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Increase of androgen-induced cell death and androgen receptor transactivation by BRCA1 in prostate cancer cells

Shuyuan Yeh*, Yueh-Chiang Hu*, Mujib Rahman*, Hui-Kuan Lin, Cheng-Lung Hsu, Huei-Ju Ting, Hong-Yo Kang, and Chawnshang Chang†

George Whipple Laboratory for Cancer Research, Departments of Urology, Pathology, Radiation Oncology, Biochemistry, Toxicology, and The Cancer Center, University of Rochester, Rochester, NY 14642

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Although mutations of the breast cancer susceptibility gene 1 (BRCA1) may play important roles in breast and prostate cancers, the detailed mechanism linking the functions of BRCA1 to these two hormone-related tumors remains to be elucidated. Here, we report that BRCA1 interacts with androgen receptor (AR) and enhances AR target genes, such as p21$^{(WAF1/CIP1)}$, that may result in the increase of androgen-induced cell death in prostate cancer cells. The BRCA1-enhanced AR transactivation can be further induced synergistically with AR coregulators, such as CBP, ARA55, and ARA70. Together, these data suggest that the BRCA1 may function as an AR coregulator and play positive roles in androgen-induced cell death in prostate cancer cells and other androgen/AR target organs.

Germ-line mutations in a gene on chromosome 17q21 known as tumor suppressor BRCA1 (breast cancer susceptibility gene 1) are responsible for a large proportion of the inherited predispositions to breast and ovarian cancers (1). Significant increases of BRCA1 mutations were also observed for prostate and colon cancers (2). Earlier reports suggested that the BRCA1 not only functions as a tumor suppressor but may also regulate other distinct cellular processes, such as mitotic cell-cycle control, DNA repair, histone acetylation, and transcription activation (3–7). Recently, BRCA1 has been correlated to the suppression of estrogen receptor (ER) transactivation (8), but the linkage of BRCA1 to the other steroid receptor remains largely unknown.

The androgen receptor (AR) is a member of the steroid receptor superfamily that interacts with androgen response elements to regulate target gene transcription. AR consists of 918 amino acid residues. The well-conserved DNA-binding domain (DBD) consists of 68 amino acids with two zinc finger structures that are involved in DNA binding. The C-terminal region of AR with 295 amino acids, including the hinge region and ligand-binding domain (LBD), is responsible for the function of dimerization and androgen binding. The N-terminal region with 555 amino acid residues contains the domain involved in the transcriptional activation of AR. The AR plays important roles in male sexual differentiation, prostate cell proliferation, and the progression of prostate cancer (9–11). The findings of transcriptional interference/squelching of steroid receptors provided the concept of the existence of transcriptional coregulators to mediate steroid receptor function (12, 13). This concept enabled us to further study AR-associated coregulators and the diverse functions of AR (14–21).

In addition to promoting cell growth, androgen signaling through AR can induce apoptosis in thymocyte (22, 23) and in AR-stable transfected PC-3 cells (24). Despite the growth-stimulating effects of androgen–AR in the prostate cancer LNCaP cell, androgen at high concentrations can also inhibit cell growth that results in biphasic growth curve of LNCaP cells (25). In addition, androgen can inhibit the growth of LNCaP-derived cells, which grow under androgen ablation condition for over 40 passages (26, 27). Moreover, mitogen-activated protein kinase kinase kinase 1 may induce prostate cell apoptosis via the induction of AR transactivation (28). Thus, it has been well documented that androgen and AR may play important roles both in cell growth and apoptosis; the detailed mechanisms of how androgen and AR signaling can play these two opposite functions, however, need further characterization.

Here, we report that BRCA1, but not p53, can function as a coregulator to enhance AR transactivation in prostate cancer cells. This BRCA1-enhanced AR transactivation is androgen-dependent and requires the integrity of the AR DBD. Furthermore, our data indicate that BRCA1 can directly interact with AR. Moreover, our results suggest that AR could cooperate with BRCA1 in inducing the expression of p21$^{(WAF1/CIP1)}$, a cyclin-dependent kinase inhibitor (29, 30). In addition, using membrane integrity assay with propidium iodide (PI) inclusion, our results also demonstrated that addition of 5α-dihydrotestosterone (DHT) and ectopically expressed BRCA1 can further increase the percentage of dead cells. Although BRCA1 can activate p21$^{(WAF1/CIP1)}$ by itself, our data suggest that the BRCA1 may also play important roles in the androgen-induced cell death via interaction and cooperation with AR to induce the p21$^{(WAF1/CIP1)}$ expression.

