are shown to be involved in the estrogen-induced apoptosis [13, 16, 28]. However, despite the

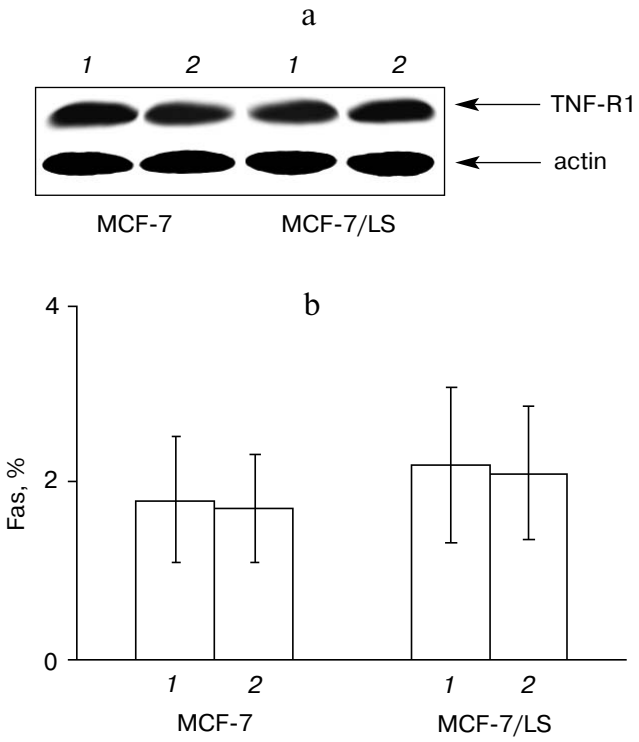

Fig. 3. Contents of TNF-R1 (a) and Fas (b) in MCF-7 and MCF-7/LS cells. a) The TNF-R1 level in the cells precultured for 24 h without additions (*1*) and in the presence of 10^{-9} M 17β-estradiol (*2*) was determined by immunoblotting. Protein loading was controlled by membrane hybridization with anti-actin antibodies. b) The percent of Fas-positive cells was determined by indirect immunofluorescence flow cytometry. The cells were cultured for 24 h without additions (*1*) and in the presence of 10^{-9} M 17β-estradiol (*2*), and then incubated with primary antibodies to CD95/Fas, stained with secondary antibodies conjugated with the fluorescent label FITC, and analyzed using a flow cytofluorimeter.

