BRCA2 cooperates with histone acetyltransferases in androgen receptor-mediated transcription

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Germ-line mutations of the *BRCA2* tumor suppressor gene greatly increase the risk of developing breast and ovarian cancers. Here, we show that wild-type BRCA2, but not a tumor-specific truncated mutant BRCA2, synergizes with the nuclear receptor coactivator p160 GRIP1 to enhance transcriptional activation by androgen receptor (AR). BRCA2 not only associates with AR and GRIP1 but also cooperates with both the histone acetyltransferase P/CAF and BRCA1 to enhance AR- and GRIP1-mediated transactivation. As such, BRCA2 can exert its tumor suppressor function, in part, by modulating androgen signaling, which has been shown to be antiproliferative in a subset of breast cancer cells and particularly implicated in male breast tumors.

Inherited mutations of the *BRCA2* tumor susceptibility gene greatly increase the risk of acquiring familial breast and/or ovarian cancers for women and also account for an increased risk of breast cancer in men (1). The *BRCA2* gene encodes a 3,418-aa-long nuclear protein with no sequence homology to any other known proteins (2). The phenotype of BRCA2-deficient cells as well as the physical and functional interaction between BRCA2 and the Rad51 recombinase indicate that one of the tumor suppressor functions of BRCA2 is linked to recombination-mediated DNA repair. Furthermore, BRCA2 has also been implicated in cell cycle control (3).

The mechanisms by which BRCA2 participates in these essential biological processes have remained elusive. How mutation of *BRCA2*, which is widely expressed in different tissues, leads predominantly to tumors of the breast and ovary, two steroid hormone-dependent tissues, is even more enigmatic. Interestingly, the gene encoding AIB1/pCIP/ACTR, a coactivator for steroid hormone receptors, has been implicated as a risk-modifying gene for *BRCA1*- and *BRCA2*-associated breast cancers (4). One steroid hormone receptor, the androgen receptor (AR), is expressed in a large proportion of primary breast cancers. In addition, androgen signaling inhibits the growth of some breast cancer cells (5–8), and germ-line mutations of the *AR* gene have been linked with male breast cancer (9–11). Therefore, we speculated that BRCA2 might be a coactivator for AR and thereby potentiates AR-mediated antiproliferative signals, which may be a novel facet of BRCA2's tumor suppressor function.

Methods

Plasmids. CR3-BRCA1 and pSG5-AR were obtained from B. Weber (University of Pennsylvania, Philadelphia) and B. Peeters (University of Leuven, Leuven, Belgium), respectively. Mouse mammary tumor virus-luciferase (pMMTV-Luc) reporter, pCMX-P/CAF, and pCMX-GRIP1 were from R. Evans (Salk Institute, La Jolla, CA). pAR-DEF was from S. Kato (University of Tokyo, Tokyo). pSG5.HA-GRIP1 (5–1,462, full length), pSG5.HA-GRIP1ΔAD1 (full-length GRIP1 with amino acid 1,057–1,109 deleted), pSG5.HA-GRIP1ΔAD2 (GRIP1 5–1,121), and pSG5.HA-GRIP1ΔAD1+ΔAD2 were gifts from M. Stallcup (University of Southern California, Los Angeles). MG-BRCA2 was described (12). Mutant MG-BRCA2L1042stop was created by site-directed mutagenesis of the BRCA2 cDNA of MG-BRCA2. FlagAR (1–919, full length) was constructed by lifting the *Bam*HI AR fragment out of pSG5-AR and insertion into the *Bam*HI site of pCMV2Flag vector (Sigma). FlagAR/AB (1–556) was constructed by lifting the 1.7-kb *Hin*dIII fragment out of FlagAR and insertion into the *Hin*dIII site of pCMV2Flag. FlagAR/DEF (632–918) was constructed by lifting the *Bam*HI (blunt-ended)–*Xba*I fragment out of pAR-DEF and insertion into the *Eco*RV-*Xba*I sites of pCMV2Flag. To make FlagAR/C(D) (552–646) and FlagAR/(D)EF (650–919), a PCR amplicon with flanking in-frame *Eco*RI–*Bam*HI sites and *Cla*I–*Xba*I sites was introduced into the corresponding sites of pCMV2Flag vector, respectively.

