ISSN 0026-8933, Molecular Biology, 2019, Vol. 53, No. 3, pp. 442–451. © Pleiades Publishing, Inc., 2019.
Russian Text © The Author(s), 2019, published in Molekulyarnaya Biologiya, 2019, Vol. 53, No. 3, pp. 502–512.

MOLECULAR CELL BIOLOGY

UDC 577.218

BRCA1 and Estrogen Receptor α Expression Regulation in Breast Cancer Cells

A. M. Scherbakov[a], E. A. Shestakova[a, *], K. E. Galeeva[a], and T. A. Bogush[a]

[a] *Blokhin National Medical Research Center of Oncology of the Ministry of Health of the Russian Federation, Moscow, 115478 Russia*

**e-mail: elenaanshestakova@mail.ru*

Received November 9, 2018; revised January 17, 2019; accepted January 23, 2019

Abstract—BRCA1 (breast cancer 1) protein is involved in the genome stability maintenance participating in homologous recombination-dependent DNA repair. Disruption of BRCA1 functioning is associated with breast and ovarian cancer. Despite the important role of BRCA1 in DNA repair in all cell types, the development of BRCA1-associated cancer takes place mainly in estrogen-dependent tissues such as breast and ovarian ones. Using breast cancer cell line MCF-7 it was demonstrated in in vitro experiments that the estrogen 17β-estradiol (E2), phytoestrogens (genistein and apigenin) and antiestrogens (tamoxifen and fulvestrant) inhibited estrogen receptor α (ERα) expression while only genistein influenced BRCA1 increasing its expression. In hypoxia, that is an important factor of solid tumors progression, the decrease of BRCA1 and ERα expression was demonstrated in MCF-7 cells. Therefore, hypoxia influences both BRCA1-dependent DNA repair and hormonal regulation of breast cancer cell growth. Taken together, obtained results demonstrate a relationship between BRCA1 and steroid hormones signal transduction pathways in breast cancer cells and point out to the importance of complex BRCA1 and ERα expression regulation mechanisms studies including epigenetic gene expression regulation.

Keywords: BRCA1 protein, estrogen receptors, breast cancer, MCF-7, flow cytometry, hypoxia, phytoestrogens, estrogens

DOI: 10.1134/S0026893319030166

INTRODUCTION

BRCA1 (breast cancer 1) gene product, BRCA1 protein, participates in genome stability maintenance being involved in DNA repair mainly through homologous recombination [1–3] as well as via non-homologous end-joining [4]. It is a protein with molecular mass of 220 kDa whose expression is regulated with the participation of epigenetic mechanisms [1, 5, 6].

BRCA1 function disruption is associated with the development of several cancers, in particular breast cancer (BC) and ovarian cancer. Despite an important role of *BRCA1* gene mutations in the development of hereditary forms of BRCA1-associated oncological diseases [7, 8], sporadic forms of BRCA1-associated cancers, except small number of cases induced by somatic mutations [9], are determined with the epigenetic regulation of *BRCA1* gene expression [5].

Epigenetic mechanisms of *BRCA1* gene expression regulation are described in comprehensive reviews [5, 6]. Currently, several major molecular mechanisms of BRCA1 function disruption are known: (1) epigenetic inhibition of BRCA1 mRNA and protein synthesis, due to CpG-islands methylation in *BRCA1* gene promoter [10, 11], (2) BRCA1 expression changes resulted from covalent histone modifications in *BRCA1* gene [12, 13] and (3) regulation with transcription factors [14]. It is worth to mention also the loss of heterozygosity (LOH) leading to complete absence of both BRCA1 mRNA and protein [15], amplification of *BRCA1* gene [16] and aberrant increase in *BRCA1* functioning [3].

The decrease of BRCA1 protein level taking place at hypoxia, epithelial-mesenchymal transition (EMT) is accompanied by histone modifications in *BRCA1* gene promoter [12, 13]. The inhibition of *BRCA1* gene expression at hypoxia depends on series of modifications in the histone H3: the demethylation of Lys in position 4 (H3K4me), the deacetylation of H3K9ac, coupled with the methylation of H3K9 [12]. At the EMT the diminution of *BRCA1* gene expression is linked with the demethylation of H3K4me2 [13].

Despite the key role of BRCA1 in DNA repair in all cell types, the development of BRCA1-associated cancer is observed mainly in estrogen-dependent tis-

Abbreviations: AB, antibodies; IF-FC, immunofluorescent method associated with flow cytometry; BC, breast cancer; ERα, estrogen receptor α; *BRCA1* (breast cancer 1), tumor suppressor gene *BRCA1*; BRCA1 (breast cancer 1), tumor suppressor protein BRCA1; TPZ, tirapazamine.

