Estrogen-dependent cell signaling and apoptosis in BRCA1-blocked BG1 ovarian cancer cells in response to plumbagin and other chemotherapeutic agents

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Background: Cellular response to chemotherapeutic drugs in the absence of BRCA1 either completely or partially had drawn less attention. The present study evaluated whether there is a differential inhibition of cell growth by selected compounds with respect to BRCA1 status in estrogen receptor (ER)-positive ovarian cancer cells.

Materials and methods: The BG1 ovarian cancer cells used in the experiments were antisensely blocked with BRCA1 gene. Growth inhibition and apoptotic induction were analyzed to evaluate the cytotoxic effects. Small interfering RNA (SiRNA) transfection, western blot analysis, RT–PCR analysis and molecular modeling were carried out to analyze the estrogen-dependent action of plumbagin.

Results: Although we found that all the compounds studied induce apoptosis, the induction was in the order of plumbagin > doxorubicin > tamoxifen > cisplatin. Plumbagin can bind to the active site of ER-α. Plumbagin, however, induced ER-α 46 kDa truncated isoform, which was found abundantly preempted in the cytoplasm compared with a 66-kDa full-length isoform. The truncated isoform is known to inhibit classical ER-α signaling pathways. SiRNA-transfected cells for ER-α exhibited lower cytotoxicity upon plumbagin treatment than the control-transfected cells.

Conclusion: Taken together, this study indicates that plumbagin has chemotherapeutic potential in BRCA1-mutated/defective ER-positive cancers.

Key words: BRCA1, cisplatin, doxorubicin, plumbagin, tamoxifen

Introduction

We had earlier found increased propensity for apoptosis in BRCA1-blocked cells (BG1, ovarian cancer cells) to plumbagin, a plant-derived naphthaquinone [1]. Since BRCA1 is involved in estrogen receptor (ER) inhibition, an increased susceptibility for these cells to antiestrogen drug, tamoxifen (TAM) was anticipated. We found that plumbagin, however, was more effective in inducing apoptosis than TAM [1]. Further, it was demonstrated that the mode of action of plumbagin was through a reactive oxygen species (ROS)-dependent mechanism [2]. At present, there is no difference in the treatment strategy for BRCA1-mutated patients, even though accumulating evidences indicate that it can be done [3].

The scheduled study is pursued by using chemotherapeutic agents such as cisplatin (CIS) and doxorubicin (DOX) in comparison to plumbagin, which are known to induce (DNA damage-mediated cell growth) inhibition of tumor cells. We have analyzed whether these compounds activate p53 (whether apoptosis induction involves p53), BARD1 (BRCA1 ring domain 1, which is a binding partner of BRCA1) and ER-α (because these cells are ER-α overexpressing).

The results of this study indicate that plumbagin is more effective (at a lower concentration) than CIS/DOX/TAM in inducing apoptosis in BRCA1-blocked cells (ER-positive ovarian cancer cells) than BRCA1-unblocked cells. Plumbagin may interfere with ER-α signaling pathway and induce apoptosis in a death receptor-mediated pathway.

Experimental Procedures

Cell lines

BG1 cells derived from a patient with stage III, poorly differentiated ovarian adenocarcinoma is an estrogen-responsive cell line with wild-type BRCA1 gene. Antisense-blocked cells (from exons 1 to 11) were termed as AS4 and control vector-transfected cells were termed NEO [2, 4]. BRCA1 repression with antisense blocking causes partial reduction in the protein expression which can be comparable to BRCA1-mutated condition where functional protein is not produced. The cells were maintained in Dulbecco’s Modified Eagle’s medium (DMEM) with 10% fetal bovine serum (FBS), with 1× antibiotic antimycotic (Invitrogen, NY). Before each
experiment, the cells were pretreated for 5 days in phenol red-free, DMEM/F-12 medium (Invitrogen, Carlsbad, CA) supplemented with 10% charcoal/dextran-treated serum (HyClone, Logan, UT) and 10^{-8} M 17β-estradiol [1,3,5(10)-estratriene 3,17β-diol; Sigma-Aldrich, St Louis, MO].

