Original Article
Effect of adriamycin on BRCA1 and PARP-1 expression in MCF-7 breast cancer cells

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Abstract: To study the effects of adriamycin on the expression of BRCA1 and PARP-1 in BRCA1 wild-type MCF-7 cells. We used Western blotting to detect BRCA1 and PARP-1 levels in MCF-7 cells treated with adriamycin, and used flow cytometry to detect apoptotic MCF-7 cells. Results showed that adriamycin can increase PARP-1 activation in a dose- and time-dependent manner. BRCA1 levels were also increased upon treatment with a high dose of adriamycin, but gradually decreased over time. Treatment of MCF-7 cells with 3-ABA inhibited PARP-1 activity, but had no effect on BRCA1 levels. Compared to adriamycin and 3-ABA treatment alone, the co-treatment can increase the apoptosis of MCF-7 cells. Compared to BRCA1-defective HCC1937 cells, adriamycin combined with 3-ABA can further induce apoptosis of MCF-7 cells ($P < 0.05$). All of these suggested that adriamycin can affect the PARP-1 activation and the expression of BRCA1. Combined with 3-ABA has an additive effect on the rate of apoptosis observed.

Keywords: MCF-7, adriamycin, BRCA1, PARP-1

Introduction

Chemotherapy is important for breast cancer treatment and drug resistance can inhibit the effectiveness of these agents. The emergence of drug resistance is closely related to the dys-regulation of the DNA-damage repair proteins [1]. Both breast cancer susceptibility 1 (BRCA1) and poly (ADP-ribose) polymerase (PARP-1) play an important role during DNA-damage repair [2].

Germline mutations in the breast cancer sus-ceptibility genes BRCA1 have been linked to the development of breast cancer [3]. Recent studies suggest that BRCA1 and PARP-1 maybe function in the sensing and/or repair of DNA damage. The first PARP enzyme was discovered over 40 years ago and PARP-1 is the most abundant and best-characterized member of the PARP family of enzymes [4]. PARP-1 has a key role in the repair of single-strand breaks (SSBs), resulting from oxidative stress via the base excision repair/SSB repair (BER/SSBR) pathway [5]. It has been reported that ADR can induce decreases in BRCA1 protein and/or mRNA in MCF-7 [6]. However, in this study, we found that the DNA-damaging agent, adriamy-cin, can upregulate BRCA1 with low does, but down-regulate BRCA1 with high does, and indu-ce PARP-1 activation.

Materials and methods

Cell culture

MCF-7 (wild BRCA1) and HCC1937 (mutant BRCA1) breast cancer cell lines were purchased from the Cell Bank of Type Culture Collection of Chinese Academy of Sciences, Shanghai, China. The cells were cultured in High glucose Dul-becco's modified Eagle's medium (DMEM), con-taining 10% heat-inactivated fetal bovine serum (FBS), 100 U/mL penicillin, 100 U/ml strepto-mycin, and 2 mmol/L L-glutamine, at 37°C in a humidified 5% CO_2 atmosphere.

Cell treatments

Cells were plated into 35 mm dishes (MCF-7, 3 × 10^5; HCC1973, 5 × 10^5) and were treated the following day with 0.05, 0.1, 0.5, and 1, 2
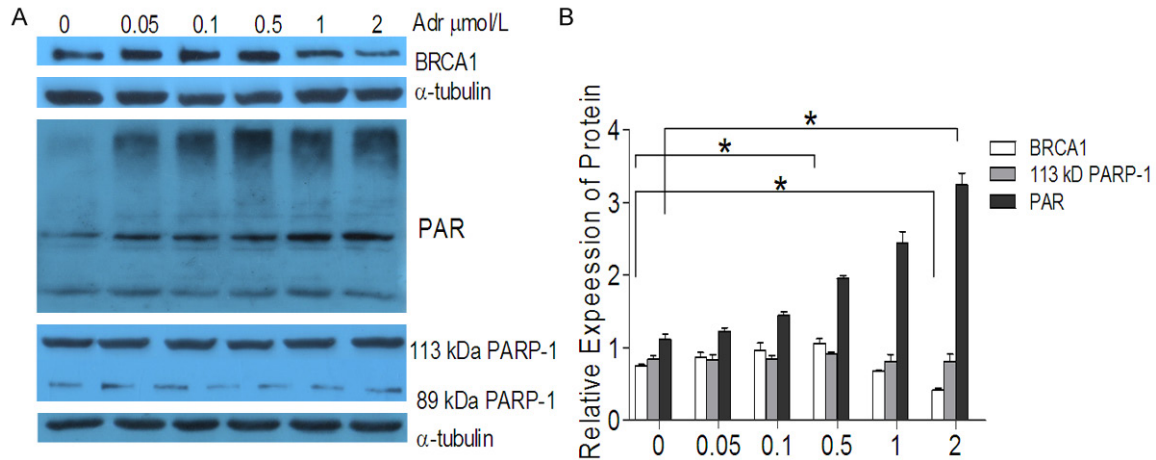
Figure 1. PARP-1 activation and the change of BRCA1 expression after different concentrations of ADR treatment with ADR 24 h, and recovery 12 h were detected by Western blotting in MCF-7 cells. A. The expression of BRCA1 performed the trend of increastion first then reduction, in the concentration of 0.05, 0.1, 0.5, 1 μmol/L, the expression of BRCA1 increased, in 2 μmol/L, the expression was less than front concentration groups and control group. ADR induced PARP-1 activation and showed dose dependent. B. Image-Pro plus 6.0 detected the relative expression of BRCA1 and PARP-1 and PARP-1 actviation. *$P < 0.05$, compared with control group (BRCA1); *$P < 0.05$, compared with control group (PAR); $P > 0.05$, compared with control group; n = 3.