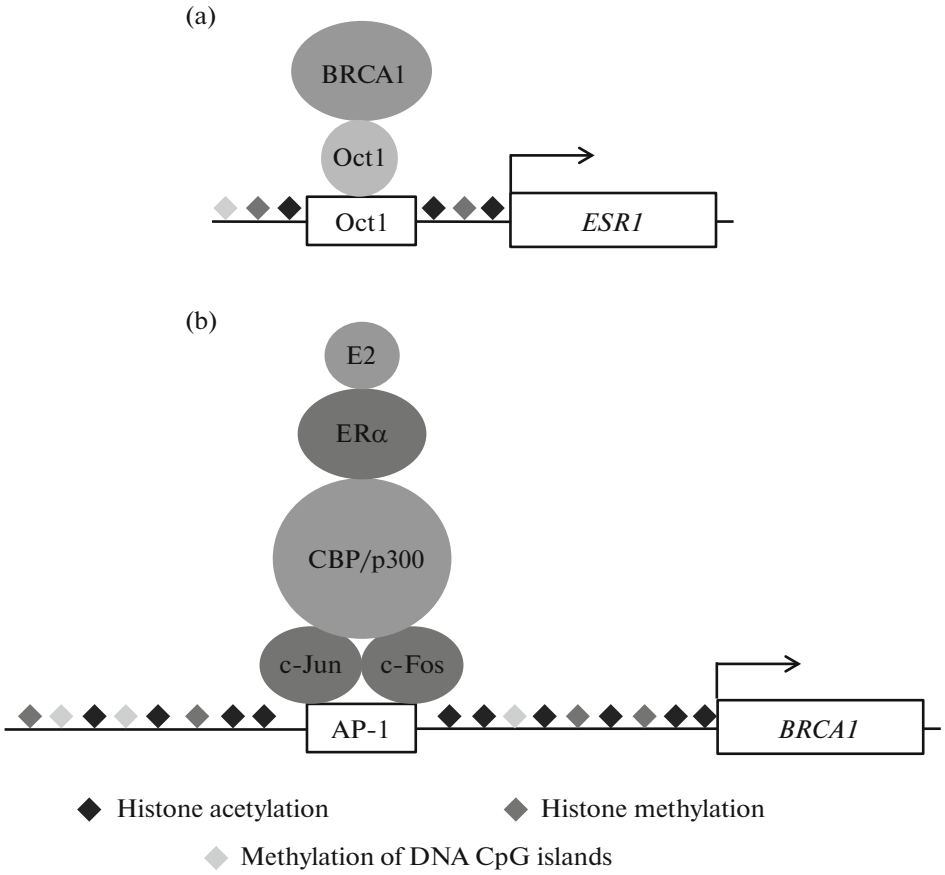

Fig. 1. Transregulation of BRCA1 and ERα expression. (a) Gene *ESR1* coding for ERα is a target of transcriptional coactivator, BRCA1. BRCA1 and transcription factor Oct-1 activate transcription of *ESR1* gene. (b) *BRCA1* gene is a target of ERα and is regulated with estrogen activated ERα in complex with transcription factor AP-1 (c-Jun/c-Fos) and transcriptional coactivator, histone acetyltransferase CBP/p300.

sues such as breast and ovarian ones. BRCA1 and estrogen receptor α (ERα) expression crosstalk described in literature both at the level of transcription and at the level of post-translation protein modifications point to possible reasons of BRCA1-associated cancer tissue specificity. From one hand, the gene coding ERα, *ESR1*, is a target of the transcription coactivator—BRCA1 (Fig. 1a). In relation with this, the quantity of ERα transcripts synthesized from *ESR1* gene is proportional to the amount of non-mutated functional BRCA1 protein. BRCA1 in complex with transcription factor Oct-1 activates *ESR1* gene transcription in MCF-7 and T47D BC cell lines (Fig. 1a) [17]. From another hand, *BRCA1* gene being a target of ERα is regulated by ERα in complex with transcription factor AP1 (c-Jun/c-Fos) and coactivators—histone acetyltransferases CBP/p300 (Fig. 1b) [18–20]. As a result, breast tumors with low level of BRCA1 protein usually contain also small quantities of ERα [17]. Therefore, BRCA1 protein function disruption caused by epigenetic inhibition of *BRCA1* gene leads to the reversal of ERα status in tumor from positive to negative and to the development of BRCA1-associated ERα-negative tumors failed to be cured with targeted antiestrogen therapy.

Besides the regulation on the gene level, BRCA1 inhibits ERα activity by protein-protein interactions – as a result of BRCA1-dependent receptor ubiquitination and inhibition of its acetylation [21]. Altogether, it leads to the deregulation of ERα-dependent genes. One of the ERα targets is a progesterone receptor (PR) gene, whose deregulation causes the development of several BC subtypes [22–24]. Moreover, *ESR1* gene is a target of its own product, ERα, as well as of ERβ [25]. It is worth to note that Oct-1 and ERβ, that regulate *ESR1* gene, have similar structure because they are recognized with monoclonal antibody (AB) 14C8 widely used for ERβ detection in tumor tissue and tissue culture cells [26].

Ligands, that bind and influence ERα activity, include estrogens in most cases activating ERα, such as 17β-estradiol (E2), and phytoestrogens, for example, genistein, as well as antiestrogens inhibiting ERα, among them are tamoxifen and fulvestrant.

Prognostic and predictive value of BRCA1 protein and of another DNA repair protein, ERCC1, was

demonstrated in recent research [5, 6, 27]. Analyzing BRCA1 expression levels in ER-negative and ER-positive tumors Bogush and co-authors [28] revealed that decreased BRCA1 expression level could be prognostic marker of unfavorable BC course. In this study for precise quantitative estimation of BRCA1 protein expression level in BC tissue we applied immunofluorescent method associated with flow cytometry (IF-FC). This method was improved and routinely used in our laboratory for quantitative estimation of protein oncomarker expression including estrogen receptors, DNA repair marker ERCC1, markers of multiple drug resistance [29–31].

In this research we studied the influence of (phyto)estrogens and antiestrogens on BRCA1 and ERα expression in MCF-7 BC cells and determined the expression of these proteins in hypoxia conditions.

EXPERIMENTAL

Cells. MCF-7 cells obtained from American Type Culture Collection (ATCC, USA) were kept in cryobank of National Medical Research Center of Oncology before experimental usage. The identity and the stability of cell line were confirmed using the analysis of short tandem repeats (GORDIZ, Russia). Cells were cultivated in DMEM media containing 10% of fetal bovine serum (HyClone, USA), 50 units/mL gentamicin (Paneco, Russia) and 0.1 mg/mL of Na pyruvate (Santa Cruz, USA) at 37°C, 5% CO_2 and 80–85% humidity. In all experiments cells in logarithmic growth phase were used.

ERα and BRCA1 expression analysis in MCF-7 cells. In the experiments on the influence of estrogens and antiestrogens on ERα and BRCA1 expression MCF-7 BC cell line was cultivated in DMEM medium without phenol red and with the addition of fetal bovine serum without steroids (Cat. # SH30068/03, HyClone, USA). Incubation of cells with estrogens and antiestrogens were carried out during 24 hours. In experiments 10-nM 17β-estradiol (E2; Cat. # E2758; Sigma-Aldrich), 5-μM tamoxifen (Cat. # 13258; Cayman Chemical, USA), 0.1 μM fulvestrant, genistein (concentration 0.5, 10, 15, 20 and 30 μM) and apigenin (concentration 15 and 30 μM) were used. Cell suspensions were incubated with primary mouse monoclonal anti-BRCA1 AB (0.006 μg/mL; Cat. # SD118, Calbiochem) or primary rabbit monoclonal anti-ERα AB (0.008 μg/mL or 0.032 μg/mL; Cat. # ab27614, clone SP-1, Abcam) during 15–20 hours and with corresponding secondary fluorescent AB (DyLight650, Cat. # ab98729 and Cat. # ab98510; Abcam) during 1.5 hour. Cell fluorescence was measured using Navios flow cytometer (Beckman Coulter, USA).