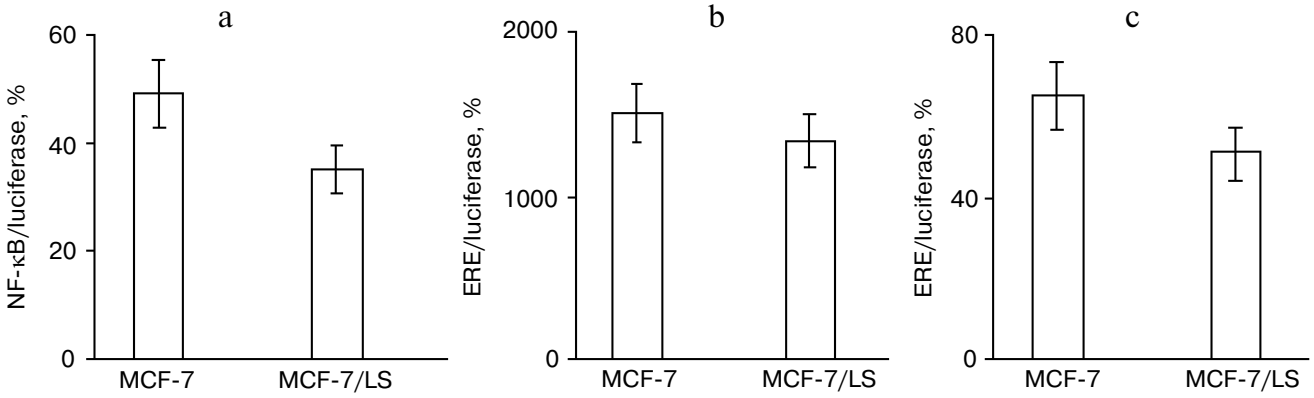

Fig. 4. Reciprocal negative regulation of NF-κB and ERs in MCF-7 and MCF-7/LS cells. a) Analysis of transcriptional activity of NF-κB in MCF-7 and MCF-7/LS cells upon treatment with estradiol. The cells were transfected with the plasmid containing the luciferase reporter gene controlled by the NF-κB-responsive element, then the cells were cultured in the absence and in the presence of 10^{-9} M 17β-estradiol for 24 h, and the luciferase activity was determined as described in "Materials and Methods". The diagram presents the luciferase activity in the estradiol-treated cells, the activity in the control cells taken as 100%. b) Effect of estradiol on the transcriptional activity of ERs in MCF-7 and MCF-7/LS cells. The cells were transfected with the ERE-TK-LUC plasmid containing the luciferase reporter gene controlled by the estrogen-responsive element, then the cells were cultured in the absence and in the presence of 10^{-9} M 17β-estradiol for 24 h, and the luciferase activity was determined. The diagram presents the luciferase activity in the estradiol-treated cells, 100% being the luciferase activity in the control cells. c) Effect of TNF-α (tumor necrosis factor α) on expression of the ERE-containing reporter plasmid. The MCF-7 and MCF-7/LS cells transfected with the ERE-TK-LUC plasmid were incubated in the absence or in the presence of TNF-α (10 ng/ml) for 24 h, and the luciferase activity was determined. The luciferase activity in the control cells is taken as 100%. The mean values ± standard deviations are given for three independent experiments.

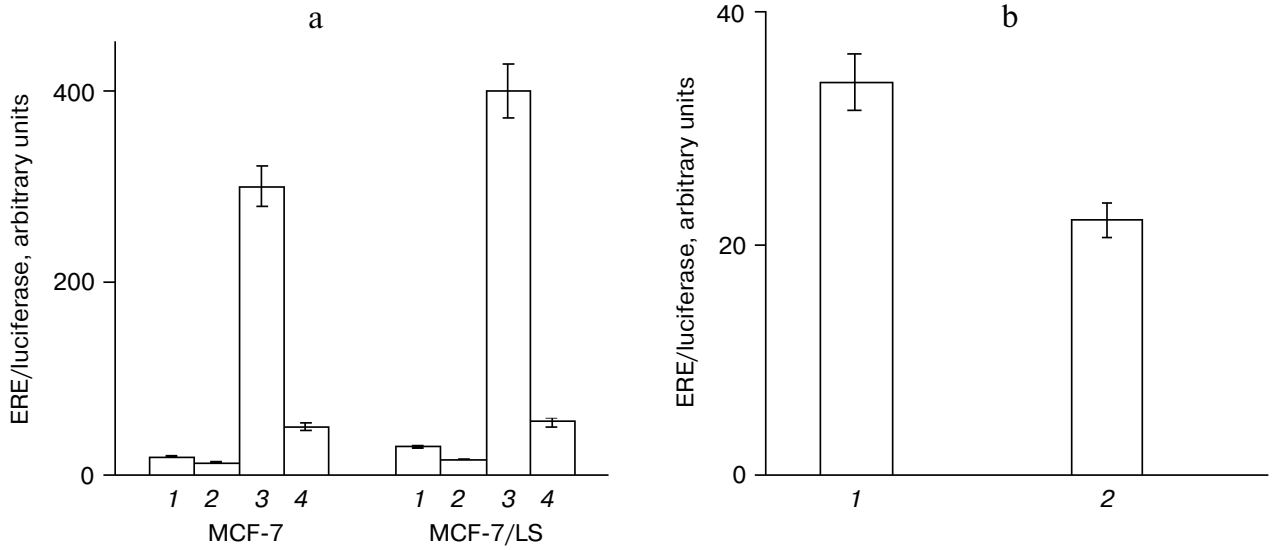

Fig. 5. Regulation of ER-dependent transcriptional activity. a) Effect of tamoxifen on the transcriptional activity of ERs. MCF-7 and MCF-7/LS cells transfected with the ERE-TK-LUC plasmid were incubated without additions (*1*), in the presence of 10^{-6} M tamoxifen (*2*), 10^{-9} M 17β-estradiol (*3*), and 10^{-9} M 17β-estradiol + 10^{-6} M tamoxifen (*4*) for 24 h, and then the activity of luciferase was determined. b) Effect of transfection with PTEN on expression of the ERE-containing reporter plasmid in the MCF-7/LS cells. The MCF-7/LS cells were co-transfected with the ERE-TK-LUC plasmid and the plasmid containing the control vector pcDNA3 (*1*) or cDNA of PTEN (*2*). The luciferase activity was determined in the cells 24 h later by the standard protocol. The mean values ± standard deviations are given for three independent experiments.

achievements in the study of the estrogen apoptotic action, the mechanism of the breast cancer cell sensitization to the estrogen-induced apoptosis remains unknown. What occurs with the tumor cells during the sensitization, what are the metabolic systems resulting in the development of the paradoxical response of the cells to estrogen and their death? These questions are still open.

