American Journal of
Biomedical Science & Research

www.biomedgrid.com
ISSN: 2642-1747

Research Article

Effect of Stress-Inducing Agents and HTLV-1 Tax on BRCA1 and Controlled Gene Expression

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To Cite This article: *George Abu-Aqil, Manal Suleiman, and Mahmoud Huleihel*, Effect of Stress-Inducing Agents and HTLV-1 Tax on BRCA1 and Controlled Gene Expression. Am J Biomed Sci & Res. 2026 31(4) AJBSR.MS.ID.004064,* **DOI:** 10.34297/AJBSR.2026.31.004064

Received: June 23, 2026 **Published:** July 03, 2026

Abstract

The primary gene implicated in hereditary Breast Cancer (BC) is the tumour suppressor gene Breast Cancer Gene 1 (BRCA1), which is activated by the Estrogen Receptor Alpha (ERα). Insufficient expression or functionality of BRCA1 may contribute to the development of BC. ERα activation leads to the activation of various genes through direct binding to Estrogen-Responsive Elements (EREs) on promoters or indirectly by interacting with other transcription factors. Previously, our research revealed that Human T-Lymphotropic Virus Type-1 (HTLV-1) Tax strongly inhibits ERα-induced BRCA1 expression, and stress-inducing agents robustly stimulate HTLV-1 expression. This study investigated the impact of Tax and stress-inducing agents on BRCA1 and ERE expression. Our results showed that both 12-O-Tetradecanoylphorbol-13-Acetate (TPA) and Taxol significantly enhanced BRCA1 expression over the course of treatment, while cisplatin showed a slight reduction. Conversely, while TPA significantly stimulated ERE expression, both Taxol and cisplatin had a marginal inhibitory effect. The induced BRCA1 expression by TPA and Taxol appears to be dependent on Protein Kinase C (PKC). Furthermore, Tax demonstrated no significant impact on TPA-induced BRCA1 expression, but it strongly inhibited the effect of Taxol on BRCA1 expression. Additionally, cisplatin and Tax exhibited an antagonistic relationship. Notably, our results suggest that the tested stress agents do not influence BRCA1 and ERE expression through the ERα pathway.

Keywords: TPA, ERα, ERE, HTLV-1, Tax, BRCA-1.

Introduction

Breast Cancer (BC) is one of the most common cancers in women, accounting for about 30% of women's cancer cases, and is a significant cause of cancer-related deaths [1,2]. Most BC cases are caused by lifestyle and environmental factors [3,4], while a minority (5-10%) are hereditary, due to genetic changes [2]. The most important gene involved in hereditary BC cases is Breast Cancer Gene 1 (BRCA1), a multifunctional tumor suppressor gene [5,6] responsible for about 40-45% of hereditary BC cases [7,8]. Although BRCA1 mutations are responsible for a minority (2-3%) of all BC cases, there is a reduced or lack of BRCA1 expression in a large part (over 30%) of sporadic cancers, suggesting a much wider role of BRCA1 in BC [5]. BRCA1 promotes many cellular activities, which are important for its tumor suppressor function, such as DNA damage repair [9], chromatin remodeling [10], induction of cell cycle arrest [11], and apoptosis [12]. Any lack of BRCA1 activity, whether due to non-expression or specific mutations, may lead to breast and ovarian cancers [2]. It found that women carrying BRCA1 dysfunctionality have a 57-65% probability of developing BC throughout their lifetime [13]. In fact, it seems that BRCA1 is involved mainly in breast and ovarian cancers due to the response of these organs to estrogen (E2) [14]. E2 activates its receptor α (ERα) by direct ligation in the nucleus of the cells. The E2-ERα complex acts as a transcriptional factor, activating various cellular genes through two pathways. The first is a classical pathway; ERα binds directly to specific sequences called E2 Responsive Elements (EREs), which are located in the promoter of target genes and this way activates the transcription of these genes after recruiting different co-factors [15]. The second is a non-classical pathway; ERα binds indirectly to the promoters of target genes lacking EREs by interacting with other transcriptional factors that have specific binding sites in these promoters. As a result, the bound ERα complex, together with the recruited co-factors, promotes the transcriptional activity of these genes [16]. For example, although the BRCA1 promoter has no EREs, its transcriptional activity is

promoted by ERα through the non-classical pathway [17] through binding of the ERα complex to Jun/Fos transcription factor, which is ligated with its binding site (AP-1) located in the BRCA1 promoter [17]. It should be noted that other co-activators and co-factors, such as CREB-binding protein p300 (CBP/p300), are recruited to the active E2-ERα complex and are required for its transcriptional activity [18-21].