Experimental Procedures

Materials and Plasmids. DHT was obtained from Sigma. pSG5-AR, ARA55, and pSG5-ARA70N were constructed as described (14, 17, 21). pCR3BRCA1, pCR3BRCA1-Δ11, and pCR3BRCA1P1749R were from B. Weber (University of Pennsylvania, Philadelphia). −291 and −2326p21-LUC were from W. S. El-Deiry (University of Pennsylvania). The plasmid construction junctions were verified by sequencing.

Cell Culture and Transfections. Human prostate cancer DU145 and PC-3 cells were maintained in DMEM containing penicillin (25 units/ml), streptomycin (25 μg/ml), and 5% FCS. Human LNCaP, T47D, and MCF-7 cells were maintained in RPMI 1640 with 10% FCS. Transfections in DU145 and PC3 cells were performed by using the calcium phosphate precipitation method, as described (14). Briefly, 4×10^5 cells were plated on 60-mm

Abbreviations: AR, androgen receptor; BRCA1, breast cancer susceptibility gene 1; DHT, 5α-dihydrotestosterone; PSA, prostate-specific antigen; ARA, androgen receptor-associated protein; LBD, ligand-binding domain; DBD, DNA-binding domain; ER, estrogen receptor; CAT, chloramphenicol acetyltransferase; LUC, luciferase; MMTV, mouse mammary tumor virus; GST, glutathione *S*-transferase; PI, propidium iodide.

*S.Y., Y.-C.H., and M.R. contributed equally to this paper.

†To whom reprint requests should be addressed. E-mail: chang@urmc.rochester.edu.

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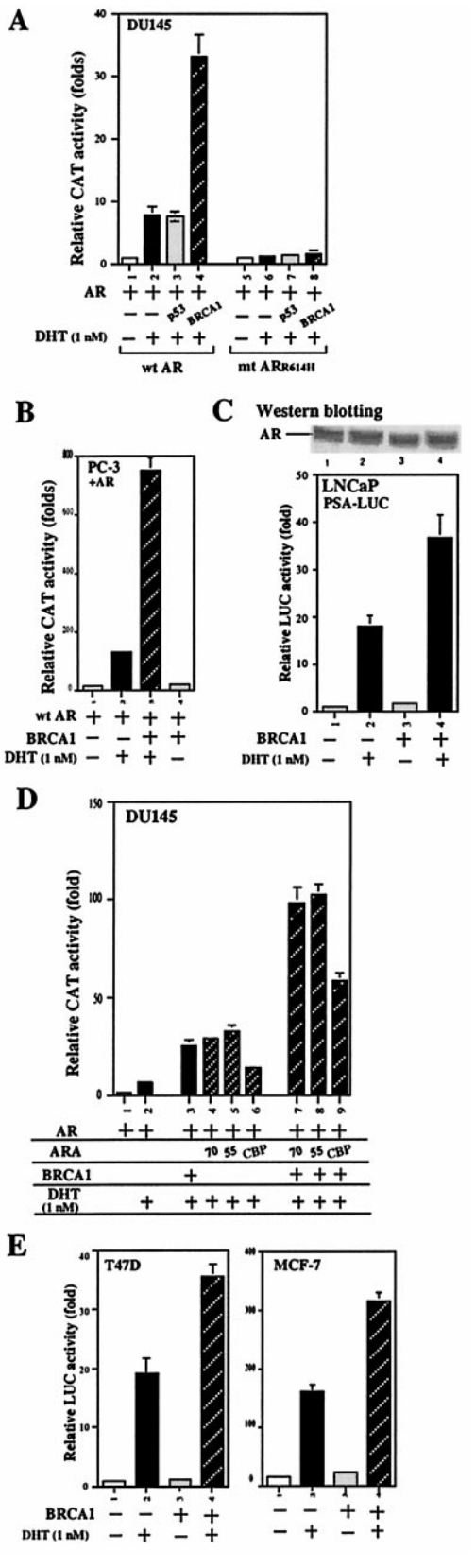