In the present work, we demonstrated that cultivation of MCF-7 cells in steroid-free medium for two months was sufficient for their sensitization to the apoptotic action of estradiol. The established cell subline, MCF-7/LS, generated through 2-month exposure in steroid-free medium, was characterized by the low sensitivity to the growth-stimulating action of estradiol if compared with wild-type MCF-7 cells. Transfer of the MCF-7/LS cells into medium containing 17β-estradiol at the physiological concentration of 10^{-9} M resulted in the development of apoptosis after eight days of the cell cultivation in the presence of estrogen.

The study on the influence of estradiol on the level and activity of the major components of the NF-κB signaling pathway revealed that estradiol had no effect on the level of the TNF-R1 and Fas receptors but significantly suppressed the NB-κB-dependent transcriptional activity of the cells. Despite the resistance to the growth-stimulating action of estradiol, the MCF-7/LS cells retained the ability to suppress the NF-κB-dependent transcription in response to estradiol. Importantly, the ability of estradiol to suppress the transcriptional activity, and, according to some data, the NF-κB expression, is well known and has been described for various experimental models [15, 38, 44, 45]. Moreover, several reports demonstrated the existence of the reciprocal antagonism between NF-κB and ERs based on the formation of inactive complexes between these proteins and/or the competition for binding with common activator proteins, such as CBP/p300 [38, 44, 46].

The further analysis of the functional activity of ERs was performed using three parameters: ER content in the cells, ligand-binding activity of ERs, and expression of the ERE-TK-LUC plasmid containing the luciferase reporter gene under the control of the estrogen-responsive element (ERE). The ERs are major and specific activators of ERE-containing promoters, and, although some receptor-related proteins can additionally activate ERE [47, 48], the determination of expression of the ERE-dependent reporter genes is a widely used approach for assessment of the transcriptional activity of ERs. We found a marked increase in the expression of the ERE-containing promoter in the MCF-7/LS cells that occurred in parallel with a slight decrease in the concentration and ligand-binding ability of the ERs. The addition of the antiestrogen tamoxifen had no effect on the transcription of the ERE-containing reporter plasmid, suggesting that the receptor could be activated in the MCF-7/LS cells independently of estrogen.

In total, our findings confirmed observations of other authors who had shown the estrogen-independent activation of ERs in cells resistant to the mitogenic action of estradiol [11, 49]. Considering the abovementioned ability of estradiol to suppress the activity of NF-κB, we suggest that the constitutive increase in the basal transcriptional activity of ERs can be one of factors providing for the functioning of the negative regulatory loop ER–NF-κB and increasing the apoptotic effect of estradiol on MCF-7/LS cells.

At present, the mechanism of the ligand-independent activation of ERs is not clear, but the basal activity of ERs is established to be maintained by some proteins of the PI3K-signaling pathway, in particular Akt/PKB, which are capable of producing active complexes with ERs in the absence of estradiol [9, 41-43]. In our experiments the transfection of MCF-7/LS cells with cDNA of PTEN (phosphatase dephosphorylating the products of the PI3K enzymatic activity, 3-OH-phosphoinositides) markedly decreased the ER-dependent transcriptional activity. In general, these findings demonstrated the possible involvement of the PI3K-signaling pathway in the maintaining of the ligand-independent activation of ERs, and further studies are required for more close characterization of the interaction mechanism of the PI3K-signaling pathway proteins with ERs, in particular, during the breast cancer cell sensitization to the estrogen-induced apoptosis.

Thus, in the present work we have shown that the increase in expression of the ERE-containing promoter, as well as unaffected cell capability for estrogen-dependent suppression of NF-κB, can be considered as a specific features which determine the sensitization of breast cancer cells to estrogen-induced apoptosis. Most likely, the unaffected ability for estrogen-dependent suppression of NF-κB is far from being the only factor determining the cell sensitization to estrogen-caused apoptosis. We suggest that an imbalance between the pro- and anti-apoptotic pathways is crucial for the cell sensitization to estrogen-induced apoptosis, and the estrogen-dependent suppression of NF-κB provokes further disturbance of such a balance and, as a result, stimulates the cell entry in apoptosis. The existence of this imbalance is indirectly confirmed by the enhanced sensitivity of the cells to some proapoptotic agents, which develops in parallel with the sensitization to the apoptotic action of estradiol [18]. We anticipate that further investigations will allow us to establish in detail the mechanism of tumor cell sensitization to the apoptotic action of estrogens and identify the major intracellular proteins involved in transmission of the apoptotic signal from estrogens.

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