Human T-Cell Leukemia Virus Type-1 (HTLV-1) is a retrovirus involved in various serious human diseases, including Adult T-Cell Leukemia (ATL) [22], tropical spastic paraparesis/HTLV-1-associated myelopathy [23], and others. It is widely agreed that the viral trans activator Tax protein is a key element in the mechanism of ATL genesis and is probably involved in other HTLV-1-related disorders [24-26]. Our previous results proved the ability of Tax to prevent BRCA1 expression and functionality in breast epithelial cells [27,28]. Therefore, HTLV-1 might also be a putative risk factor for BC development. It was found that different stress-inducing agents, such as the carcinogen 3-methylcholanthrene (3-MC), 12 O-Tetradecanoylphorbol-13- Acetate (TPA), cisplatin, and Taxol, can strongly activate HTLV-1 Long Terminal Repeat (LTR) independently of HTLV-1 Tax [29,30]. These results suggest that stress agents may activate the latent virus in the carriers' infected cells [31]. These stress agents are potentially accessible to human contact or consumption, and some of them, such as TPA, are plant products [32]. Therefore, these agents may be considered as possible environmental cofactors related to HTLV-1 pathogenicity [33]. Additionally, our previous results demonstrated a strong stimulatory effect of TPA on both BRCA1 and EREs expression without treatment with E2 [33]. TPA, a potent activator of Protein Kinase C (PKC), exerts most of its biological effects through PKC [34,35].

This study examined the effect of these stress agents on BRCA1 and EREs expression, as well as the involvement of HTLV-1 Tax in this expression.

Materials and Methods

Cells

MCF-7 cells were used (weakly invasive ER-α positive BC cells) [36] for the different analyses. The cells were grown and maintained in Dulbecco's Modified Eagle's Medium (DMEM) with two mM L-glutamine, 10% Fetal Bovine Serum (FBS), and 1% penicillin/streptomycin.

Reagents

TPA, Cisplatin, and Taxol were obtained from Sigma Chemicals Israel Ltd., Holon, Israel. The cells were treated when indicated with 50 nM TPA. When indicated, the cells were treated with 50 nM TPA, Cisplatin, and Taxol. One mM of BI (Calbiochem, La Jolla, CA), which is a Protein Kinase C (PKC) inhibitor, was used. 20 nM estrogen (E2, Sigma-Aldrich Chemical Co.) was added to the cells, where it is indicated, at 5 hr before cell harvest. Monoclonal antibodies against BRCA1, Tax, ER-α, CBP, CREB, and Ps2 were all purchased from Santa Cruz Biotechnology Inc (Santa Cruz, CA, USA).

Plasmids

Plasmids that expressed the reporter firefly luciferase (Luc) under the control of BRCA1 promoter (BRCA1-Luc) or under the control of estrogen response elements (EREs-Luc) were obtained from Haim Werner (Clinical Biochemistry, Tel-Aviv University, Israel). Plasmids that expressed the different PKC isoforms (α, β1, β2, δ, £ and η) were obtained from Etta Livne (from our department) [37]. HTLV-1 Tax expressing plasmid through hCMV promoter was provided by Francoise Bex (Laboratorie de Microbiologie, Universite Libre de Bruxelles, B-1070, Brussels, Belgium). The plasmid expressing the Renilla luciferase was purchased from Promega (Madison, WI, USA) [38].

Plasmid Transfection

To insert the various plasmids into the cells, the jetPRIMTM transfection kit (Polyplus-transfection company, France) was used according to the manufacturer's instructions. Briefly, one μg of each plasmid DNA was transfected into about 5 × 10^5 cells/well of a 6-well plate. One μg of each plasmid DNA was transfected up to 3 μg total DNA in each transfection mixture (missing amount of DNA was completed with an empty plasmid (dLTR). Transfection efficiency, determined with a GFP-expressing plasmid, was found by FACS analysis in our cells to be about 70%. 0.2μg of pRL-renilla plasmid was added to the transfection mixture as a control for efficiency. Cells were extracted, and luciferase enzymatic activity was measured using a 20/20 Luminometer (Promega) at 24 hours post-transfection. The Luc activity was normalized to Renilla and presented as a fold change relative to the relevant control.

Western Blot Analyses and Co-Immunoprecipitation

Whole cell extracts were prepared by NucBuster Kit (Calbiochem, Catalog No. 71183-3) according to the Manufacturer's protocol. 80μg protein of the tested extracts was analyzed by Western analysis as previously described [39] using the appropriate monoclonal antibodies. Aliquots of the nuclear extracts (200 μg protein) were immunoprecipitated with the specified mouse antibodies and analyzed by Western blot for co-precipitated proteins with the respective rabbit antibodies as previously described [40].