Fig. 1. Potentiation of the AR transactivation by BRCA1. (*A*) BRCA1, but not p53, potentiates the wild-type AR transactivation in prostate cancer cells. In each 60-mm dish of DU145 cells, 1 μg of pSG5-AR, 3 μg of MMTV-CAT, and/or 4.5 μg of pCR3-BRCA1, or 4.5 μg of p53 were transfected into cells by calcium phosphate method (14). The total plasmid amount was adjusted with pSG5, pCR3, or pCMV parent vector to 11 μg for each 60-mm transfection by calcium phosphate precipitation method. (*B*) BRCA1 can potentiate the AR transactivation in PC-3 cell. Cells were transfected as mentioned above. (*C*) BRCA1 can potentiate the AR transactivation in LNCaP cells in the presence of androgen without changing the expression of AR. 0.5 μg of PSA-LUC and 1.0 μg of pCR3 or pCR3-BRCA1 were transfected into LNCaP cells in 35-mm dish for 2 h by *SuperFect*. Cells were then treated with 1 nM DHT for an additional 24 h and harvested for LUC assay. The relative LUC activity was normalized against *Renilla* LUC activity (Promega). Data represent an average of three independent experiments. Duplicate LNCaP cells were harvested, and 60 μg of whole-cell extract was assayed with Western blotting for the detection of AR protein. The ectopically expressed BRCA1 cannot affect the expression of endogenous AR in LNCaP cells. (*D*) AR coregulators could cooperate with BRCA1 to synergistically enhance the AR transactivation. DU145 cells were cotransfected with 3 μg of MMTV-CAT, 1 μg of pSG5-AR, and 3 μg of alone or together with 3 μg CBP, ARA70N, ARA55, or BRCA1, in the absence or presence of 1 nM DHT. The error bars represent the mean $\pm$ SD of four independent experiments. (*E*) BRCA1 can potentiate the AR transactivation in MCF-7 and T47D cells. 0.5 μg of PSA-LUC reporter plasmid or 1.0 μg of pCR3-BRCA1 were transfected into T47D and MCF-7 cells. Cells were treated with 1 nM DHT after transfection as mentioned above.

dishes 24 h before transfection, and the medium was changed to DMEM with 5% charcoal—dextran-stripped FCS (CS-FBS) 1 h before transfection with precipitate containing AR expression plasmid, and mouse mammary tumor virus–chloramphenicol acetyltransferase (MMTV-CAT) reporter gene derived by long-terminal repeat of MMTV promoter (31). A β-galactosidase expression plasmid, pCMV-β-gal, was used as an internal control for transfection efficiency. The total amount of DNA was adjusted to 11 μg with pSG5 in all transcriptional activation assays. After 24 h of transfection, the medium was changed again, and the cells were treated with DHT, antiandrogen, or other treatment. After another 24 h, the cells were harvested for CAT assay as described (14). The CAT activity was visualized by PhosphorImager (Molecular Dynamics) and quantitated by IMAGEQUANT software (Molecular Dynamics). LNCaP T47D and MCF-7 cells were transfected by using *SuperFect* (Qiagen, Chatsworth, CA). At least three independent experiments were carried out in each case.

Glutathione *S*-Transferase (GST) Pull-Down Assay. GST–BRCA1 fusion proteins and GST control protein were purified as described by the manufacturer (Amersham Pharmacia). The purified GST proteins were then resuspended in 100 μl of interaction buffer [20 mM Hepes, pH 7.9/150 mM KCl/5 mM $MgCl_2$/0.5 mM EDTA/0.5 mM DTT/0.1% (vol/vol) Nonidet P-40/0.1% (wt/vol) BSA/1 mM PMSF/10% glycerol] and mixed with 5 μl of ^{35}S-labeled TNT proteins (TNT coupled reticulocyte lysate system, Promega) in the presence or absence of 1 μM DHT at 4°C for 3 h. After several washes with NETN buffer (20 mM Tris, pH 8.0/100 mM NaCl/6 mM $MgCl_2$/1 mM EDTA/0.5% Nonidet P-40/1 mM DTT/8% glycerol/1 mM PMSF), the bound proteins were separated on an SDS/8% PAGE and visualized by using autoradiography.

Thiazolyl Blue (MTT) Assay. The MTT assay is a quantitative colorimetric assay for mammalian cell survival and proliferation. PC-3(AR2) cells were grown at 4×10^5 in 60-mm plates in RPMI 1640 with 5% charcoal-stripped FCS. After 24 h of transfection with BRCA1, the medium was changed with or without 10 nM DHT for another 48 h. Then 200 μl of thiazolyl blue (MTT, 5 mg/ml, Sigma) was added into each plate with 1 ml of medium for 3 h at 37°C. After incubation, 2 ml of 0.04 N HCl in isopropanol was added into each well. After several rounds of pipetting and 5 min of incubation at room temperature, the absorbency was read at a test wavelength of 570 nm.