μmol/L ADR, respectively, for 24 hours, followed by 12 h of recovery. Cells were then collected, and total proteins were extracted with 1 × cell lysis buffer (20 mM Tris-HCl pH 7.5, 150 mM NaCl, 1 mM Na_2EDTA, 1 mM EGTA, 1% Triton, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na_3VO_4, and 1 μg/mL leupeptin). The expression levels of BRCA1, PARP-1 and PARP activation were detected by Western blot with corresponding antibodies. The control cells were handled similarly without ADR treatments. Each treatment had 3 replicates for each experiment. Cells treated with 1 μM ADR for 24 h and recovered for 6, 12, 24, and 48 h were collected for Western blot analysis. The control cells had 0 h recovery. Each treatment had 3 replicates for each experiment. All cells were collected for Western blot analysis to detect the expression levels of related proteins. Each treatment had 3 replicates for each experiment. Cells were plated and treated with 2.5, 5, 10, and 20 μM PARP-1 inhibitor (3-Aminobenzamide, 3-ABA) (Amresco) dissolved in DMSO for 24 h, followed by 12 h of recovery. Cells were collected for Western blot analysis of BRCA1 and PARP-1 proteins. Cells without 3-ABA treatment were used as control. Each treatment had 3 replicates for each experiment. Flow cytometry was used to detect cell apoptosis after the treatment of 5 μM

3-ABA with 1 μM ADR for 24 h, followed by 12 h of recovery.

Western blot analysis

Cells were detached from the culture dish, washed with PBS, and lysed in 100 μL lysis buffer for 10 min on ice. The pellet was removed, and sample buffer was added to the supernatant. Proteins were resolved by SDS/PAGE (8% gel) and transferred onto poly-vinylidene difluoride (PVDF) membrane. The blot was blocked with 5% (w/v) non-fat powdered milk in PBS containing 0.1% Tween for 1 h, washed with PBS/Tween, incubated overnight with antibodies against BRCA1 (Santa Cruz), PARP-1 (Cell Signaling), PAR (SAB) and a-tubulin (Sigma), and incubated for 2 h with appropriate secondary antibody. Protein bands were visualized by ECL-plus (Amersham Biosciences), and pictures were taken with the Chemi-Doc XPS imaging system.