**chemicals**

DOX, CIS and TAM citrate were obtained from Sigma-Aldrich. Plumbagin used in the experiments was isolated from the Indian medicinal plant, *Plumbago zeylanica* as mentioned elsewhere [5]. Annexin staining kit and mouse monoclonal antibodies to ER-α (D-12, sc-8005) were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA) and ER-β (E1276) and p53 (P5813) from Sigma-Aldrich. The ApoAlert™ Mitochondrial Membrane Sensor Kit was purchased from Clontech (Palo Alto, CA). The spectrophotometric assay substrates for caspase-3, -6 and -8, AcDEVD-AFC, AcVEID-AFC and AcIEHD-AFC, respectively, were bought from BD PharMingen, BD Biosciences, San Jose, CA. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from USB Amersham Biosciences (Cleveland, OH).

**[H]thymidine incorporation**

Thymidine incorporation was carried out to determine the growth differences of the AS4 and NEO cells. For [H]thymidine incorporation, cells were treated with [H]thymidine for 6 h (0.5 μCi per well), and proteins were precipitated in ice-cold 5% trichloroacetic acid (100 μl per well). The cells were solubilized with 0.2 N NaOH. The radioactivity was counted using a liquid scintillation counter. The extent of thymidine incorporation was the measure of the growth proficiency of the cells.

**MTT assay**

This assay was done as described elsewhere [2]. The drugs DOX and CIS were added at different concentrations in quadruplicate samples. A concentration lower than the LD50 was used for further experiments. The concentrations used for further experimentations were plumbagin (2.5 μM), TAM (18.5 μM), DOX (15 μM) and CIS (200 μM).

**Annexin V binding**

Annexin V-fluorescein isothiocyanate (FITC) (200 μg/ml) was used for staining as described previously [2]. The apoptotic cells were viewed using LEICA DIMRE-2 inverted confocal laser scanning microscope equipped with Ar/He/Ne laser (488 nm) with x20 magnification green filter (tetra methyl rhodamine isothiocyanate 540 nm) and photographed.

**assessment of mitochondrial membrane potential ΔΨm**

This assay was done with ApoAlert™ Mitochondrial Membrane Sensor Kit (Clontech) [1]. The cells were viewed under confocal laser scanning microscope (488 and 540 nm) and photographed.

**caspase activation**

The caspase-3, -6 and -8 activity was assessed spectrofluorimetrically (Perkin Elmer LS-50, Waltham, MA) (excitation and emission wavelengths 400 and 505 nm, respectively) as described [6].

**analysis of p53, BARD1 and RAD51 expression profile by RT–PCR**

Total RNA was isolated from the cells using TRIZOL reagent (Invitrogen). For cDNA isolation, 2 μg/μl of total RNA and random hexamers were incubated at 70°C for 2 min. To the reaction, 1× Moloney Murine Leukemia Virus (MMLV) RT buffer, 1 mM deoxy nucleotide triphosphate 200 U of MMLV RT and 10 U of RNasin Ribonuclease inhibitor were added to make up the final reaction volume to 30 μl and incubated at 42°C for 1.5 h, 4°C for 2 min and 70°C for 2 min. All reagents were from Promega Corporation (Madison, WI) except when otherwise specified. Gene-specific primers are listed in Table 1.

The PCR profile for p53 was 95°C for 1 min, 60°C for 2 min and 72°C for 2 min; and BARD1 95°C for 10 s, 60°C for 30 s and 72°C for 1.3 min and for β-actin the cycle condition was 95°C for 30 s, 50°C for 30 s and 72°C for 45 s. The products of amplification were resolved in a 2% agarose gel and photographed using a gel documentation system.

**western blotting for ER**

The whole-cell lysate was isolated as described before [1]. Cytosolic and nuclear extracts were isolated and 50 μg of protein was loaded for western blots and probed with respective antibodies.