Cell Membrane Integrity Assay. Dead cells were indicated as loss of cell membrane integrity assayed by PI inclusion. PC-3(AR2)

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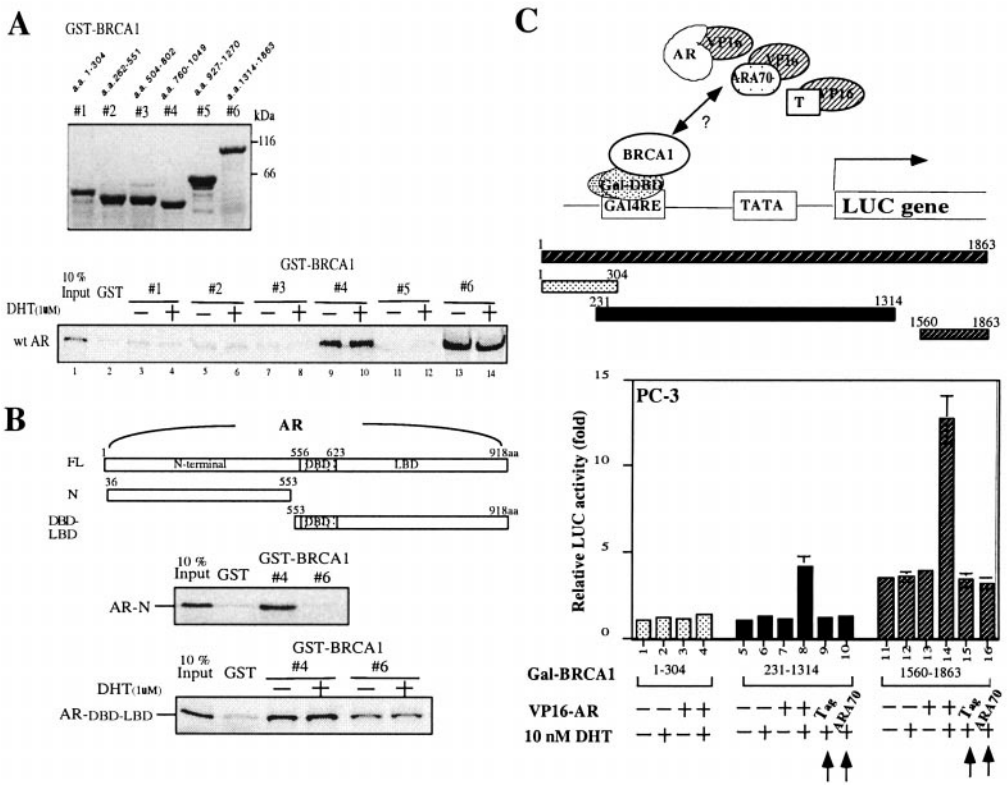

Fig. 2. The interaction between BRCA1 and AR. (*A*) Mapping the domains of BRCA1 that are responsible for AR interaction. Six recombinant GST–BRCA1 fusion proteins, fragments #1, #2, #3, #4, #5, and #6, were generated in *Escherichia coli* as described. BRCA1 residues are marked relative to the translation initiation site. The Coomassie blue-stained SDS polyacrylamide gel, showing the relative abundance of each fusion protein, was used in the GST pull-down assay as described. The 110-kDa protein bound to GST–BRCA1 #4 and #6 is a product of ^{35}S-methionine-labeled full-length AR. (*B*) Mapping the domain of AR that is required for BRCA1 interaction. 20 μl of *in vitro* translated ^{35}S-methionine-labeled AR N (from amino acids 36–553) and AR DBD-LBD (from amino acids 553–918) protein was used to perform the pull-down assay. The results indicated that GST–BRCA1 fragment #4 (amino acids 758-1064) can interact both with the N-terminal and DBD-LBD of AR. In contrast, GST–BRCA1 #6 (amino acids 1314–1863) can associate with only the DBD- LBD of AR. Our data indicate that there are two contact pockets between BRCA1 and AR. (*C*) The interaction between AR and BRCA1 by mammalian two-hybrid assay. PC-3 cells in 35-mm dishes were transiently cotransfected with 0.5 μg of reporter plasmid pG5-LUC, and 0.75 μg of Gal4DBD fused BRCA1 constructs, amino acids 1–304, amino acids 231-1314, amino acids 1560–1863, with or without 0.75 μg of VP16 fused AR (VP16-AR) construct for 2 h by *SuperFect*. 1 nM DHT was added for another 24 h, and then the cells were harvested for LUC assay. Arrows indicate VP16-fused SV40 large T antigen and ARA70 were applied here to assure the interaction specificity between BRCA1 and AR. The results indicate that BRCA1 amino acids 231-1314 and amino acids 1560–1863 are responsible for AR interaction; these results are consistent with the results from GST pull-down assay.

cells were transfected with 4 μg of BRCA1. After 2 h of transfection, the medium was changed, and 10 nM DHT or vehicle were applied for another 4 days. The medium was changed on day 2 to remove the floating cells resulting from transfection reagent effect. On day 4, the media with floating cells were first set aside. Attached cells were trypsinized, combined with floating cells, and then stained with 20 μg/ml PI for 10 min. The PI-positive cells were then counted under a fluorescent microscope.