Detection of apoptosis

The treated cells were washed twice with cold PBS, before being harvested and resuspended in 1 × binding buffer. Five μL FITC Annexin V and 5 μL PI (propidium iodide) were added to the suspension and incubated for 10 min at RT in
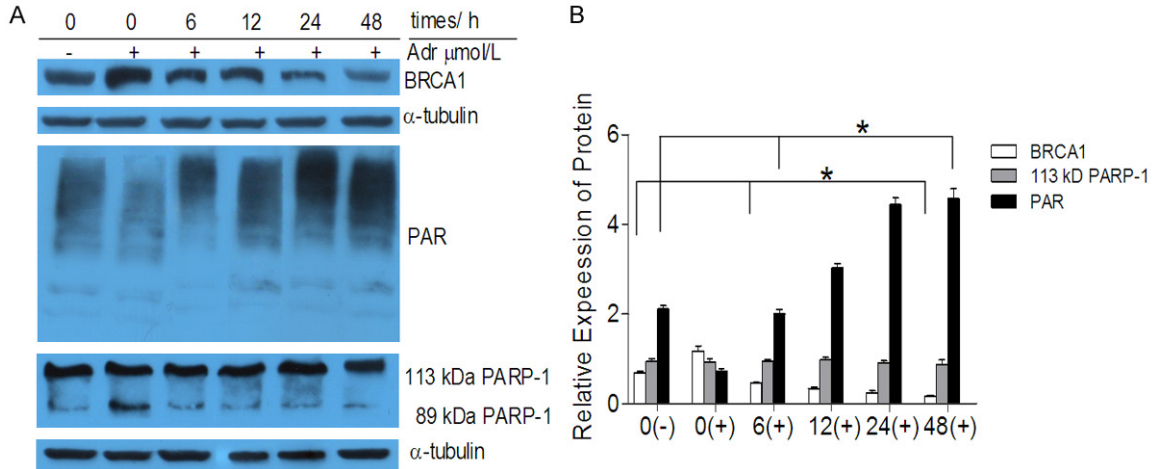
Figure 2. PARP-1 activation and the change of BRCA1 expression after 1 µmol/L ADR treatment with recovery different time. After treatment with 1 µmol/L ADR (24 h) and recovery different time (0, 6 12, 24, 48 h), cells were harvested and analyzed by Western blot for PAR formation and BRCA1expression. A. Decreased level for BRCA1 were detected as early as 6 h after ADR treatment and recovery, but these alterations were much more prominent after long times. PARP-1 activation showed time dependence, but with a prominent increase observed by 6 h after ADR treatment and recovery. B. Relative expression levels of the three proteins determined by Image-Pro plus 6.0 in MCF-7. *$P < 0.05$, compared with the 0 µmol/L doxorubicin group & the 0 recovery time group (BRCA1); *$P < 0.05$, compared with the 0 µmol/L doxorubicin group & the 0 recovery time group (PAR); $P > 0.05$, compared with the 0 µmol/L doxorubicin group & the 0 recovery time group (113 kDa PARP-1); n = 3.
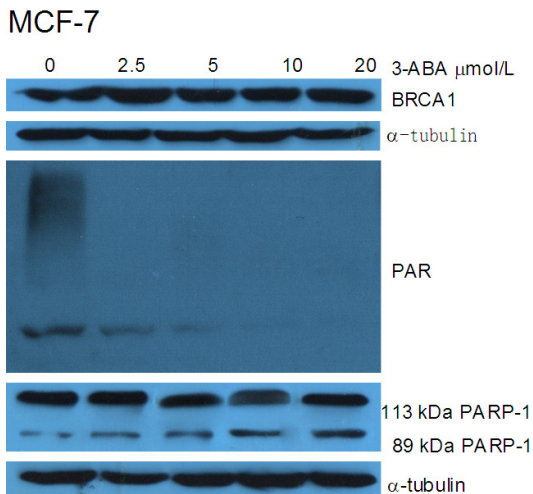

Figure 3. PARP-1 inhibitor 3-ABA treatment inhibits PARP-1 activation. 3-ABA significantly inhibited PAR formation after 24 h treatments of 2.5, 5, 10, and 20 µmol/L of 3-ABA for 24 h and 24 h of recovery in MCF-7 cells. And the results also showed that 3-ABA didn't affect the expression of BRCA1 and the full-length PARP-1. Cells were harvested and analyzed by Western blot, and α-tubulin was used as the loading control.

the dark, before 400 µL 1 × binding buffer was added to each tube for flow cytometry analysis (Becton Dickinson) within 1 h.

Statistical analysis

Image-Pro plus 6.0 was used to quantify the bands from western blotting. Results were presented as means ± S.D. Thet-test was performed using the Statistical Package for Social Science (SPSS for Windows package release 17.0) and indicated in Results and Figure legends. $P < 0.05$ was considered as statistically significant. *$P < 0.05$; **$P < 0.01$.