In experiments analyzing the influence of hypoxia on marker expression the cells were incubated in DMEM media containing 10% fetal bovine serum during 24, 96, 144 or 240 hours in the atmosphere containing 1% O_2.

In experiments studying the effect on marker expression of tirapazamine (TPZ)—substance activated in hypoxia conditions and inducing DNA double strand breaks—cells were incubated in hypoxia conditions during 72 hours, then in the presence of 5 or 10 μM TPZ in hypoxia during 24 hours. The cells incubated in normal conditions with the same TPZ concentrations and during the same time were used as a control. Marker expression levels in cells were determined using IF-FC method as described earlier.

Results treatment. BRCA1 and ERα expression parameters were obtained using FlowJo 10.0 program (https://www.flowjo.com) and statistical method of Kolmogorov–Smirnov [32]. Marker expression was estimated according to following parameters: (1) the level (L) was determined as a content (in percentages) of specifically fluorescent cells comparatively to the control (incubation with secondary AB); (2) the intensity (I)—as the ratio of specific cell fluorescence in treated sample to the control; (3) the index/the product (Pr)—as the product of marker expression L and I divided by 100.

RESULTS

Phytoestrogen Influence on BRCA1 and ERα Expression

The research of phytoestrogens (genistein and apigenin) influence on BRCA1 and ERα expression was performed on MCF-7 hormone-dependent BC cell line. BRCA1 protein expression was analyzed in conditions characterized with the changes in gene expression and chromatin epigenetic modifications (histone proteins and DNA). Phytoestrogens dependent *BRCA1* gene activation belongs to these conditions [33–35].

It was demonstrated that BRCA1 and ERα expression changes depending on the dose of phytoestrogen genistein. Genistein in low concentration (0.5 μM) induced the increase of BRCA1 expression index in 1.7 fold (Table 1). At higher genistein concentration (10 μM) BRCA1 expression index increased in 1.3 fold, whereas the increase of genistein concentration up to 20 μM did not change BRCA1 expression index.

The effect of genistein on ERα expression was reverse (Table 1). At phytoestrogen concentration changes 0.5 → 10 → 20 μM the diminution of ERα expression index in 2.9, 3.9 and 4.0 folds, respectively, was revealed with the maximal genistein influence at the concentration of 20 μM. Therefore, the genistein action on ERα expression is opposite and more pronounced than the one on BRCA1.

It is important to note also the qualitative differences of genistein influences on BRCA1 and ERα expression indexes. As it was mentioned earlier, the expression index is an integral parameter of marker expression that includes the content (in percentages)

Table 1. (Phyto)estrogen and antiestrogens influences on BRCA1 and ERα expression in MCF-7 BC cells

Compound, concentration	Changes of marker expression parameter*					
	BRCA1			ERα		
	L	I	Pr	L	I	Pr
Genistein, μM						
0.5	1.2↑	1.4↑	1.7↑	1.2↓	2.4↓	2.9↓
10.0	1.1↑	1.2↑	1.3↑	1.3↓	3.0↓	3.9↓
20.0	1.0	1.0	1.0	1.3↓	3.1↓	4.0↓
Apigenin, μM						
15.0	1.1↓	1.1↓	1.2↓	1.6↓	2.9↓	4.6↓
Tamoxifen, μM						
5.0	1.0	1.1↑	1.1↑	1.0	1.2↓	1.2↓
17β-Estradiol, nM						
10.0	1.0	1.1↑	1.1↑	1.1↓	3.7↓	4.1↓
Fulvestrant, μM						
0.1	1.1↓	1.0	1.1↓	1.2↓	4.5↓	5.4↓

* Cells were incubated with all compounds during 24 hours. Designations in this and other tables: L, level; I, intensity; Pr, product (index); ↓, the decrease of expression parameters comparatively to control; ↑, the increase of expression parameters comparatively to control. Typical results of three experiments are presented.

of marker expressing cells and marker average quantity in particular cells. The decrease of ERα expression index was caused by the change of the marker expression intensity and to a lesser extend—of the marker expression level. In case of BRCA1 the effect of phytoestrogen on both parameters was revealed. In other words, the genistein induced increased protein expression not only in BRCA1-positive cells but also in initially BRCA1-negative ones. This observation corresponds to earlier published results obtained with the method of immunoblotting and concerning BRCA1 expression activation induced with the genistein at low concentrations [33]. In Table 1 it is shown that the apigenin (the phytoestrogen of flavone type) did not change BRCA1 expression but considerably decreased the ERα one. The incubation of cells with apigenin at 15 μM concentration led to the decrease of ERα expression index in 4.6 folds; the increase of apigenin concentration up to 30 μM did not change ERα expression index.

Based on the obtained results it is possible to suggest that the mechanisms of two phytoestrogens influence on BRCA1 expression are different, because the genistein augmented BRCA1 expression, whereas the apigenin did not cause the effect on it. Both the genistein and the apigenin diminished ERα expression.

On the grounds of recent literature data and of our results it is possible to conclude that phytoestrogens are highly cytotoxic for BC cells [36–38]. For example, the phytoestrogen genistein has cytotoxic activity towards BC cells of different molecular subtypes and is considered as perspective cytostatic and proapoptotic agent [36, 37]. Currently, the question of genistein usage in complex cancer therapy is discussed [39]. In research centers all over the world the clinical trials are held dealing with this phytoestrogen usage as a perspective antitumor compound and a mean of supportive care (following number of studies www.clinicaltrials.gov: NCT01126879, NCT02336087, NCT00078923, NCT00276835).

In this study we have shown that the genistein influence on hormone-dependent BC cells is partially realized through the enhancement of tumor suppressor BRCA1 expression.

Estrogen and Antiestrogen Influence on BRCA1 and ERα Expression

The research of the effect of the estrogen 17β-estradiol (E2) and antiestrogens, tamoxifen and fulvestrant, on BRCA1 and ERα expression was carried out on the same MCF-7 hormone dependent BC cell line. BRCA1 expression was analyzed in the conditions accompanied with the activation or the inhibition of ERα.

As it is shown in Table 1, the antiestrogen tamoxifen slightly changed ERα and BRCA1 expression parameters. The incubation of the cells with E2 or with the fulvestrant—ERα irreversible inhibitor inducing receptor degradation—did not reveal significant changes of the level, the intensity and the index of BRCA1 expression comparatively to the control (Table 1, Fig. 2).