RNA Extraction

Total RNA was extracted using the commercial kit "GenElute Mammalian Total RNA miniprep", Sigma, according to the kit's instructions.

cDNA synthesis

RNA was reverse transcribed into DNA using a commercial cDNA synthesis kit, qScriptTM, Quanta bio company. The amount of the BRCA1 and pS2 mRNA was determined by RT-PCR using the following primers:

BRCA-1:

Forward: 5′-ACAGCTGTGTGGTGCTTCTGTG-3′

Reverse: 5′-CATTGTCCTCTGTCCAGGCATC-3′

PS2:

Forward: 5′-CATCGACGTCCCTCCAGAAGAG-3′

Reverse: 5′-CTCTGGGACTAATCACCGTGCTG-3′

Chromatin Immunoprecipitation (ChIP)

MCF-7 (2 × 10^7) cells were transfected with Tax-expressing plasmid by the jetPRIMTM kit, and at 24 hours post-transfection, the cells were treated with E2 for 5 hours. The binding of the tested proteins to the appropriate promoters was examined by ChIP assay using an EZ Chip kit (Millipore) according to the manufacturer's instructions. BRCA1 promoter region flanking the Sp/AP-1/CRE binding sites (171 base pairs) in the examined DNA was amplified by real-time PCR, using the following primers: Forward: 5′-GACAGATGGGTATTCTTTGACG-3′

Reverse: 5′-GCATATTCCAGTTCC TATCACGAG-3′ [18].

The ERE region of the pS2 promoter was amplified using the following primers:

Forward pS2: 5′-TATGAATCACTTCTGCAGTGAG-3′

Reverse pS2: 5′- GAGCGTTAGATAACATTTGCC-3′

Results

Time Effect of Treatment with Stress-Inducing Agents on the Expression of BRCA1 And Genes Controlled by Eres

Previous studies showed that stress-inducing agents strongly induced HTLV-1 expression [28,41-43]. In our previous study, we proved the ability of HTLV-1 Tax to block the E2-ERα induced expression of BRCA1 while stimulating the E2-ERα induced expression of genes that contain EREs in their promoters [28]. These results may indicate HTLV-1 involvement in BC development. Thus, based on these findings, we investigated in the present study the effect of various stress-inducing agents (TPA, cisplatin, and Taxol) on BRCA1 and EREs expression. This was done by examining the effect of these stress-inducing agents (cisplatin and Taxol compared with TPA) on the expression of the reporter (Luc) driven by either the BRCA1 promoter (BRCA1-Luc) or ERE region (ERE-Luc) in MCF7 cells.

In our previous study, we showed the stimulatory effect of TPA on both BRCA1 and ERE expression [33]. Our results in this study show that TPA strongly activated both BRCA1 and ERE-Luc expression (17- and 10-fold increases over their basal levels, respectively) when treatment was performed after transfection with the appropriate plasmids (Figures 1a and 1b, respectively, column 7). However, when treatment began 24 hours before transfection, there was a significant reduction in BRCA1 and ERE-Luc expression (Figures 1a and 1b, respectively, column 5). On the other hand, while cisplatin slightly reduced the basal expression of both BRCA1 and ERE regardless of the treatment time (Figures 1a and 1b, respectively, columns 5 and 8), Taxol strongly induced the activation of BRCA1 as a function of treatment time (Figure 1a, columns 6 and 9) and has no notable effect on ERE expression (Figure 1b, columns 6 and 9) (Figure 1).

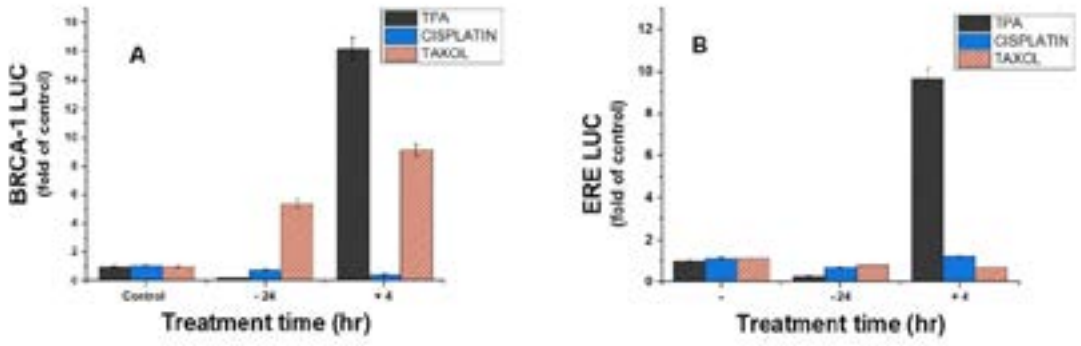

Figure 1: Effect of time of treatment with stress inducing agents on the expression of BRCA1 and genes controlled by ERE. MCF-7 were transfected with a plasmid expressing BRCA1-Luc (1μg) (A) or ERE-Luc (1μg) (B) and treated with TPA (50 nM), cisplatin (2μM) or Taxol (5nM) at different times before or after the transfection. The cells were harvested at 24h post transfection for analyzing the reporter expression. The presented results are an average of three repeated experiments ± SE.