Results

ADR treatment induces PARP-1 activation and decreases BRCA1 expression in MCF-7 cells

It has been shown in many different cells that infringing DNA damage activates PARP-1 [7]. The impact of DNA damaging agent, ADR, on PARP-1 activity remains unknown. Additionally, BRCA1 has been implicated in the process of apoptosis and both BRCA1 and BRCA2 may be involved in DNA repair [8]. We examined the expression of BRCA1 and PARP-1 following treatment of MCF-7 human breast cancer cells with ADR. Cells were treated with 0, 0.05, 0.1, 0.5, 1, 2 µM ADR for 24 h, and examined by Western blot. No obvious cytotoxicity was observed in cells treated with ADR. ADR initially increased BRCA1 levels, but its level was
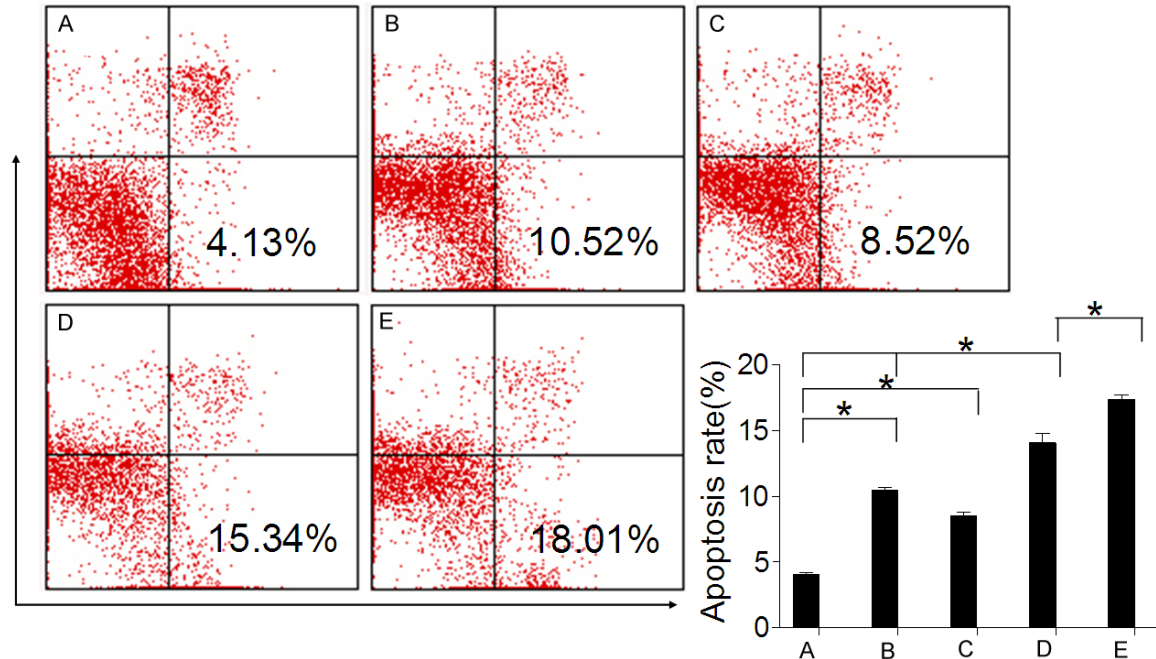
Figure 4. Effect of PARP-1 inhibition on cell apoptosis after ADR treatment. Cell apoptosis of MCF-7 cell lines, after co-treatment 24 h with 1 µM ADR and 5 µM 3-ABA, and 12 h recovered, measured by FCM. D. The PARP-1 inhibitor 3-ABA significantly increased the cell apoptosis induced by ADR inMCF-7 cells; E. and 3-ABA can further increase the HCC1937 cells apoptosis compared with MCF-7 cells induced by ADR. *$P < 0.05$, compared with the control group (MCF-7); *$P < 0.05$, compared with the ADR MCF-7 cell lines group & the control group; *$P < 0.05$, compared with the ADR + 3-ABA MCF-7 cell lines group; n = 3. (A: Control group MCF-7, B: ADR-treated MCF-7, C: 3-ABA-treated MCF-7, D: ADR and 3-ABA co-treated HCC1937, E: ADR and 3-ABA co-treated MCF-7).

reduced over time. Low BRCA1 expression was induced using high dose ADR compared to low dose ADR (**Figure 1A**). We hypothesized that the activation of PARP-1 (PAR) may counteract the ADR-induced cytotxicity by promoting DNA repair. First, we demonstrated that ADR induced PARP-1 activation, PAR ($P < 0.05$) (**Figure 1A**), but did not effect levels of full-length and cleaved PARP-1 ($P > 0.05$) (**Figure 1A**).