Results and Discussion

Potentiation of the AR Transactivation by BRCA1. To test the effect of the BRCA1 in AR transactivation, human prostate cancer DU145 cells, which lack the AR, were cotransfected with the androgen-response reporter MMTV-CAT, AR, and BRCA1. As shown in Fig. 1*A*, addition of the BRCA1 enhances the activity of wild-type AR (lane 2 vs. lane 4). Notably, BRCA1 cannot potentiate the transactivation of mutant ARR614H, which has lost the DNA binding capacity by an arginine-to-histidine substitution at amino acid residue 614 (lane 6 vs. lane 8) (11). Moreover, p53, the universal tumor suppressor (32), showed no effect on the AR transactivation (lane 2 vs. lane 3). Similar induction was observed when we replaced DU145 with prostate cancer PC-3 cells (Fig. 1*B*). In the prostate cancer LNCaP cells, the BRCA1 could also enhance transactivation of the endogenous AR on prostate-specific antigen-luciferase (PSA-LUC) (33) in the presence of 1 nM DHT (Fig. 1*C*). In the absence of DHT, BRCA1, however, showed little effect on the AR transcriptional activity (Fig. 1*C,* lane 3). The results from Western blotting further indicate the expression of AR was not affected by the ectopically expressed BRCA1 (Fig. 1*C Upper*). In addition, our data also suggested that the enhancement of activities of androgen response element-containing promoter is not mediated by the transactivation of BRCA1 itself (Fig. 1 *B*, lane 4, and *C*, lane 3). Together, our results suggest that BRCA1 could function as a coregulator to enhance AR transactivation from either endogenous AR or ectopically expressed AR. This BRCA1-enhanced AR transactivation is androgen-dependent and requires the integrity of the AR DBD (Fig. 1*A*, lanes 5–8).

As our earlier reports showed that AR transactivation could be further enhanced by several AR-associated coregulators (ARAs), we were interested in determining the potential linkage between the BRCA1 and these ARAs (13–19). As shown in Fig. 1*D*, the addition of the BRCA1, ARA70N (ARA70 amino acids 1–401) (13, 14), ARA55 (16), or CBP could increase AR transactivation from 5-fold to 25-fold, 28-fold, 33-fold, and 12-fold, respectively. Simultaneous addition of the BRCA1 and ARA70N could increase AR transactivation synergistically from

5-fold to 90-fold. Similar synergistic induction results were also obtained when we replaced ARA70N with ARA55 (from 5-fold to 97-fold) or CBP (from 5-fold to 55-fold). These data therefore demonstrate that the BRCA1 not only could enhance AR transactivation but could also cooperate with other AR coregulators to synergistically induce AR transactivation.

When we replaced AR with ER in our functional assay, our results showed that the BRCA1 could moderately enhance the ER transactivation in all three prostate cancer cells (LNCaP, PC-3, or DU145) (S.Y. and C.C., unpublished observation). These data are therefore in contrast to a recent report that showed the BRCA1 functioning as a repressor to inhibit the ER transactivation (8). The discrepancy between our data and earlier reports about induction vs. repression of ER transactivation by BRCA1 remains unclear. One possibility could be our assay uses a physiological estrogen concentration (10^{-8} M) and the earlier report used a supraphysiological concentration (10^{-6} M). Another possibility could be due to the use of different transfection methods and/or normalizing the transfection efficiency in different ways. Thus, in addition to reporter gene assay, it is essential to investigate the effect of BRCA1 on expression of endogenous AR target gene.

BRCA1 Enhanced the AR Transactivation in Breast Cancer Cells. Of the primary mammary tumors tested, approximately 65% are positive for ER and progesterone receptor and 75–80% are positive for AR (34). Although AR is expressed in 75–80% of the primary breast tumors, the role of AR in breast tumors remains largely unknown.

Other studies also suggested that androgen and AR might play some roles in the inhibition of breast tumor growth (35). The detailed mechanism, again, remains unclear. To test whether BRCA1 can also influence the AR transactivation in breast cancer cells, BRCA1 and AR as well as reporter gene were cotransfected in two different breast cancer cells. As shown in Fig. 1*E*, our data indicated BRCA1 could also enhance the AR transcriptional activity in breast cancer MCF-7 and T47D cells (lane 2 vs. lane 4). Although it is not clear if this finding has direct linkage to the androgen-mediated suppression of breast tumor growth, our data provide evidence to connect two important molecules, BRCA1 and AR, in breast cancer.