On the contrary, ERα expression parameters in these conditions considerably decreased. Upon the incubation with E2, ERα expression intensity and index diminished in 3.7 and 4.1 folds, respectively, and

at the fulvestrant influence—in 4.5 and 5.4 folds, respectively. It is necessary to underline that in both cases the decrease of ERα expression index was caused by the reduction of ERα expression intensity; at the same time the marker expression level was slightly lowered (Table 1). In other words, the incubation of cells with the estrogen or irreversible ERα inhibitor does not change the number of cells expressing ERα, but leads to the pronounced decrease of intracellular marker content.

Taken together, E2 and antiestrogens, fulvestrant and tamoxifen, did not influence BRCA1 expression determined using IF-FC method in MCF-7 cell line. Concerning ERα expression, E2 and the fulvestrant considerably reduced its expression, whereas the tamoxifen inducing the stabilization of inactive ERα in the cytoplasm almost did not change the marker expression parameters.

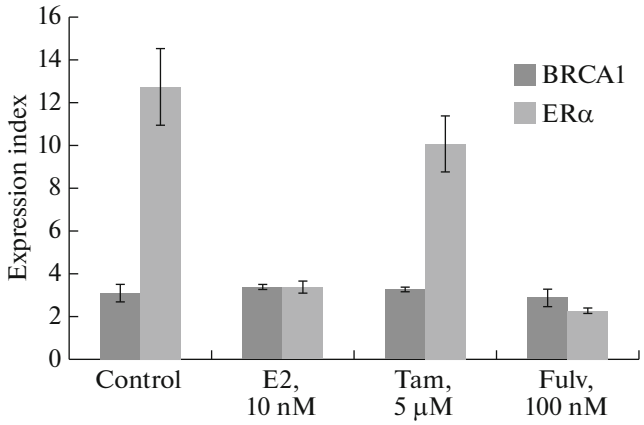

Fig. 2. Estrogen 17β-estradiol (E2), antiestrogens tamoxifen (Tam) and fulvestrant (Fulv) influence on BRCA1 and ERα expression in MCF-7 cells. E2, tamoxifen and fulvestrant did not change BRCA1 expression, whereas E2 and fulvestrant considerably decreased ERα expression.

Hypoxia Influence on BRCA1 and ERα Expression

Usually solid tumor progression is accompanied with gradual decrease of tumor tissue oxygenation called hypoxia. According to the literature, the growth of solid tumors in hypoxia conditions is characterized by cell metabolism changes and their reduced sensitivity to radio- and chemotherapy [40–43]. We demonstrated that the transfer of MCF-7 cells in hypoxia conditions (1% O_2), at the diminution of oxygen concentration from 21 to 1%, the reduction of BRCA1 expression parameters depended on the time of incubation in hypoxia (Table 2). Comparatively to the control MCF-7 cells (normoxia), in cells placed in hypoxia conditions for 24 hours BRCA1 expression level was decreased in 1.3 fold and expression index—in 1.6 fold. Incubation time increase led to the diminution of these parameters: in 96 hours BRCA1 expression index lowered in 1.7 fold, in 144 hours—in 1.8 fold and in 240 hours—in 2.1 fold.

In hypoxia conditions we also observed the decrease of ERα expression index and intensity comparatively to control cells. When cells were incubated during 240 hours in hypoxia, this integral parameter was reduced in 3.2 folds (Table 2). Summarizing obtained results, it is worth to note that the diminution of BRCA1 expression index in hypoxia conditions was caused, mainly, by the decrease of the marker expression level (that is, the content (in percentages) of cells expressing the marker), and not by that of the expression intensity which remained approximately the same at the increase of hypoxia duration (Table 2). In case of ERα the reduction of expression index in hypoxia conditions was related to the decrease of both the level and the intensity of marker expression (Table 2). Such influence of hypoxia on BRCA1 expression differs in essence from the effect of phytoestrogen genistein on the expression of this marker: in hypoxia conditions the tendency of the BRCA1 expression decrease due, mainly, to the diminution of the BRCA1 expression level was revealed, whereas at the influence of phytoestrogen genistein the increase of BRCA1 expression dependent on the augmentation of the marker expression intensity was observed. In other words, in hypoxia conditions the reduction of BRCA1 expression was caused, in major part, by the decrease of the quantity of cells expressing the marker and at the influence of phytoestrogen genistein the increase of

Table 2. BRCA1 and ERα expression in MCF-7 cells in hypoxia conditions

Time*, h	Expression parameters changes (comparative to the control)					
	BRCA1			ERα		
	L	I	Pr	L	I	Pr
24	1.3↓	1.2↓	1.6↓	1.1↓	1.3↓	1.4↓
96	1.4↓	1.2↓	1.7↓	1.0	1.0	1.0
144	1.5↓	1.2↓	1.8↓	1.1↓	1.2↓	1.3↓
240	1.9↓	1.1↓	2.1↓	1.5↓	2.1↓	3.2↓

* Time of cell incubation in hypoxia conditions (1% O_2).

Table 3. TPZ influence on BRCA1 and ERα expression in MCF-7 cells

Conditions	[TPZ], μM	Expression parameters changes (comparative to the control)					
		BRCA1			ERα		
		L	I	Pr	L	I	Pr
Normoxia	5	1.2↓	1.1↓	1.3↓	1.3↑	1.1↑	1.4↑
	10	1.1↓	1.1↓	1.2↓	1.2↑	1.0	1.2↑
Hypoxia	5	1.2↑	1.0	1.2↑	1.1↓	1.1↓	1.2↓
	10	1.4↑	1.1↑	1.5↑	2.7↓	1.5↓	4.1↓

* Cells were incubated in normoxia or hypoxia conditions during 72 hours, then TPZ was added in corresponding concentration and cells were incubated for the additional 24 hours (control cells were not subjected to TPZ treatment).

BRCA1 expression was mainly dependent on the augmentation of the marker quantity in already expressing cells.