ADR treatment decreases BRCA1 and induces PARP-1 activation

MCF-7 cells were treated with ADR (1 µM) and cells were collected at different time points to assess BRCA1 level and PARP-1 activation. Decreased levels of BRCA1 were detected as early as 6 h after ADR treatment and recovery, but these alterations were much more prominent with time (**Figure 2A**). PARP-1 activation occurred in a time-dependent manner, with a prominent increase by 6 h after ADR treatment and recovery (**Figure 2A**). In MCF-7 cells containing WT p53, signifcant down-regulation of BRCA1 was observed by 6 h and with time after ADR treatment and recovery. Signifcant up-reg-

ulation of PARP-1 activation was also observed. In all treatment conditions, PARP-1 cleavage fragments were observed without significant changes in full-length PARP-1 ($P > 0.05$) (**Figure 2A**).

No effects of PARP-1 inhibitor on BRCA1 expression

PARP-1 inhibitor, 3-aminobenzamide (3-ABA), inhibits the activity of poly (ADP-ribose) transferase enzymes, and can prevent ADR-induced PARP-1 activation. PARP-1 inhibitor has potential clinical application [9]. We used 2.5, 5, 10, and 20 µM 3-ABA to treat MCF-7 cells for 24 h, followed by 12 h of recovery. As shown in **Figure 3**, 3-ABA inhibited the expression of PAR but did not affect the expression of BRCA1 and the full-length PARP-1.

Co-treatment of MCF-7 and HCC1937 cells with doxorubicin and 3-ABA potentiates apoptosis cell death

MCF-7 cells normally express WT BRCA1. To determine the different in the ability of DNA-

damage repair between BRCA1 and PARP-1, we compared HCC1937 cells with mutant BRCA1 (BRCA1$^{-/-}$) [10]. We further determined whether changes in BRCA1 and PARP-1 activation induced by adriamycin might be directly related to its cytotoxicity. MCF-7 and HCC1937 cells were co-treated with 5 µM 3-ABA and 1 µM ADR for 24 h and recovered for 12 h, and apoptosis was detected by FCM. We observed that the degree of apoptosis was greater in MCF-7/WT BRCA1 cells than in HCC1937 parental cells (**Figure 4**).

Discussion

Breast cancer is one of the most common and important diseases affecting women. More than 40%-50% of the hereditary breast cancers are linked to germ-line breast cancer (BRCA1/2) gene mutations [11]. The breast cancer susceptibility gene, *BRCA1*, an important determinant of response to DNA-damaging agents, has been is linked to the resistance of tumor cells to ionizing radiation (IR) or other DNA-damaging agents due to up-regulation of DNA-damage repair and damage-induced checkpoint controls [12]. BRCA1 is a tumor suppressor gene. It has been shown that upregulation of BRCA1 expression leads to increased resistance to CDDP in ovarian cancer cells, but it was different to DNA helix intercalator and topoisomerase II inhibitor ADR [13]. Poly (ADP-ribose) polymerases (PARPs) play a key role in the repair of base damage via the base excision repair pathway [14]. Pharmacological inhibition of PARP induces cell death in tumors with mutations in certain DNA repair pathways, such as the BRCA pathways of double-strand break repair, and when combined with chemotherapies that cause DNA damage [15]. ADR has long been considered one of the most active agents in the treatment of breast cancer [16]. ADR causes DNA double strand and single strand breaks by inhibiting topoisomerase II [17]. It is also a powerful iron chelator. Iron induces cell death by binding to DNA and producing free radicals that immediately cleave DNA and disrupt cell membranes.

Although, it has been reported that ADR can induce decreases in BRCA1 protein and/or mRNA in MCF-7 [6, 18], in this study, we first demonstrated that BRCA1 levels decrease upon treatment with high dose cytotoxic agents ADR but increase upon treatment with low does

ADR in MCF-7 cells. Time-dependent decrease of BRCA1 was detected as early as 6 h after 1 µmol/L ADR treatment and recovery, but these alterations were much more prominent over time. Dose and time dependent up regulation of PARP activation protein were first observed as early as 6 h after treatment and 6 h recovery. The ADR-induced down-regulation of BRCA1 did not appear to be directly related to the induction of apoptotic DNA fragmentation or cell death in HCC1937 cells (BRCA1$^{-/-}$). Prominent apoptosis of MCF-7 cells with wild-type BRCA1 was observed with ADR.