The Interaction Between BRCA1 and AR. To test whether BRCA1 can enhance AR transactivation via the interaction with AR, we used GST pull-down assay to map the interaction sites between the BRCA1 and AR. The relative abundance of six different GST–BRCA1 peptides was indicated as Coomassie blue staining (Fig. 2*A Upper*). As shown in Fig. 2*A* (*Lower*), recombinant GST-BRCA1–peptide 4 (amino acids 758–1064) and −peptide 6 (amino acids 1314–1863) were found to bind specifically to *in vitro* translated ^{35}S-methionine-labeled AR in the presence or absence of DHT (lanes 9, 10, 13, and 14). Further mapping studies showed that the GST-BRCA1–peptide 4 interacted with AR N terminus (amino acids 36–553) as well as DNA and ligand-binding domains (DBD-LBD, amino acids 553–918), and the GST-BRCA1–peptide 6 interacted only with AR DBD-LBD (Fig. 2*B*).

Results from a mammalian two-hybrid assay also indicated that two regions (amino acids 231–1314 and amino acids 1560–1863) of the BRCA1 were required for AR interaction (Fig. 2*C*). The interaction between BRCA1 and AR is specific, as VP16-fused SV40 large T antigen or ARA70 could not interact with Gal4DBD-fused BRCA1 constructs (Fig. 2*C*, lane 5 vs. lanes 9 and 10; lane 11 vs. lanes 15 and 16). Overall, data from GST pull-down and mammalian two-hybrid assays indicated that there are two possible contact pockets between BRCA1 and AR.

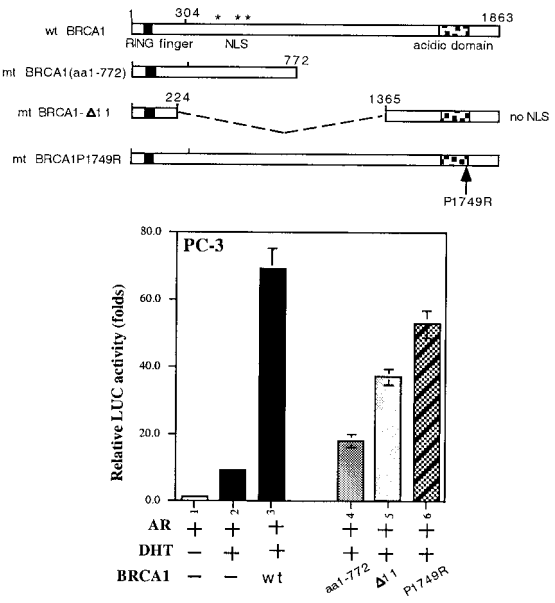

Fig. 3. Mutations of BRCA1 reduce the enhancement of AR transactivation. PC-3 cells in 35-mm dishes were cotransfected with 0.5 μg of reporter plasmid MMTV-LUC, 0.5 μg of pSG5AR, and 1 μg of BRCA1 construct for 2 h. 1 nM DHT was then added for 24 h before cells were harvested for LUC assay. As compared with the wild-type BRCA1, the cotransfection of three mutant BRCA1s, one of the constructs lacks the C terminus of BRCA1 (BRCA1 amino acids 1–772), one has a point mutation at its C terminus (BRCA1-P1749R), and the other lacks exon 11 (BRCA1-Δ11), reducing the enhancement of AR activity (lane 3 vs. lanes 4–6).

Mutations of BRCA1 Reduce the Enhancement of AR Transactivation. The BRCA1 gene encodes a 1,863-amino acid (220 kDa) protein. Truncation of BRCA1, even at position 1853, which lacks only 10 C-terminal residues, abolishes the ability of BRCA1 to suppress breast cancer growth (36). Truncations in the BRCA1 3′ terminal position also are among the most frequent mutations in familial breast cancers (37). Furthermore, missense mutations associated with cancers mapped to the C terminus of BRCA1 (38, 39). As we have established the functional connection between wild-type BRCA1 and AR, we are curious about how these mutant BRCA1s can affect the AR transactivation.

As shown in Fig. 3, androgen response luciferase (MMTV-LUC) assays indicated that mutations of BRCA1, lacking the C-terminal activation domain from amino acids 773 to 1863 (BRCA1 amino acids 1–772), deleting the exon 11 from amino acids 224 to1365 (BRCA1-Δ11), or a C-terminal point mutation at amino acid 1749 from proline to arginine (BRCA1-P1749R), could reduce the enhancement of AR transactivation. These mutation studies further strengthen the above interaction data (Fig. 2) and suggest that two regions, amino acids 758–1064 and amino acids 1560–1863, within the BRCA1 might play important roles for the BRCA1-enhanced AR transactivation.