TPZ is a cytotoxin selectively activated with the enzymes in cells at lowering of the oxygen level (hypoxia). TPZ induces DNA double strand breaks followed with the apoptosis in hypoxia conditions. This substance is considered as a candidate remedy for the therapy of solid tumor range [44]. Cell incubation with TPZ in normoxia conditions did not change considerably the BRCA1 expression index (Table 3). However, TPZ in 5 μM concentration influenced ERα expression in normoxia with the increase of expression index in 1.4 fold. Cell incubation with 5 μM TPZ in hypoxia conditions did not induce considerable changes in ERα and BRCA1 expression indexes. The augmentation of TPZ concentration up to 10 μM led to the increase of BRCA1 expression index in 1.5 fold with simultaneous decrease of ERα expression index in 4.1 fold (Table 3). The increase of BRCA1 expression index was mainly caused by the augmentation of BRCA1 expression level and not of the marker expression intensity. It means that the augmentation of BRCA1 expression during TPZ application depends, mainly, on the increase of the number of new cells expressing BRCA1. Therefore, the appearance of DNA double strand breaks in hypoxia leads not only to the translocation of BRCA1 in the DNA damage loci [3], but also to the increase of the number of cells expressing this DNA repair protein.

In case of ERα the decrease of expression index, in major part, was due to the diminution of the expression level and to a lesser extend—of the expression intensity. Therefore, at the incubation with TPZ the decrease of ERα expression is associated, mainly, with the reduction of the number of cells expressing the marker (Table 3).

DISCUSSION

Our experiments carried out in vitro demonstrated several particularities of BRCA1 and ERα expression regulation in MCF-7 BC cells. It was revealed that two studied phytoestrogens, genistein and apigenin, influenced differently on BRCA1 and ERα expression: the first one induced the increase of BRCA1 expression and the reduction of ERα expression, whereas the second one diminished ERα expression without the effect on BRCA1.

The estrogen 17β-estradiol (E2) and antiestrogen fulvestrant decreased ERα expression but did not influence BRCA1. According to the literature data [45, 46], E2 induces the diminution of ERα protein level simultaneously increasing the expression of ERα target genes: progesterone receptor (*PR*), *pS2*, *GREB1* and *SDF1*. Concerning the mechanism of E2 effect it is worth to note that ERα, as many other transcription factors, undergoes the proteolysis associated with the transcription of its target genes [47]. It is known that E2 induces Src-dependent phosphorylation of ERα at Tyr 537 leading to the association of ERα with the coactivator E6-AP, activation of ERα target genes, ubiquitination of ERα at *pS2* and *GREB1* gene promoters due to the ubiquitin-ligase activity of E6-AP and following proteasome degradation of ERα [47]. In earlier works it was demonstrated that E2 activates ERα ubiquitination and its proteasome degradation [48], whereas proteasome inhibitors increase the level of ERα protein but, at the same time, disturb transcription of some ERα target genes [49]. Moreover, deubiquitinase OTUB1 deubiquitinates ERα and decreases its transcription activity [50]. Therefore, the ubiquitination of ERα is necessary for the efficient transcription of ERα target genes, at the same time it leads to consequent degradation of activated receptor. In relation with this, in estrogen-negative tumors high quantities of ERα mRNA but not of translated protein are observed [51]. Obviously, the reason for this could be found in ubiquitination of ERα coupled with the transcription of target genes and following proteasomal degradation of ubiquitinated ERα [47].

It is worth to note that ligands influencing gene expression with the participation of epigenetic mechanisms, including histone modifications, not necessarily induce proportional changes in the synthesis of corresponding mRNA and proteins. Observed discrepancy of mRNA and protein synthesis could be

related to the regulation on mRNA translation level under the influence of different factors including the unbalanced cell metabolism, development processes and cell transformation [52, 53]. *BRCA1* gene expression regulation at the level of mRNA translation, possibly, could explain our data on the changes of BRCA1 protein synthesis (approximately in 2 fold) during phytoestrogens and hypoxia influences. According to literature data, in some cases the mRNA level increases essentially more, than the protein one, for example at E2 effect on BRCA1 mRNA and protein synthesis in MCF-7 BC cells [54]. In some cases the influences of certain compounds could be registered only on the mRNA but not on the protein level, as it was demonstrated upon phytoestrogen resveratrol effect on *BRCA1* gene expression [55]. It seems that in these cases the molecular mechanisms function inhibiting protein synthesis at different stages of translation (RNA interference, inhibition of initiation and elongation of translation) that could, in turn, lead to its post-translation modification and degradation. This system could be useful for the studies of histone modifications direct effect on changes of *BRCA1* mRNA synthesis without considering the quantity of final product, BRCA1 protein.

Dagdemir and coauthors [34] studied changes in histone modifications in *BRCA1* and *ESR1* (coding for ERα) promoters upon the addition of phytoestrogens and estrogens. Using the method of chromatin immunoprecipitation (ChIP) the authors demonstrated that at the influence of genistein, daidzein, equol or E2 at the promoters of *BRCA1* and *ESR1* genes the level of transcription inhibiting histone modifications (H3K9me3 and H3K27me3) is decreased and the quantity of transcription activating histone modifications (H3K4ac and H4K8ac) is augmented. However, other histone modifications, that are important at the regulation of genes activated with E2-ERα, such as H3K14ac, H3K18ac, H3K23ac, H3R17me, were left out of the scope of the authors view [56]. Despite the lack of comprehensive studies in this field, it is possible to suggest that, as in case of *pS2* gene regulation in MCF-7 BC cells, *BRCA1* promoter activated also with E2-ERα could be similarly regulated with the involvement of histone acetyl-transferase CBP and Arg (R) specific histone methyl-transferase CARM1 [56]. It is possible that in *BRCA1* gene promoter at E2 application histone H3 located at the *BRCA1* promoter undergo consequent modifications of amino acid residues Lys (K) and Arg (R) starting with the acetylation of Lys in position 18 in histone H3 (H3K18ac) followed with the acetylation of Lys in position 23 in histone H3 (H3K23ac) and, finally, with the methylation of Arg in position 17 also in histone H3 (H3R17me), while amino acid residue Lys in position 14 in histone H3 remains acetylated both in E2-induced and non-induced conditions (H3K14ac) [56].

Taken together, it is possible to suggest several mechanisms of phytoestrogen-dependent regulation of *BRCA1* and *ESR1* expression that we observed. From one hand, the phytoestrogen genistein could activate ERα that leads to the formation of the complex with HAT CBP/p300, the acetylation of histones in *BRCA1* promoter and the increase of BRCA1 mRNA and protein. From another hand, the genistein being an inhibitor of DNA methyltransferases (DNMTs) [57, 58] could diminish the DNA hypermethylation in the *BRCA1* promoter and, therefore, could induce the increase in *BRCA1* mRNA transcription and the augmentation of the quantity of protein translated from this mRNA.