BRCA1 encodes the tumor suppressor protein that acts as a negative regulator of tumor growth [19]. The functional BRCA1 protein is present in normal breast epithelial tissue and is reduced or absent in some breast tumors. Therefore, BRCA1 may function in monitoring genomic integrity and in DNA repair [20]. Many chemotherapeutic agents including ADR induce DNA damage in mammalian cells and eventually deregulate the growth control or cause distractions in DNA metabolisms. Our results show that ADR can down-regulation BRCA1 at high dose, but with time dependent at 1 µmol/L ADR treatment and increased PARP-1 activation in a dose- and time-dependent manner. Low expression of BRCA1 is associated with decrease DNA-damage repair activity which may allow cells to be more susceptible to undergo apoptosis. PARP-1 activation can enhance DNA-damage repair and decrease cell apoptosis. For ADR treatment, compared with DNA repair, DNA damage plays a more important role. Thus, if BRCA1 is mutated or deleted, PARP-1 activation would play a more important role in DNA damage repair. Inhibiting PARP-1 activation will increase tumor cells apoptosis. Poly (ADP-ribose) polymerase inhibition enhances DNA damage responses induced by DNA damaging agent, especially with BRCA1 mutation or deletion. PARP inhibitors are being investigated as a monotherapy for the treatment of patients with BRCA1/2 mutations [21]. It is also investigated for triple-negative breast cancers because of its molecular similarities to BRCA1-mutated malignancies [22]. PARP inhibitors may be a potential therapeutic strategy to potentiate the DNA-damaging effects of chemotherapy and radiation [23].

In conclusion, we observed that the DNA-damaging agent, ADR, induced down-regulation

of BRCA1 and up-regulation PARP-1 activation in MCF-7 cell lines. The sensitivity of MCF-7 cells with BRCA1$^{-/-}$ to adriamycin-induced cell apoptosis. Abrogating the cell-cycle arrest induced by PARP inhibition plus chemotherapies may be a strategy in the treatment of BRCA-proficient cancer. Further research is required to establish the mechanism(s) and functional significance of down-regulation of BRCA1 and up-regulation of PARP-1 activation.

Acknowledgements

Disclosure of conflict of interest

None.

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References

[1] Chitikova ZV, Gordeev SA, Bykova TV, Zubova SG, Pospelov VA and Pospelova TV. Sustained activation of DNA damage response in irradiated apoptosis-resistant cells induces reversible senescence associated with mTOR down-regulation and expression of stem cell markers. Cell Cycle 2014; 13: 1424-1439.

[2] Jacot W, Thezenas S, Senal R, Viglianti C, Laberenne AC, Lopez-Crapez E, Bibeau F, Bleuse JP, Romieu G and Lamy PJ. BRCA1 promoter hypermethylation, 53BP1 protein expression and PARP-1 activity as biomarkers of DNA repair deficit in breast cancer. BMC Cancer 2013; 13: 523.

[3] Gabaldo Barrios X, Sarabia Meseguer MD, Alonso Romero JL, Marin Vera M, Marin Zafra G, Sanchez Henarejos P, Sanchez Bermudez AI and Ruiz Espejo F. Novel BRCA1 deleterious mutation (c.1918C>T) in familial breast and ovarian cancer syndrome who share a common ancestry. Fam Cancer 2014; [Epub ahead of print].

[4] Morales J, Li L, Fattah FJ, Dong Y, Bey EA, Patel M, Gao J and Boothman DA. Review of poly (ADP-ribose) polymerase (PARP) mechanisms of action and rationale for targeting in cancer and other diseases. Crit Rev Eukaryot Gene Expr 2014; 24: 15-28.

[5] Tinker AV and Gelmon K. The role of PARP inhibitors in the treatment of ovarian carcinomas. Curr Pharm Des 2012; 18: 3770-3774.

[6] Andres JL, Fan S, Turkel GJ, Wang JA, Twu NF, Yuan RQ, Lamszus K, Goldberg ID and Rosen EM. Regulation of BRCA1 and BRCA2 expression in human breast cancer cells by DNA-damaging agents. Oncogene 1998; 16: 2229-2241.

[7] Weaver AN and Yang ES. Beyond DNA Repair: Additional functions of PARP-1 in cancer. Front Oncol 2013; 3: 290.