**nuclear lysate preparation**

After incubating the cells with the compounds for 1 h at 37°C, the cytosolic and nuclear extracts were isolated using the cytosolic extraction buffer [solution A (1 M Hepes, 2 M KCl, 0.1 M EGTA) (0.976 μl), 0.1 M diithiothreitol (DTT) (10 μl), 100 mM PMSF (5 μl), 1 mg/ml leupeptin (2 μl), 1 mg/ml aprotinin (2 μl) and 250 mg/ml benzamidine (2 μl)] and the nuclear isolation buffer [solution B (1 M Hepes, 5 M KCl, 0.1 M ethylene glycol tetra acetic acid (EGTA)) (0.976 μl) (0.1 M DTT(10 μl), 100 mM phenyl methyl sulfonyl fluoride (PMSF) (10 μl), 1 mg/ml leupeptin (2 μl), 1 mg/ml aprotinin (2 μl)]

**Table 1. The primer pairs used in this study for RT–PCR**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward</th>
<th>Reverse</th>
</tr>
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<tbody>
<tr>
<td>p53</td>
<td>5′ CAG AAG GAC CCA GGA CTT CCA 3′</td>
<td>5′ TGA AAT CTT CCA GGG TGG GGM 3′</td>
</tr>
<tr>
<td>BARD1</td>
<td>5′ ATGGATCCAAATGTTAAGAGC 3′</td>
<td>5′ CCCATCTGTTGCTGATC 3′</td>
</tr>
<tr>
<td>ER-α</td>
<td>5′ TGGACTGCGCCCACTTCTG 3′</td>
<td>5′ TGGACGCTGCGCCCACTTCTG 3′</td>
</tr>
<tr>
<td>ER-α exon 2 Common reverse</td>
<td>5′ CCGCAAGCTGCTGAGCC 3′</td>
<td></td>
</tr>
<tr>
<td>RADS1</td>
<td>5′ GGC GGT CAA GGT AAC TGT TGG 3′</td>
<td>5′ CGC TGA GCT GAT ACC AAA CTT 3′</td>
</tr>
<tr>
<td>β-Actin</td>
<td>5′ GGAGCTGCGGCGGCGGCGTTG 3′</td>
<td>5′ GAAATGTAATGGGAGCCGCTG 3′</td>
</tr>
</tbody>
</table>

BARD1, BRCA1 ring domain 1; ER, estrogen receptor.
and 250 mg/ml benzamidine (2 μl). For western blot, 50 μg protein were loaded.

**Transient transfection studies with siRNA**

A 21–23 bp siRNA [ER-α shortcut siRNA mix, New England Biolabs, Inc. (NEB) Ipswich, MA] was used for silencing ER-α. Cells were cultured in 96-well plates in 200 μl DMEM/Ham’s F-12 medium supplemented with 10% FBS. After 24 h, siRNA was transfected (20 nM per well) using TransPass R1 transfection reagent (NEB). Posttransfected cells (48–50 h) were treated with plumbagin (2.5 μM), TAM (18.5 μM) and CIS (200 μM) and after 24 h cell viability was carried out by MTT assay as described earlier.

**Results**

**Plumbagin is most effective in cell growth inhibition than CIS, DOX or TAM in BRCA1-blocked cells**

We confirmed BRCA1 gene silencing by western blot analysis (data not shown) and determined the growth advantage of BRCA1 antisense (AS4) cells over control (NEO) cells by [H]thymidine incorporation assay. AS4 cells incorporated up to five-fold more [H]thymidine than NEO cells, indicative of their higher proliferative nature (Figure 1A).

Differential inhibition of cell growth by the above agents was analyzed by growth inhibitory MTT assay. Both AS4 and NEO cells were less sensitive to CIS for a wide range of concentrations as evident from Figure 1B. The calculated LD₅₀ value for DOX was 15 and 25 μM for AS4 and NEO cells, respectively (Figure 1C). The calculated LD₅₀ value for CIS was 200 μM for both AS4 and NEO cells (Table 2), but that of plumbagin and TAM were as reported earlier [1]. These results indicate that plumbagin was most effective in inducing cell growth inhibition when compared with CIS, DOX or TAM.

**Plumbagin induces apoptosis more effectively than TAM, CIS and DOX in BRCA1-blocked cells**

Flip-flop of phosphatidyl serine to the outer surface of the membrane represents early stage of apoptosis and was detected as bright green fluorescence when stained with annexin V-FITC. No significant difference, in annexin binding, was observed between AS4 and NEO cells, on treatment with CIS, DOX and TAM. In contrast, the cells treated with plumbagin showed more late apoptotic changes in AS4 cells than NEO cells (Figure 2A). Propidium iodide (PI) staining showed late apoptotic behavior in treated cells.