Our research on the effect of hypoxia at BRCA1 and ERα expression in MCF-7 cells revealed the tendency towards the decrease of these two proteins synthesis. Hypoxic cytotoxin TPZ did not influence considerably the BRCA1 expression index in normoxia conditions. In hypoxic conditions the augmentation of BRCA1 expression was observed at the incubation with TPZ, that being activated to the form of cytotoxic radical upon the diminution of oxygen level, induces DNA double strand breaks. These are new mechanisms of tumor cell response to hypoxic cytotoxins influence. Antiestrogen effects of TPZ in BC cells revealed at hypoxia are of considerable interest for further research. It is not possible to exclude that the hypoxia influences BRCA1-associated processes of DNA repair and simultaneously regulates hormone-dependent BC cell growth.

On the basis of obtained data concerning the influence of biologically active substances including phytoestrogens, estrogens, antiestrogens at BRCA1 and ERα expression in BC cells it is possible to conclude that the changes of integral parameter of marker expression, index, occurred, mainly, due to the changes of the expression intensity and not of the protein expression level. On the contrary, in hypoxia conditions and at the influence of hypoxic cytotoxin TPZ, changes of BRCA1 and ERα expression indexes were caused, in major part, with the changes of the levels and not of the markers expression intensities. In other words, phytoestrogens, estrogens and antiestrogens application almost does not influence the quantity of cells expressing these tumor markers but changes the quantity of markers in already expressing cells; and, on the contrary, at the influence of hypoxia and TPZ the number of cells expressing BRCA1 or ERα changes, and the quantity of the marker in already expressing cells almost does not change. Taking together, the results of presented research revealed coordinated regulation of BRCA1-associated DNA repair and steroid hormones signaling pathways. Moreover, it was demonstrated that in BC cells in hypoxia conditions hormone-dependent cell proliferation and growth as well as the expression of the key tumor suppressor, BRCA1, simultaneously decreased. In future studies it is planned to determine the role of these molecular mechanisms in the formation of the chemoresistance of the tumors with considerable hypoxic regions.

In conclusion, it is worth to note that obtained results will become a basis for the future complex studies of DNA repair marker, BRCA1, and proliferation marker, ERα, expression regulation molecular mechanisms including epigenetic regulation of their gene expression.

FUNDING

This research is supported with the grant Russian Fund for Basic Research (RFBR) 18-29-09017 (experiments with cell cultures).

COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflict of interest. This article does not contain any studies involving animals or human participants performed by any of the authors.

ADDITIONAL INFORMATION

The article was translated by the authors.

REFERENCES

1. Clark S., Rodriguez A.M., Snyder R.R., Hankins G.D.V., Boehning D. 2012. Structure-function of the tumor suppressor BRCA1. *Comput. Struct. Biotechnol. J.* **1**, 1–8. pii: e201204005
2. Silver D.P., Dimitrov S.D., Feunteun J., Gelman R., Drapkin R., Lu S.D., Shestakova E., Soundarapandian V., DeNunzio N., Dragomir S., Mar J., Liu X., Rottenberg S., Jonkers J., Ganesan S., Livingston D.M. 2007. Further evidence for BRCA1 communication with the inactive X chromosome. *Cell.* **128**, 991–1002.
3. Hu Y., Scully R., Sobhian B., Xie A., Shestakova E., Livingston D.M. 2011. RAP80-directed tuning of BRCA1 homologous recombination function at ionizing radiation-induced nuclear foci. *Genes Dev.* **25**, 685–700.
4. Saha J., Davis A.J. Unsolved mystery: The role of BRCA1 in DNA end-joining. 2016. *J. Radiat. Res.* **57**, Suppl. 1, i18–i24.
5. Shestakova E.A. 2016. Epigenetic regulation of BRCA1 expression and its role in breast cancer stem cell development. *Turk. J. Biol.* **40**, 981–989.
6. Bogush T.A., Shestakova E.A., Vikhlyantseva N.O., Bogush E.A., Chemeris G.Yu., Davydov M.M. 2017. Epigenetic mechanisms of *BRCA1* regulation. *Onkoginekologiya*. **22** (2), 4–11.
7. Sokolenko A.P., Imyanitov E.N. 2017. Molecular tests for the choice of cancer therapy. *Curr. Pharm. Des.* **23**, 4794–4806.
8. Iyevleva A.G., Imyanitov E.N. 2016. Cytotoxic and targeted therapy for hereditary cancers. *Hered. Cancer Clin. Pract.* **14**, 17.
9. Hosking L., Trowsdale J., Nicolai H., Solomon E., Foulkes W., Stamp G., Signer E., Jeffreys A. 1995. A somatic BRCA1 mutation in an ovarian tumour. *Nat. Genet.* **9**, 343–344.
10. Esteller M., Silva J.M., Dominguez G., Bonilla F., Matias-Guiu X., Lerma E., Bussaglia E., Prat J., Harkes I.C., Repasky E.A., Gabrielson E., Schutte M., Baylin S.B., Herman J.G. 2000. Promoter hypermethylation and BRCA1 inactivation in sporadic breast and ovarian tumors. *J. Natl. Cancer Inst.* **92**, 564–569.
11. Esteller M. 2006. CpG island methylation and histone modifications: Biology and clinical significance. In: *Ernst Schering Research Foundation Workshop. The Histone Code and Beyond. New Approaches to Cancer Therapy*, vol. 57. Eds Berger S.L., Nakanishi O., Haendler B. Springer, pp. 115–126.
12. Lu Y., Chu A., Turker M.S., Glazer P.M. 2011. Hypoxia-induced epigenetic regulation and silencing of the BRCA1 promoter. *Mol. Cell. Biol.* **31**, 3339–3350.
13. Wu Z.Q., Li X.Y., Hu C.Y., Ford M., Kleer C.G., Weiss S.J. 2012. Canonical Wnt signaling regulates Slug activity and links epithelial–mesenchymal transition with epigenetic Breast Cancer 1, Early Onset (*BRCA1*) repression. *Proc. Natl. Acad. Sci. U. S. A.* **109**, 16654–16659.
14. McCoy M.L., Mueller C.R., Roskelley C.D. 2003. The role of the breast cancer susceptibility gene 1 (*BRCA1*) in sporadic epithelial ovarian cancer. *Reprod. Biol. Endocrinol.* **1**, 72.
15. Russel P.A., Pharoah P.D., De Foy K., Ramus S.J., Symmonds I., Wilson A., Scott I., Ponder B.A., Gayther S.A. 2000. Frequent loss of *BRCA1* mRNA and protein expression in sporadic ovarian cancers. *Int. J. Cancer.* **87**, 317–321.
16. Ribeiro I.P., Marques F., Caramelo F., Pereira J., Patricio M., Prazeres H., Ferrao J., Juliao M.J., Castelo-Branco M., de Melo J.B., Baptista I.P., Carreira I.M. 2014. Genetic gains and losses in oral squamous cell carcinoma: Impact on clinical management. *Cell Oncol.* (Dordr.). **37**, 29–39.
17. Hosey A.M., Gorski J.J., Murray M.M., Quinn J.E., Chung W.Y., Stewart G.E., James C.R., Farragher S.M., Mulligan J.M., Scott A.N., Dervan P.A., Johnston P.G., Couch F.J., Daly P.A., Kay E., et al. 2007. Molecular basis for estrogen receptor alpha deficiency in *BRCA1*-linked breast cancer. *J. Natl. Cancer Inst.* **99**, 1683–1694.
18. Jeffy B.D., Hockings J.K., Kemp M.Q., Morgan S.S., Hager J.A., Beliakoff J., Whitesell L.J., Bowden G.T., Romagnolo D.F. 2005. An estrogen receptor-αp300 complex activates the *BRCA-1* promoter at an AP-1 site that binds Jun/Fos transcription factors: Repressive effects of p53 on *BRCA-1* transcription. *Neoplasia.* **7**, 873–882.
19. Di L.J., Fernandez A.G., De Siervi A., Longo D.L., Gardner K. 2010. Transcriptional regulation of *BRCA1* expression by a metabolic switch. *Nat. Struct. Mol. Biol.* **17**, 1406–1413.
20. Suba Z. 2015. DNA stabilization by the upregulation of estrogen signaling in *BRCA* gene mutation carriers. *Drug Des. Dev. Ther.* **9**, 2663–2675.
21. Ma Y., Fan S., Hu C., Meng Q., Fuqua S.A., Pestell R.G., Tomita Y.A., Rosen E.M. 2010. *BRCA1* regulates acetylation and ubiquitination of estrogen receptor-alpha. *Mol. Endocrinol.* **24**, 76–90.