Is The Stress-Agent Effect on BRCA1 And ERE Expression Mediated by PKC Activity?

It was previously shown that the activation of HTLV-1LTR by TPA is antagonized by the PKC activity [44], and thus, we wondered whether the activity of the other tested stress-inducing agents on HTLV-I LTR and BRCA1 is related to PKC activity. As mentioned in the introduction section, TPA is a potent activator of the PKC [34,35], and long exposure to TPA causes down-regulation of PKC expression [41]. As shown in Figure 1, the activity of both TPA and Taxol on BRCA1 was affected by the time of treatment, whereas that of cisplatin was not. To examine the possible involvement of PKC in the activity of these agents on BRCA1 and ERE expression, MCF-7 cells were transfected with either BRCA1-Luc or ERE-Luc plasmids and treated with TPA, cisplatin, or Taxol alone or with BI at the time of transfection. Our results showed that both TPA and Taxol significantly induced BRCA1 expression, while cisplatin reduced its basal expression (Figure 2A). In addition, BI completely abolished both TPA and Taxol-induced activation with no effect on cisplatin activity. On the other hand, only TPA significantly induced ERE expression, whereas both Taxol and cisplatin slightly reduced basal ERE expression (Figure 2bB). BI completely prevented TPA-induced activation of ERE expression, with no significant effect on Taxol- or cisplatin-induced ERE expression.

Differential Effects of PKC Isoforms on BRCA1 And ERE Expression in Cells Treated with the Examined Stress-Inducing Agents

The above results showed that TPA- and Taxol-induced activation of BRCA1 is mediated by PKC activity (Figure 2). It was therefore important to determine which PKC isoforms are responsible for this activity. To achieve that, MCF-7 cells (containing BRC A1-Luc or ERE-Luc) were treated with TPA for 48 hours to deplete all cellular PKCs, then transfected with plasmids expressing different PKC isoforms and treated with TPA, Taxol, or cisplatin at the time of transfection.