One of the initial events in apoptosis is loss of mitochondrial membrane potential. MitoSensor™ gets aggregated in the mitochondria of healthy cells and fluoresce red. In contrast, when mitochondrial membrane potential is lost it shows only green fluorescence due to the presence of unpolymerized

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**Figure 1.** (A) Cells were treated with [H]thymidine (0.5 μCi per well) and incubated for 6 h. The extent of thymidine incorporation indicated the growth proficiency of the cells. (B and C) Cell viability was assessed in AS4 and NEO cells using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenytetrazolium bromide assay with both cisplatin and doxorubicin.
dye in the cytoplasm. The number of cells, which showed green fluorescence, was less in the case of CIS and DOX when compared with cells treated with plumbagin and TAM. The extent of loss of mitochondrial membrane potential was more in plumbagin-treated AS4 cells than NEO cells (Figure 2B).

Caspases are a family of cysteine proteases that are activated during different phases of the apoptosis and once activated, they cleave and activate downstream caspases. As shown in the Figure 3A, drug treatment in AS4 cells activated all the three caspases (6, 3 and 8) at 6 h. There was no statistically significant difference between the caspase activities of AS4/NEO when treated with CIS/DOX/TAM at 6 h. DOX showed a relatively increased caspase-3 activity than caspase-6 and -8 activities at 6 h. Caspase activity (at 6 h) was found to be higher in AS4 cells than in NEO when treated with plumbagin, even though the mean difference never reached statistical significance. At 12 h, only caspase-8 showed ~6.5-fold increase for plumbagin-treated AS4 cells (Figure 3B).

plumbagin-induced p53 and BARD1 expression is dependent on the presence of estradiol

All the four compounds increased p53 mRNA (messenger RNA) levels in NEO cells at 1.5 h as shown in Figure 4A and B. In contrast, in AS4 cells these drugs reduced p53 mRNA levels. In the absence of estradiol, p53 mRNA expression however, was high in AS4 control cells as well as when treated with plumbagin or TAM in comparison to NEO cells (Figure 5A). RT–PCR results point to the fact that BARD1 expression was also high in AS4 cells when treated with plumbagin at 4 h and was estrogen dependent (Figure 5B). Basal level expression of p53 and ER-α was high in AS4 cells (Figure 6). These results indicate that plumbagin-induced cell death was enhanced in the absence of estradiol, which may be because the cells are estrogen responsive.

plumbagin causes increased expression of 46 kDa truncated ER-α isoform in the cytoplasm than TAM

In order to further explore the expression of ER, we investigated the expression of ER-α in nuclear and cytosolic extracts after plumbagin treatment. It was found that a truncated form of ER-α was expressed more in the cytoplasm of plumbagin-treated AS4 cells, in comparison to cells treated with TAM (Figure 7A). Cytosolic and nuclear extracts of plumbagin-treated cells show abundant ER-α 46 kDa protein expression, however, ER-β expression does not seem to be changed in response to plumbagin (Figure 7B and C). Higher expression of ER-α 46 kDa isoform reveals that plumbagin induces apoptosis by activation of this isoform, which is known to inhibit classical ER-α signaling pathway [7]. RAD51, which is a binding partner of BRCA1, was found to be decreased in BRCA1-blocked cells treated with plumbagin (Figure 7D).

The expression of ER-α transcripts in response to plumbagin was studied by RT–PCR. Both truncated and full-length ER-α transcripts were present in control cells when amplified by primers corresponding to exon 1F region at C-terminal end of ER-α. When amplified by primers specific to exon 1A region, which will be spliced from 5’ untranslated region resulting in human estrogen receptor (hER-α) 46 kDa transcript, we observed corresponding bands in plumbagin-treated cells (Figure 7D).

Molecular docking studies using docking tools (Gold 2.12 Academic Version) to identify whether there is any prospective interaction between plumbagin and ER-α which revealed that plumbagin may bind to ER-α with higher affinity to the active site where estradiol bind (with a Gold Score of 35) (Figure 8).

siRNA-mediated repression of ER-α decrease the effect of plumbagin

To establish ER-α dependence for plumbagin action in AS4 and NEO cells, growth inhibitory assay was carried out in ER-α