The AR and BRCA1 Could Cooperatively Regulate the Expression of p21$^{(WAF1/CIP1)}$ and Stimulate the Cell Death in PC-3(AR2). Whereas most studies suggest that the BRCA1 can function as a tumor suppressor, several recent studies also point out that BRCA1 could play important roles in cell differentiation and proliferation. For example, the BRCA1 could be detected during cell differentiation in the mammary gland, and targeted disruption of the BRCA1 at exon 5–6 results in a mouse embryo that is unable to develop beyond day 7.5 (40). The detailed mechanism linking the BRCA1 function to cell differentiation, however, remains unclear. One possible explanation may involve the

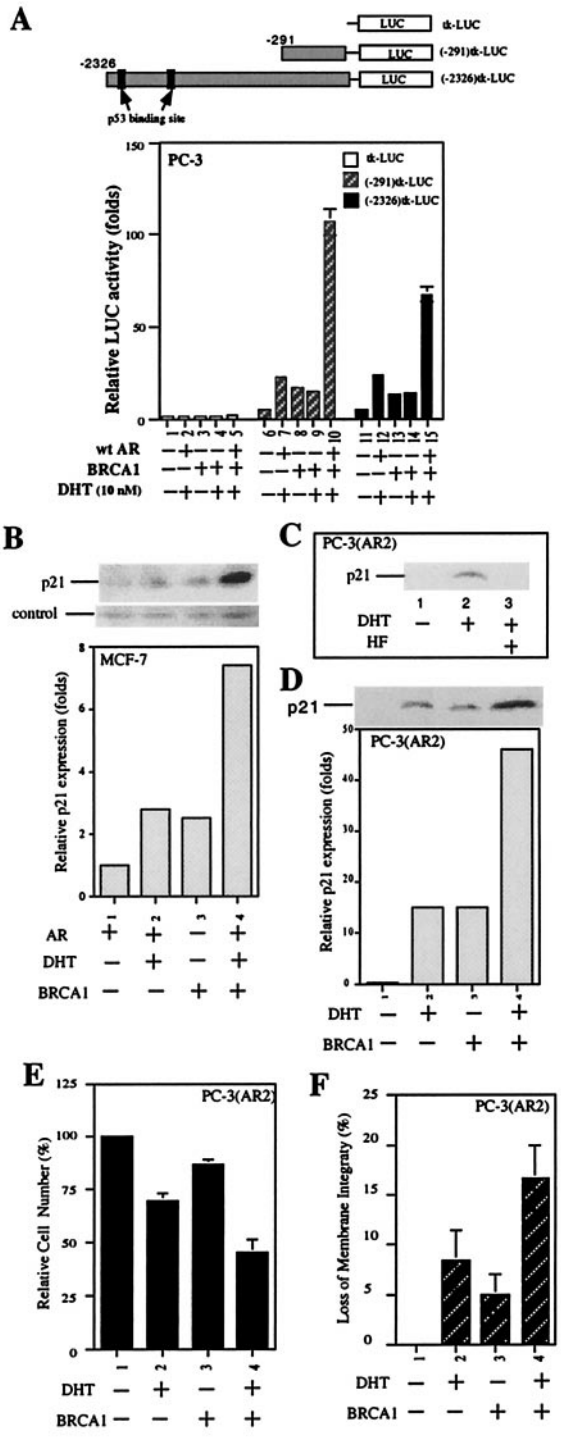

Fig. 4. The AR and BRCA1 could cooperatively regulate the promoter activity and protein expression of p21$^{(WAF1/CIP1)}$ and stimulate the cell death in PC-3(AR2). (*A*) The coexpression of AR and BRCA1 cooperatively induces the −291 and −2326 p21$^{(WAF1/CIP1)}$ promoter but not the basal promoter activity. In each transfection, 0.5 μg of reporter gene, 0.5 μg of AR, with or without 1 μg of BRCA1, were cotransfected into PC-3 cells. After 2 h of transfection, the medium was changed, and 10 nM DHT was added for another 30 h. (*B*) The endogenous p21$^{(WAF1/CIP1)}$ expression was induced by DHT-AR and BRCA1 in MCF-7 cells. MCF-7 cells were seeded on 60-mm wells and cotransfected with 2 μg of AR with or without 4 μg of BRCA1 by *SuperFect*. After 2 h of transfection, the medium was changed, and 10 nM DHT was applied for another 30 h. In each experiment, 60 μg of whole-cell extract was applied for the Western blotting. (*C*) The p21$^{(WAF1/CIP1)}$ protein is enhanced by DHT/AR and inhibited by hydroxyflutamide (HF) in PC-3(AR2) cells. The expression of protein p21$^{(WAF1/CIP1)}$ can be induced by DHT, and this induction can be inhibited by 1 μM HF (lane 2 vs. lane 3). (*D*) The p21$^{(WAF1/CIP1)}$ protein is enhanced by DHT/AR and BRCA1 in PC-3(AR2). PC-3(AR2) cells were cotransfected with or without 4 μg of BRCA1. After 2 h of transfection, the medium was changed, and 10 nM DHT was applied for another 30 h. In each experiment, 60 μg of whole-cell extract was applied for the Western blotting. (*E*) Cell growth is regulated by DHT/AR and BRCA1 in PC-3(AR2) cells. Duplicate PC-3(AR2) cells (as in *D*) were applied to MTT assay for the relative cell number determination. (*F*) Dead cells were indicated as loss of cell membrane integrity assayed by PI inclusion. PC-3(AR2) cells were transfected with 4 μg of BRCA1. After 2 h of transfection, the medium was changed, and 10 nM DHT or vehicle was applied for another 4 days. The medium was changed on day 2. Attached cells were trypsinized and collected with floating cells, stained with 20 μg/ml PI on day 4. After 10 min of staining, the PI-positive cells were then counted under fluorescent microscope.