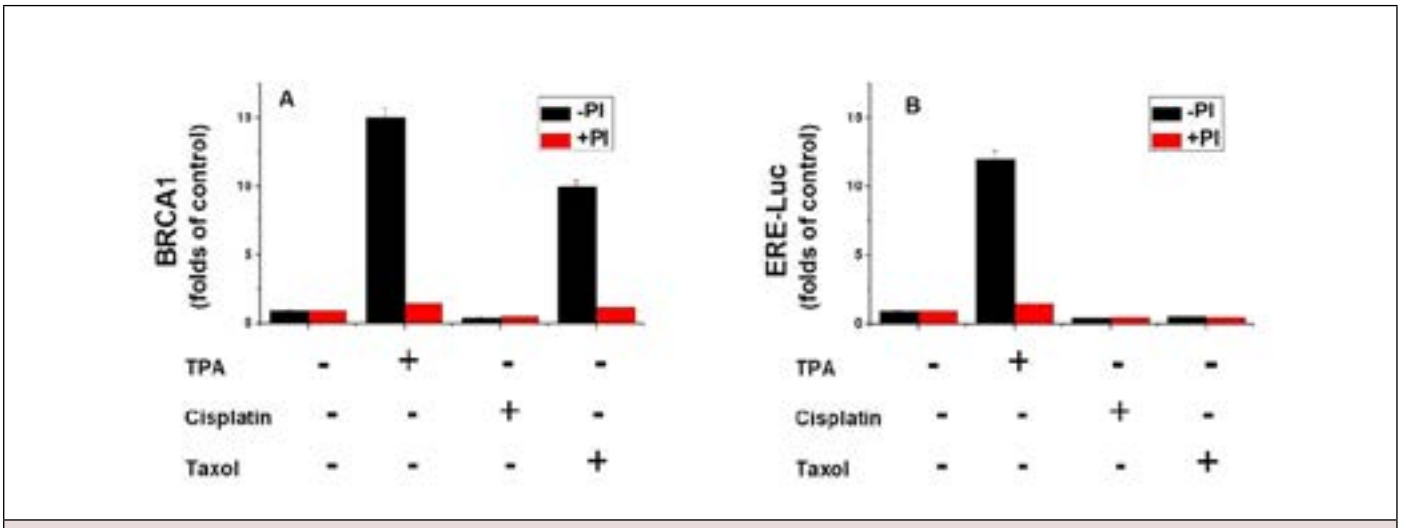

Figure 2: Involvement of PKC in TPA, cisplatin and Taxol effect on BRCA1 and ERE expression MCF-7 cells were transfected with plasmids expressing either BRCA1-Luc (1µg) (A) or ERE-Luc (1µg) (B) and treated with 50nM TPA, 2µM cisplatin or 5nM Taxol alone or with 2nM BI (a potent inhibitor of PKC activity) at the time of transfection. The cells were harvested at 24h post transfection for analyzing the reporter expression. The presented results are an average of three repeated experiments ± SE.

The results presented in Figure 3A shows that PKCα, β1, δ, and η highly induce the expression of BRCA1 in cells that were treated with TPA at and post-transfection (columns 4, 7, 13, and 18, respectively), while PKCβ2 and ε have no stimulatory effect and even they reduce the basal expression of BRCA1 (Figure 3A, columns 10, and 16, respectively). In the cells that were treated with Taxol, PKCα, δ, and η strongly induced the expression of BRCA1 (Figure 3A, columns 6, 15, and 21, respectively), while the other tested isoforms did not show any stimulatory effect on BRCA1expression. In cells treated with cisplatin, none of the tested PKC isoforms showed a stimulatory effect on BRCA1 expression (Figure 3).

In the case of ERE, it can be seen in Figure 3B that PKCα, PKCβ2, PKCε, and PKCη significantly induced its expression in cells that were treated with TPA at and post-transfection (columns 4, 10, 16, and 19, respectively) while PKCβ1 and PKCδ reduced its basal expression (Figure 3B, columns 7, and 13, respectively). In cells that were treated with either cisplatin or Taxol, all tested PKC isoforms did not show any stimulatory effect on ERE expression (Figure 3B).

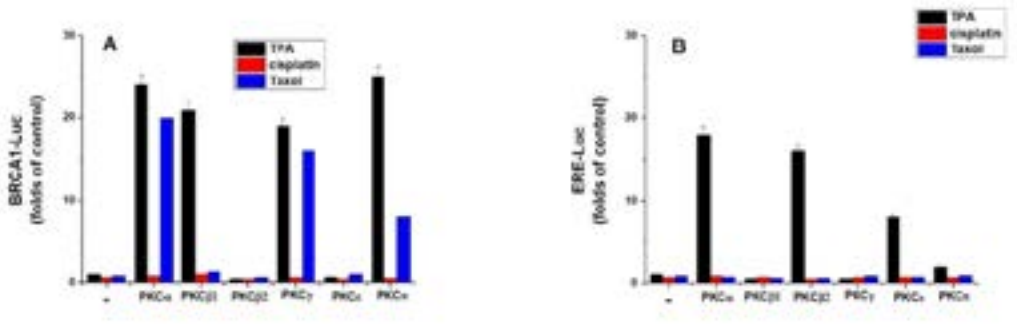

Figure 3: Effect of PKC isoforms on BRCA1 and ERE expression in cells treated with the examined stress inducing agents. Cells were transfected with plasmids expressing either BRCA1-Luc (1µg) (A) or ERE-Luc (1µg) (B) respectively, treated with 50nM TPA for 48hs in order to deplete all cellular PKCs. Then these cells were transfected with plasmids expressing different PKC isoforms (1µg) and treated with 50nM TPA, 2µM Taxol or 5µM cisplatin at the time of transfection. The cells were harvested at 24h post transfection for analyzing the reporter expression. The presented results are an average of three repeated experiments ± SE.

HTLV-1 Tax Involvement in the Effect of The Examined Stress-Inducing Agents on The Expression of BRCA1 And ERE