<table>
<thead>
<tr>
<th>Drug (µM)</th>
<th>AS4</th>
<th>NEO</th>
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<tbody>
<tr>
<td>Cisplatin</td>
<td>&gt;200</td>
<td>&gt;200</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>15</td>
<td>25</td>
</tr>
<tr>
<td>Tamoxifen</td>
<td>25.7</td>
<td>29.8</td>
</tr>
<tr>
<td>Plumbagin</td>
<td>2.68</td>
<td>5.1</td>
</tr>
</tbody>
</table>

Table 2. LD_{50} values for the drugs tested
siRNA-transfected cells. In response to plumbagin, cell death was decreased in ER-α siRNA-transfected cells compared with untransfected cells (Figure 9), indicating that the depletion of ER-α expression decreases plumbagin-mediated cell growth inhibition. In the NEO cells, ER-α blocking completely protected the cells from cytotoxic effects of plumbagin.
treatment. TAM was more effective in ER-a-positive AS4 and NEO cells as expected. When both ER-a and BRCA1 were blocked, the effect of TAM is further reduced indicating that TAM will not be effective in ER-negative BRCA1-mutated cells. Cells remained insensitive to CIS, irrespective of ER-a and BRCA1 status. The above results indicate that plumbagin has specific growth inhibitory activity in BRCA1-blocked ER-a overexpressed condition.

discussion

BRCA1, a protein involved in DNA repair and apoptosis, its mutation results in defective cellular functions. Genomic instability resulting in mutations in the other genes cause higher rates of tumorigenesis in BRCA1 mutation carriers [8]. Several lines of evidence indicate a cardinal role for steroid hormones and their receptors in the genesis of BRCA1 mutant cancers. BRCA1 overexpression can inhibit estrogen and progesterone-stimulated cell proliferation in vitro [9, 10]. BRCA1 has been proved to physically interact with ER-a and inhibit its transcriptional activity. Thus, one of the major mechanisms of tumorigenesis in BRCA1-mutated cancers may be through increased activity of ER-a rather than its overexpression. This may be the reason why most BRCA1-related cancers are immunohistochemically negative for ER-a and progesterone receptor. We had earlier reported that plumbagin is more effective in inducing apoptosis than TAM in AS4 cells [1]. Since sensitivity of BRCA1-deficient cells to DOX is highly controversial [11, 12] and CIS is reported to regulate the expression of BRCA1 [13], a comparative study was done to decipher the effect of CIS/DOX with respect to plumbagin/TAM in BRCA1-blocked BG1 ovarian cancer cells.

There was no specific cell growth inhibitory capacity for BRCA1 blocked cells in comparison to NEO cells in response to CIS, a cross-linker that can interfere with DNA repair pathways. BARD1 expression was not altered by CIS treatment irrespective of BRCA1 status, indicating that BARD1-induced apoptosis is not related to CIS response. Hence, it can be
indicated that BG1 ovarian cancer cells are resistant to CIS, irrespective of BRCA1 status.

DOX, an anthracycline class of anticancer drug generates free radicals that induce DNA strand breaks thereby interfering in the DNA repair pathways. Our results show that in comparison to CIS, DOX was more sensitive to BRCA1-blocked cells with a 1.7-fold increase in growth inhibition than NEO cells. Annexin staining and loss of mitochondrial membrane potential reveal the efficacy of DOX in inducing apoptosis in BRCA1-blocked cells. The level of caspase-3 was found to be increased two-fold at 6 h when compared with caspase-6 and -8 in response to DOX treatment.

TAM, a well-known antiestrogen, did not show a difference in its sensitivity towards BRCA1-blocked cells [1]. Apoptosis-inducing capacity was higher for TAM, when collated to CIS. Zhang et al. [14] reported that TAM-induced apoptosis in breast cancer cells are without alteration of p53 protein levels. At 4 h in the absence of estrogen, p53 expression was high in control as well as in plumbagin- and TAM-treated BRCA1-blocked cells.

There was no significant increase in BARD1 expression in response to TAM in AS4 cells. The result points to the fact that there was no much difference in induction of apoptosis in BRCA1-blocked or -unblocked cells, however, the mode of action of TAM-induced apoptosis is different with respect to BRCA1 status.