induction of cyclin-dependent kinase inhibitor, p21$^{(WAF1/CIP1)}$ (29, 30). In addition to its function as a key molecule in the regulation of the cell-cycle arrest, p21$^{(WAF1/CIP1)}$ is an AR target gene (41). Recent reports further linked p21$^{(WAF1/CIP1)}$ to the induction of apoptosis (42, 43). Using LUC reporter assay, our data showed that the 5′-promoter activity of p21$^{(WAF1/CIP1)}$ was cooperatively induced by the DHT-AR and BRCA1 (Fig. 4*A*, lanes 7–9 vs. lane 10; lanes 12–14 vs. lane 15). Addition of the BRCA1 can synergistically induce the p21$^{(WAF1/CIP1)}$ promoter activity in the presence or absence of the p53 response element in PC-3 cells (Fig. 4*A*, promoter −2326 vs. −291). Similar results also occurred in MCF-7 or LNCaP cells (data not shown).

Western blot analysis of endogenous p21$^{(WAF1/CIP1)}$ expression in MCF-7 cells, or PC-3 cells stably transfected with AR, PC-3(AR2) (24), also confirmed that DHT-AR could induce endogenous p21$^{(WAF1/CIP1)}$ protein expression (Fig. 4 *B–D*). This induction could be repressed by hydroxyflutamide, a popular antiandrogen for the treatment of prostate cancer, suggesting p21$^{(WAF1/CIP1)}$ induction is mediated through the AR (Fig. 4*C*, lane 2 vs. lane 3). Furthermore, addition of the BRCA1 could further increase the DHT-induced p21$^{(WAF1/CIP1)}$ expression (Fig. 4*D*, lane 4).

To correlate DHT/AR- and BRCA1-mediated p21$^{(WAF1/CIP1)}$ induction to cell-cycle arrest or cell death, an MTT assay was performed simultaneously during Western blot analysis. As shown in Fig. 4*E*, whereas DHT alone or BRCA1 alone can reduce cell numbers to 70% and 86%, respectively, simultaneous addition of DHT and BRCA1 can further reduce cell numbers to 42%. Using membrane integrity assay with PI inclusion, our results (Fig. 4*F*) also demonstrated that addition of DHT and ectopically expressed BRCA1 can further increase the percentage of dead cells, suggesting that the BRCA1 may play important roles in the androgen-induced cell death via interaction and cooperation with the AR to induce the p21$^{(WAF1/CIP1)}$ expression.

Although most of the reported AR functions have been linked to cell proliferation, it is also well documented that androgen–AR plays important roles in thymic atrophy by acceleration of apoptosis in the thymocytes (22, 23). Other reports also suggested that mitogen-activated protein kinase kinase kinase 1 may induce prostate cell apoptosis via the induction of AR transactivation (28) and that addition of 10 nM DHT to PC-3 cells transfected with AR will accelerate the cell apoptosis (24). As BRCA1 is an integral component of the RNA polymerase II holoenzyme (44), it is possible that androgen–AR may need BRCA1 to communicate properly with RNA polymerase II for the transactivation of some AR target genes related to cell growth arrest, death, or proliferation. Although it is presently not clear how to link BRCA1 function to the AR-mediated cell proliferation, the cooperative induction of p21$^{(WAF1/CIP1)}$ expression by AR and BRCA1 may provide a key connection for the BRCA1 to function with positive roles in the androgen/AR-mediated cell arrest or death.

In conclusion, our finding that the BRCA1 may function as

AR coregulator and play positive roles in androgen-induced cell death by cooperative modulation of AR transactivation represents evidence that links both the BRCA1 and AR in the prostate cancer. As the BRCA1 and AR can cooperatively induce $p21^{(WAF1/CIP1)}$ expression and thereby affect the growth of prostate cancer cells, the expression level and function of BRCA1 in the prostate cancer patients may be part of a regulatory pathway to modulate the prostate cancer progression. Further studies may help us to better understand the connection between BRCA1 and AR in prostate cancer as well as in breast cancer and other androgen target organs.

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