As published previously, HTLV-1 Tax strongly blocks the activation of BRCA1 and enhances the activation of ERE-dependent genes induced by E2 [28]. In this study, we examined the possible involvement of HTLV-1 Tax in the effects of stress-inducing agents on the expression of BRCA1 and ERE. For that, MCF-7 cells were treated with either TPA, cisplatin, or Taxol at the time of transfection with BRCA1-Luc or ERE-Luc. Some of these cells were also transfected with a plasmid expressing HTLV-1 Tax, with or without E2 treatment. The results showed that, as expected, E2 strongly induced BRCA1 expression (Figure 4A, column 5), and HTLV-1 Tax strongly inhibited this E2-induced expression (Figure 4A, column 13). It can also be seen that both TPA and Taxol have an additive effect with E2 (Figure 4A, columns 6 and 8), while cisplatin completely inhibited E2-induced BRCA1 expression (Figure 4A, column 7). While HTLV-1 Tax has no significant effect on TPA-induced activation of BRCA1, it strongly inhibited Taxol-induced activity on BRCA1 (Figure 4A, columns 10 and 12, respectively). In addition, it seems that both cisplatin and Tax have antagonistic effects on each other. While each of them strongly inhibits E2-induced activation of BRCA1 (Figure 4A, columns 7 and 13, respectively), they had no effect on E2-induced activation of BRCA1 when the cells were simultaneously treated with both of them (Figure 4A, column 15) (Figure 4).

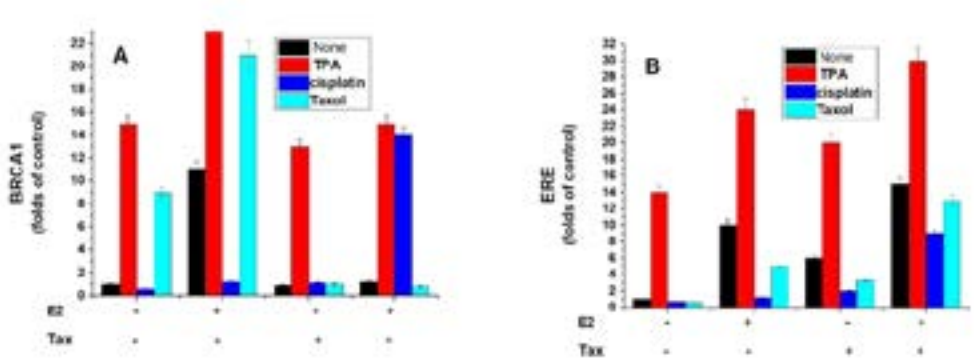

Figure 4: HTLV-1 Tax involvement in the effect of the examined stress-inducing agents on the expression of BRCA1 and ERE. MCF-7 were treated with either TPA, cisplatin, or Taxol at the time of transfection with BRCA1-Luc (A) or ERE-Luc (B). Some of these cells were transfected also with a plasmid expressing HTLV-1 Tax with or without treatment with estrogen (E2). The presented results are an average of three repeated experiments ± SE. (C) MCF-7 cells were treated with cisplatin or Taxol for 24 hours. The cells were then harvested, and the protein level was measured by Western Blot analysis using appropriate monoclonal antibodies.

In the case of ERE, TPA, E2, and Tax significantly induced ERE expression (Figure 4B, columns 2, 5, and 9, respectively), with synergistic activity between them (Figure 4B, columns 6 and 14). In contrast, both cisplatin and Taxol have significant inhibitory effects on the basal activity of ERE-regulated genes (Figure 4B, columns 3 and 4, respectively) and on the induced activation of ERE either by E2 or Tax, as can be seen in Figure 4Bb, columns 7, 8 and 11, 12, respectively. It is also worth noting that, in the case of ERE, an antagonistic effect between cisplatin and Tax can be observed when both are added to cells together with E2, and neither influences E2-induced activity (Figure 4B, column 15).

Effect Of the Examined Stress-Inducing Agents on the Mrna Expression of BRCA1 And Ps2 Genes

Trying to modify which step of BRCA1 and pS2 activation is affected by these stress agents, the effect of these agents on BRCA1 and pS2 mRNA expression was examined. For that, control and Tax-transfected MCF-7 cells were treated with the tested stress agents (TPA, cisplatin, or Taxol) for 24h, and their RNA was extracted and their mRNA reverse-transcribed into cDNA. The levels of BRCA1 and pS2 mRNA were determined by RT-PCR. The results showed that, in agreement with those presented in Figure 4, both TPA and Taxol significantly increased the mRNA levels of BRCA1, while cisplatin reduced its level (Figure 5A, lanes 2, 4, and 3, respectively). While HTLV-1 Tax, as expected, caused a significant reduction both in the basal and the Taxol-induced expression of BRCA1mRNA, it did not affect the stimulatory effect of TPA on BRCA1 mRNA expression (Figure 5A, lanes 5, 8, and 6, respectively). On the other hand, and due to the mentioned above antagonistic effect between Tax and cisplatin, adding both together did not significantly affect the basal expression of BRCA1 mRNA (Figure 5A, lane 7). In the case of pS2, TPA significantly induced its mRNA expression, while Taxol had only a minor stimulatory effect on pS2 mRNA expression, and Cisplatin significantly reduced its basal expression (Figure 5B, lanes 2, 4, and 3, respectively). Tax and TPA showed a synergistic effect on pS2 mRNA expression, while Tax significantly reduced the stimulatory effect of Taxol on pS2 mRNA expression (Figure 5B, lanes 6 and 8, respectively). As expected, Tax and cisplatin antagonized each other also in this case (Figure 5B, lane 7).