The LD50 values were less for the BRCA1-blocked cells when compared with NEO cells with plumbagin as already reported (Table 2). Plumbagin treatment triggers the mitochondrial apoptotic pathway indicated by mitochondrial membrane potential loss and caspase-9 activation [1, 15]. In BRCA1-blocked plumbagin-treated cells, caspase-8 activity was increased to ~6.5-fold, indicating that BRCA1 blockage sensitizes BG1 cells to plumbagin more effectively and induces apoptosis mainly by death receptor pathway. We found that irrespective of BRCA1 status all the drugs induced apoptosis in...
the order plumbagin > DOX = TAM > CIS as evidenced by MTT, PI staining, annexin binding and loss of mitochondrial membrane potential. BARD1 expression was increased to ~nine-fold in BRCA1-blocked cells, which was highly dependent in the presence of estradiol in response to plumbagin. BARD1 induces apoptosis by catalyzing phosphorylation of p53 by DNA damage response kinase [16]. It has also been reported that BARD1 has preapoptotic functions independent of its association with BRCA1 [17]. Therefore, BARD1 induction occurred as a result of plumbagin treatment and may be one of the mechanisms by which it induces apoptosis in BRCA1-deficient cells. RAD51 is reported to be cleaved by caspase-3 during apoptosis [18]. The decrease in the RAD51 expression in BRCA1-blocked cells indicates that caspases 3 cleavage is more active in these cells in response to plumbagin. Blockade of p53 partially decreased plumbagin-induced apoptosis [15]. In our study, we found that when BRCA1 is blocked, p53 expression is increased which may result in increased sensitivity to plumbagin compared with NEO cells.

We have also detected a 46-kDa protein that is present abundantly than the full-length 66 kDa ER-α protein by western blotting in plumbagin-treated cells. This shorter isoform is reported to be generated by alternative splicing of the 66-kDa ER-α. RT–PCR analysis also revealed that the full-length ER-α is absent when treated by plumbagin in both AS4 and NEO cells. Human ER-α 46 kDa is a powerful inhibitor of hER-α 66 kDa in a cell context where the transactivation function of AF-1 predominates over AF-2. This truncated ER-α acts as a competitive inhibitor of hER-α 66 kDa-mediated transactivation [19]. Western blot analysis of ER-β expression does not seem to be varied much by plumbagin or TAM treatment (Figure 7C).

It was also shown that siRNA-mediated repression of ER-α decrease the effect of plumbagin. In the BRCA1-unblocked cells, ER-α blocking almost completely recovers the decreased viability by plumbagin treatment which indicates that plumbagin has specific growth inhibitory activity in BRCA1-blocked ER-α overexpressed cells.

To conclude, even though CIS, DOX and TAM can cause a growth inhibitory effect on BRCA1-blocked ER-positive ovarian cancer cells; they cannot be used specifically for ER-α positive BRCA1-mutated cancers, whereas plumbagin will be a more effective chemotherapeutic agent that can be used for the above-mentioned type of cancers. This may be due to at least three reasons as depicted in Figure 10.

(i) The generation of ROS by plumbagin can create DNA damage [2]. BRCA1 may be physiologically important for mounting normal response to xenobiotic insults and oxidative stress [20–22]. (ii) Inhibiting ER signaling pathway by
induction of truncated ER-α 46 kDa isoform which acts as a competitive inhibitor of hER-α 66 kDa mediated transactivation. BRCA1 is known to inhibit ER-α activation [23]. (iii) Plumbagin down-regulates the expression of angiogenic factor [vascular endothelial growth factor (VEGF)] [24]. N-terminus of BRCA1 is essential for binding to ER-α and for down-regulating VEGF transcription via the estrogen signaling pathway [25]. In addition to this, our own results show that RAB18, M41 and certain heat shock proteins were activated by plumbagin in BRCA1-blocked cells (K. A. Thasni, T. Ratheesh Kumar, G. Rojini et al., unpublished data). The above cellular effects due to plumbagin would have normally been taken care of by the native BRCA1 protein, which is absent in AS4 cells resulting in increased apoptosis. This throws light on the fact that plumbagin has chemotherapeutic potential as an anticancer agent in estrogen-responsive BRCA1-mutated ovarian cancer patients. More research, however, has to be done to elucidate cell signaling pathways induced by plumbagin with respect to BRCA1 status.

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**references**


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**Figure 10.** A proposed model for the action of plumbagin in BRCA1-blocked ovarian cancer cells. Single-headed arrow indicates activation and closed heads indicate inhibitory pathways.