[8] Rosen EM and Pishvaian MJ. Targeting the BRCA1/2 tumor suppressors. Curr Drug Targets 2014; 15: 17-31.

[9] Sinha G. Downfall of iniparib: a PARP inhibitor that doesn't inhibit PARP after all. J Natl Cancer Inst 2014; 106: djt447.

[10] Tassone P, Blotta S, Palmieri C, Masciari S, Quaresima B, Montagna M, D'Andrea E, Eramo OP, Migale L, Costanzo F, Tagliaferri P and Venuta S. Differential sensitivity of BRCA1-mutated HCC1937 human breast cancer cells to microtubule-interfering agents. Int J Oncol 2005; 26: 1257-1263.

[11] Bernard-Gallon DJ, Dechelotte PJ, Le Corre L, Vissac-Sabatier C, Favy DA, Cravello L, De Latour MP and Bignon YJ. Expression of BRCA1 and BRCA2 in male breast cancers and gynecomastias. Anticancer Res 2003; 23: 661-667.

[12] Thompson LH. Recognition, signaling, and repair of DNA double-strand breaks produced by ionizing radiation in mammalian cells: the molecular choreography. Mutat Res 2012; 751: 158-246.

[13] Tassone P, Tagliaferri P, Perricelli A, Blotta S, Quaresima B, Martelli ML, Goel A, Barbieri V, Costanzo F, Boland CR and Venuta S. BRCA1 expression modulates chemosensitivity of BRCA1-defective HCC1937 human breast cancer cells. Br J Cancer 2003; 88: 1285-1291.

[14] Das BB, Huang SY, Murai J, Rehman I, Ame JC, Sengupta S, Das SK, Majumdar P, Zhang H, Biard D, Majumder HK, Schreiber V and Pommier Y. PARP1-TDP1 coupling for the repair of topoisomerase I-induced DNA damage. Nucleic Acids Res 2014; 42: 4435-4449.

[15] May T, Yang J, Shoni M, Liu S, He H, Gali R, Ng SK, Crum C, Berkowitz RS and Ng SW. BRCA1 expression is epigenetically repressed in sporadic ovarian cancer cells by overexpression of

C-terminal binding protein 2. Neoplasia 2013; 15: 600-608.

[16] Mittal N, Gupta M and Singla M. Cutaneous adverse drug reactions notified by pharmacovigilance in a tertiary care hospital in north India. Cutan Ocul Toxicol 2014; [Epub ahead of print].

[17] Lage H, Aki-Sener E and Yalcin I. High antineoplastic activity of new heterocyclic compounds in cancer cells with resistance against classical DNA topoisomerase II-targeting drugs. Int J Cancer 2006; 119: 213-220.

[18] Su J and Ciftci K. Changes in BRCA1 and BRCA2 expression produced by chemotherapeutic agents in human breast cancer cells. Int J Biochem Cell Biol 2002; 34: 950-957.

[19] Elia AE and Elledge SJ. BRCA1 as tumor suppressor: lord without its RING? Breast Cancer Res 2012; 14: 306.

[20] Savage KI, Matchett KB, Barros EM, Cooper KM, Irwin G, Gorski JJ, Orr KS, Vohhodina J, Kavanagh JN, Madden AF, Powell A, Manti L, McDade SS, Park BH, Prise KM, McIntosh S, Salto-Tellez M, Richard DJ, Elliot CT and Harkin DP. Brca1 Deficiency Exacerbates Estrogen Induced DNA Damage and Genomic Instability. Cancer Res 2014; 74: 2773-84.

[21] Horton JK and Wilson SH. Strategic Combination of DNA-damaging agent and PARP inhibitor results in enhanced cytotoxicity. Front Oncol 2013; 3: 257.

[22] Al-Ejeh F, Shi W, Miranda M, Simpson PT, Vargas AC, Song S, Wiegmans AP, Swarbrick A, Welm AL, Brown MP, Chenevix-Trench G, Lakhani SR and Khanna KK. Treatment of triple-negative breast cancer using anti-EGFR-directed radioimmunotherapy combined with radiosensitizing chemotherapy and PARP inhibitor. J Nucl Med 2013; 54: 913-921.

[23] Gilardini Montani MS, Prodosmo A, Stagni V, Merli D, Monteonofrio L, Gatti V, Gentileschi MP, Barila D and Soddu S. ATM-depletion in breast cancer cells confers sensitivity to PARP inhibition. J Exp Clin Cancer Res 2013; 32: 95.