22. Petz L.N., Ziegler Y.S., Loven M.A., Nardulli A.M. 2002. Estrogen receptor alpha and activating protein-1 mediate estrogen responsiveness of the progesterone receptor gene in MCF-7 breast cancer cells. *Endocrinology*. **143**, 4583–4591.
23. Schultz J.R., Petz L.N., Nardulli A.M. 2003. Estrogen receptor alpha and Sp1 regulate progesterone receptor gene expression. *Mol. Cell. Endocrinol*. **201**, 165–175.
24. Petz L.N., Ziegler Y.S., Schultz J.R., Kim H., Kemper J.K., Nardulli A.M. 2004. Differential regulation of the human progesterone receptor gene through an estrogen response element half site and Sp1 sites. *J. Steroid Biochem. Mol. Biol*. **88**, 113–122.
25. Donaghue C., Westley B.R., May F.E.B. 1999. Selective promoter usage of the human estrogen receptor-alpha gene and its regulation by estrogen. *Mol. Endocrinol*. **13**, 1934–1950.
26. Andersson C., Sundberg M., Pristovsek N., Ibrahim A., Jonsson P., Katona B., Clausson C.M., Zieba A., Ramstrom M., Soderberg Williams C, Asplund A. 2017. Insufficient antibody validation challenges oestrogen receptor beta research. *Nat. Commun*. **8**, 15840.
27. Bogush T.A., Popova A.S., Dudko E.A., Bogush E.A., Tyulyandina A.S., Tyulyandin S.A., Davydov M.I. 2015. ERCC1 as a marker of ovarian cancer resistance to platinum preparations. *Antibiotiki Khimioterap.* **60**, 42–50.
28. Bogush T.A., Dudko E.A., Shestakova E.A., Grishanina A.N., Bogush E.A., Kirsanov V.Yu., Ryabinina O.M., Vikhlyantseva N.O. 2016. Quantitative assessment of BRCA1 protein expression level in breast cancer tissue using flow cytometry. *Ross. Bioterapevt. Zh*. **15**, 49–52.
29. Bogush T.A., Shaturova A.S., Dudko E.A., Dzhuraev E.E., Polotskii B.E., Ungiadze G.V., Davydov M.I. 2011. Quantitative immunofluorescent estimation of estrogen receptor β expression in human solid tumors using flow cytometry. *Moscow Univ. Chem. Bull*. **66** (4), 253–258.
30. Bogush T.A., Shaturova A.S., Dudko E.A., Bogush E.A., Polotskii B.E., Tyulyandin S.A., Davydov M.I. 2014. Comparative assessment of the estrogen receptor β expression in the tissues of non-small-cell lung carcinoma and lung metastases of tumors of other primary localizations. *Dokl. Biochem. Biophys.* **454**, 29–33.
31. Bogush T.A., Dudko E.A., Semakov A.V., Bogush E.A., Tyulyandina A.S., Zarkua V.T., Tyulyandin S.A., Davydov M.I. 2014. Immunofluorescent assay of ERCC1 and estimation of clinical significance of the protein expression in ovarian cancer tissue. *Dokl. Biochem. Biophys*. **457**, 141–145.
32. Rebrova O.Yu. 2002. *Statisticheskii analiz meditsinskikh dannykh. Primenenie paketa prikladnykh programm STATISTICA* (Statistical Processing of Medical Data Using the STATISTICA Applications Program Package). Moscow: MediaSfera.
33. Fan S., Meng Q., Auborn K., Carter T., Rosen E.M. 2006. BRCA1 and BRCA2 as molecular targets for phytochemicals indole-3-carbinol and genistein in breast and prostate cancer cells. *Br. J. Cancer*. **94**, 407–426.
34. Dagdemir A., Durif J., Ngollo M., Bignon Y.J., Bernard-Gallon D. 2013. Histone lysine trimethylation or acetylation can be modulated by phytoestrogen, estrogen or anti-HDAC in breast cancer cell lines. *Epigenomics*. **5**, 51–63.
35. Bosviel R., Dumollard E., Dechelotte P., Bignon Y.J., Bernard-Gallon D. 2012. Can soy phytoestrogens decrease DNA methylation in BRCA1 and BRCA2 oncosuppressor genes in breast cancer? *OMICS*. **16**, 235–244.
36. Ono M., Ejima K., Higuchi T., Takeshima M., Wakimoto R., Nakano S. 2017. Equol enhances apoptosis-inducing activity of genistein by increasing Bax/Bcl-xL expression ratio in MCF-7 human breast cancer cells. *Nutr. Cancer*. **6**, 1300–1307.
37. Scherbakov A.M., Andreeva O.E. 2015. Apigenin inhibits growth of breast cancer cells: The role of ERα and HER2/neu. *Acta Naturae*. **7**, 133–139.
38. Kaushik S., Shyam H., Sharma R., Balapure A.K. 2016. Genistein synergizes centchroman action in human breast cancer cells. *Indian J. Pharmacol*. **48**, 637–642.
39. Messina M. 2014. Soy foods, isoflavones, and the health of postmenopausal women. *Am. J. Clin. Nutr*. **100** (Suppl. 1), 423S–430S.
40. Shcherbakov A.M., Vavilov N.E., Andreeva O.E., Tyaglov B.V., Mironov A.S., Shakulov R.S., Lobanov K.V., Yarotskii S.V., Shtil' A.A. 2017. Effect of acadesine on breast cancer cells under hypoxia. *Usp. Mol Onkol*. **4**, 60–64.
41. Sorokin D.V., Scherbakov A.M., Yakushina I.A., Semina S.E., Gudkova M.V., Krasil'nikov M.A. 2016. The mechanism of adaptation of breast cancer cells to hypoxia: Role of AMPK/mTOR signaling pathway. *Bull. Exp. Biol. Med*. **160**, 555–559.
42. Gilkes D.M. 2016. Implications of hypoxia in breast cancer metastasis to bone. *Int. J. Mol. Sci*. **17**, E1669.
43. Milani M., Harris A.L. 2008. Targeting tumour hypoxia in breast cancer. *Eur. J. Cancer*. **44**, 2766–2773.
44. Lin W.H., Yeh S.H., Yeh K.H., Chen K.W., Cheng Y.W., Su T.H., Jao P., Ni L.C., Chen P.J., Chen D.S. 2016. Hypoxia-activated cytotoxic agent tirapazamine enhances hepatic artery ligation-induced killing of liver tumor in HBx transgenic mice. *Proc. Natl. Acad. Sci. U. S. A*. **113**, 11937–11942.
45. Ferrand N., Stragier E., Redeuilh G., Sabbah M. 2012. Glucocorticoids induce CCN5/WISP-2 expression and attenuate invasion in oestrogen receptor-negative human breast cancer cells. *Biochem. J*. **447**, 71–79.
46. Rae J.M., Johnson M.D., Scheys J.O., Cordero K.E., Larios J.M., Lippman M.E. 2005. GREB 1 is a critical regulator of hormone dependent breast cancer growth. *Breast Cancer Res. Treat*. **92**, 141–149.
47. Sun J., Zhou W., Kaliappan K., Nawaz Z., Slingerland J.M. 2012. ERα phosphorylation at Y537 by Src triggers E6-AP-ERα binding, ERα ubiquitylation, promoter occupancy, and target gene expression. *Mol. Endocrinol*. **26**, 1567–1577.
48. Nawaz, Z., Lonard D.M., Dennis A.P., Smith C.L., O'Malley B.W. 1999. Proteasome-dependent degradation of the human estrogen receptor. *Proc. Natl. Acad. Sci. U. S. A*. **96**, 1858–1862.
49. Reid G., Hubner M.R., Metivier R., Brand H., Denger S., Manu D., Beaudouin J., Ellenberg J., Gannon F. 2003.