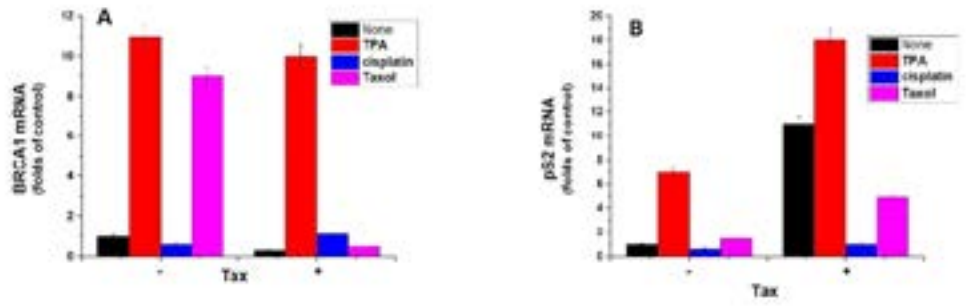

Figure 5: Effect of the examined stress inducing agents on the mRNA expression of BRCA1and pS2 genes. Control and Tax transfected MCF-7 were treated with the tested stress agents (TPA, cisplatin or Taxol) for 24h and their RNA was extracted and their mRNA was reverse transcribed into cDNA. The amount of BRCA1 and pS2 mRNA was determined by RT-PCR. The presented results are an average of three repeated experiments ± SE.

Effect of the Examined Stress-Inducing Agents On Erα Binding to ERE And BRCA1 Promoter

Trying to find out whether ERα is involved in the effect of the examined stress-inducing agents (TPA, cisplatin, and Taxol) on the activation of BRCA1or ERE expression, we examined the effect of these agents on the binding of ERα to the AP-1 DNA site at the BRCA1 promoter and to the ERE region by ChIP analysis. For that, MCF-7 cells were treated with each of the tested stress-inducing agents and with E2 as a positive control. 24h after treatment, cells were harvested, and ERα binding to either the BRCA1 promoter or the ERE was determined by CHIP assay. Our results showed that while E2, as expected, strongly induced the binding of ERα to either the BRCA1 promoter (Figure 6a) or to the ERE region (Figure 6B), all examined stress-inducing agents (TPA, cisplatin, and Taxol) had no effect on the binding of ERα to these promoters.

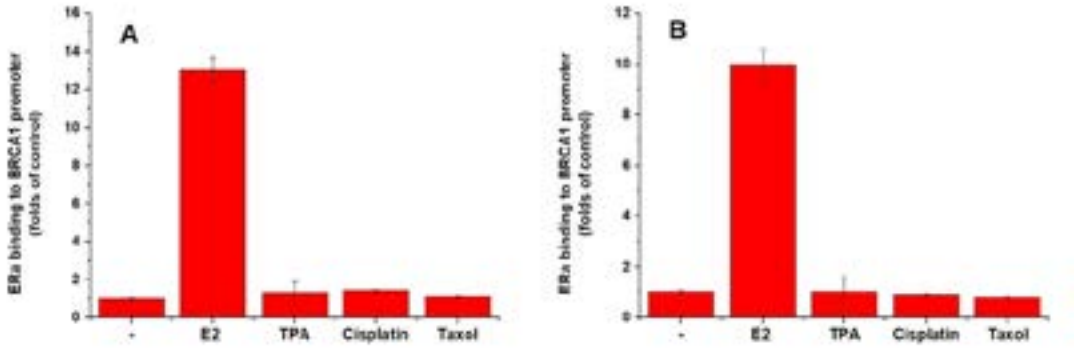

Figure 6: Effect of the examined stress inducing agents on ERα binding to ERE and BRCA1 promoter. MCF-7 cells were treated with each of the tested stress inducing agents and with E2 and at 24h after treatment the cells were extracted and the binding of ERα protein to either BRCA1 promoter (A) or to ERE (B) was determined by CHIP assay as described in Materials and Methods section. The presented results are an average of three repeated experiments ± SE.