Cyclic, proteasome-mediated turnover of unliganded and liganded ERalpha on responsive promoters is an integral feature of estrogen signaling. *Mol. Cell*. **11**, 695–707.

50. Stanisic V., Malovannaya A., Qin J., Lonard D.M., O'Malley B.W.J. 2009. OTU Domain-containing ubiquitin aldehyde-binding protein 1 (OTUB1) deubiquitinates estrogen receptor (ER) alpha and affects ERalpha transcriptional activity. *Biol. Chem*. **284**, 16135–16145.
51. Prat A., Adamo B., Cheang M.C., Anders C.K., Carey L.A., Perou C.M. 2013. Molecular characterization of basal-like and non-basal-like triple-negative breast cancer. *Oncologist*. **18**, 123–133.
52. Hentze M.W. 1995. Translational regulation: Versatile mechanisms for metabolic and developmental control. *Curr. Opin. Cell Biol*. **7**, 393–398.
53. Silvera D., Formenti S.C., Schneider R.J. 2010. Translational control in cancer. *Nat. Rev. Cancer*. **10**, 254–266.
54. Gudas J.M., Nguyen H., Li T., Cowan K.H. 1995. Hormone-dependent regulation of BRCA1 in human breast cancer cells. *Cancer Res*. **55**, 4561–4565.
55. Fustier P., Corre L.L, Chalabi N., Vissac-Sabatier C., Communal Y., Bignon Y.J., Bernard-Gallon D.J. 2003. Resveratrol increases *BRCA1* and *BRCA2* mRNA expression in breast tumour cell lines. *Br. J. Cancer*. **89**, 168–172.
56. Daujat S., Bauer U.M., Shah V., Turner B., Berger S., Kouzarides T. 2002. Crosstalk between CARM1 methylation and CBP acetylation on histone H3. *Curr. Biol*. **12**, 2090–2097.
57. Fang M.Z., Chen D., Sun Y., Jin Z., Christman J.K., Yang C.S. 2005. Reversal of hypermethylation and reactivation of p16INK4a, RARbeta, and MGMT genes by genistein and other isoflavons from soy. *Clin. Cancer Res*. **11**, 7033–7041.
58. King-Batoon A., Leszczynska J.M., Klein C.B. 2008. Modulation of gene methylation by genistein or lycopene in breast cancer cells. *Environ. Mol. Mutagen*. **49**, 36–45.