Discussion

Stress-inducing agents, such as TPA, are known to induce HTLV-1 expression [28,41-43]. Our previous studies indicated that HTLV-1 is involved in BC development, as HTLV-1 Tax can block BRCA1 expression and stimulate genes that enhance cell replication [28]. Thus, based on these findings, we investigated, in the present study, the effects of various stress-inducing agents (TPA, cisplatin, and Taxol) on BRCA1 and ERE expression. TPA, as expected, strongly stimulated the expression of both BRCA1 and genes regulated by promoters containing ERE sequences when treatment was short (2-24h), whereas longer treatment inhibited their expression. These results agree with our previous findings [33], suggesting that this activity is PKC-dependent. They are interesting because TPA is involved in various tumor types [34,35,45], whereas BRCA1 is a strong tumor suppressor, mainly in ovarian or mammary tumors [46,47]. Therefore, exploring the possible benefits of TPA as a preventive or therapeutic treatment against ovarian or mammary tumors is highly important. It should be emphasized that TPA, on the other hand, enhances the expression of genes regulated by ERE sequences, which can promote cell replication and, by various mutations, probably tumor development [48]. These results are supported by previous studies, which showed that TPA enhanced mRNA expression of pS2 and other E2-responsive genes [48-50]. Also, Taxol strongly induced BRCA1 activation over time, without a significant effect on ERE expression. In contrast, cisplatin slightly reduced basal expression of both BRCA1 and ERE, regardless of treatment time. Thus, both TPA and Taxol may be potential candidates against the development of breast and ovarian cancers. These findings may be supported by the potential use of Taxol as an anticancer drug [51-53]. In contrast, cisplatin slightly inhibits BRCA-1 promoter expression, a finding that may not be supported by its use as an anticancer drug against BC [52,54,55] and other cancers [56]. The antitumor effect of cisplatin seems unrelated to the BRCA-1 pathway. Nonetheless, it is worth noting that cisplatin inhibits the expression of genes regulated by ERE sequences, which may help prevent the development of cancer cells, as some of these genes strongly stimulate cell proliferation [57,58]. It is interesting that both TPA and Taxol-induced activation of BRCA-1 is mediated by PKC activity. TPA, in contrast to Taxol, is known as a potent PKC activator [35]; therefore, it is not surprising that its effect on BRCA-1 expression is related to PKC activity. The mechanism by which PKC is involved in Taxol activation of BRCA-1 expression appears to differ from that of TPA. It is interesting that previously, it was shown that a PKC inhibitor promotes the antitumor activity of Taxol in Triple-negative breast cancer (TNBC), which is a subtype of BC lacking targeted therapy [59]. Most interesting is that the PKC isoforms (PKCα, δ, and η) highly induce the expression of BRCA1 in cells that were treated with either TPA or Taxol (Figure 3).

Due to the fact that the HTLV-1 virus can reach the breast of infected women through breast-feeding milk, which is rich with HTLV-1-infected lymphocytes, it may infect the epithelial cells of the breast [60] and interfere with the activity and possibly with the expression of important genes in the breast cells, such as the BRCA-1 gene. Indeed, in our group's previous study, Tax was found to strongly inhibit E2-induced BRCA-1 expression while stimulating E2-induced ERE-dependent genes in breast epithelial cells and inhibiting at least part of BRCA-1 activities [27,28]. Also, environmental stressors such as TPA, cisplatin, and Taxol can activate HTLV-1 from its latent state [32,44]. Our results in the present study showed that Tax was unable to inhibit the TPA-induced acceleration of BRCA-1 expression, whereas it strongly inhibited Taxol-stimulated BRCA-1 expression. Based on our

previous findings, which showed that Tax was able to completely prevent E2-induced BRCA1 expression through the non-classical pathway of ERα, there is a possibility that the Taxol effect on BRCA1 expression is also through the ERα pathway, while the TPA effect is not through this pathway. However, it should be mentioned that, in addition to the pathway of ERα activation by E2, there are other possible transduction pathways that can activate ERα without E2 [61-63]. Accordingly, it is important to mention that signals that activate receptors may lead to phosphorylation or change the phosphorylation of the receptors or associated proteins, which may be important for ligand-independent activation [48].

On the other hand, it seems that cisplatin and Tax have antagonistic effects on each other, while each, separately, inhibits the E2-induced acceleration of BRCA-1 expression; together, they fail to eliminate this effect. To determine whether the effects of the examined stress agents on BRCA1 and ERE activation are mediated by ERα pathways, we examined their effects on ERα binding to the BRCA1 promoter and to ERE sequences. Our results showed that none of the tested stress-inducing agents (TPA, cisplatin, and Taxol) has any effect on the binding of ERα to these promoters. These results exclude the possible effects of these agents on BRCA1 and ERE expression via ERα signaling pathways. In conclusion, both TPA and Taxol may be considered potential candidates for the prevention and treatment of breast and ovarian cancer.

Acknowledgement

None.

Conflicts of Interest

None.

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