Transfection and Luciferase Assays. Human embryonal kidney 293T or 293 cells were transfected by using FuGENE6 (Roche) according to the manufacturer's recommendation. Where indicated, transfections were done according to the modified calcium phosphate coprecipitation method (13). Twelve to sixteen hours after transfection, medium was changed to DMEM containing 10% charcoal-dextran stripped FBS (HyClone) with or without 10^{-6} M 5α-dihydrotestosterone (DHT). After another 48 h, cells were harvested for luciferase assays, immunoprecipitation, and/or Western blot analysis. Luciferase assays were performed employing the Luciferase assay kit (Promega).

Coimmunoprecipitation Assays. To prepare total cell lysates, cells were washed with PBS and then lysed in 50 mM Tris·HCl (pH 8)/170 mM NaCl/0.5% Nonidet P-40/50 mM NaF containing complete protease inhibitors (Roche) at 4°C. The cell lysates were clarified by centrifugation at 15,000 × *g* for 5 min. For immunoprecipitation, total cell lysates were precleared for >1 h by incubation with protein G-Sepharose (Amersham Biosciences). Then, supernatants were incubated for >4 h with anti-Flag (M2, Sigma) or anti-hemagglutinin (HA) (12CA5, Roche) primary antibodies as well as protein G-Sepharose beads at 4°C. Finally, these were washed four times with 10 mM Tris·HCl, pH 8/250 mM NaCl/5 mM EDTA/0.5% Nonidet P-40 containing complete protease inhibitors.

Western Blot Analyses. Proteins were separated on 3–8% polyacrylamide/Tris-acetate gels with Tris-acetate-SDS buffer or 4–12% polyacrylamide/Bis-Tris gels with Mops-SDS buffer according to the manufacturer's instructions (Invitrogen). Proteins were transferred to poly(vinylidene difluoride) (PVDF) membranes (Immobilon-P, Millipore), which were blocked in PBS containing 0.2% Tween 20 and 5% nonfat dry milk. Membranes were hybridized with anti-AR (N-20, Santa Cruz Biotechnology), anti-BRCA2 (BRCA2-A; gift from J. Chen and D. Livingston, Harvard Medical School, Cambridge, MA), anti-BRCA2 (3E6, GeneTex, San Antonio, TX), anti-HA (12CA5), or anti-Flag (M2) primary antibodies in 1% nonfat dry milk-PBS/0.2% Tween 20. Goat anti-mouse IgG $F(ab)_2$ (Pierce), anti-mouse IgG (Santa Cruz Biotechnology), or donkey anti-rabbit Ig (Amersham Biosciences), horseradish peroxidase-conjugated antibody was used as a secondary antibody in 1%

Abbreviations: AR, androgen receptor; DHT, 5α-dihydrotestosterone; HA, hemagglutinin.

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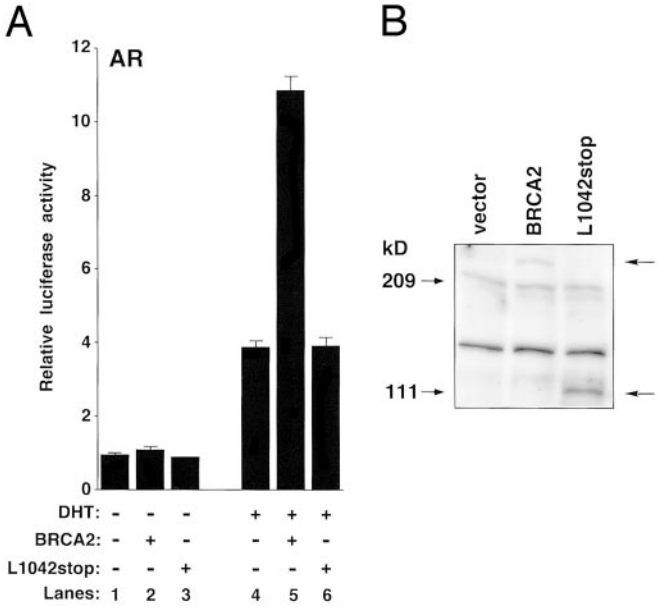

Fig. 1. Wild-type but not a tumor-specific truncated mutant BRCA2 synergizes with GRIP1 to enhance AR function. (*A*) 293 cells in 6-cm dishes were transiently transfected by using FuGENE6 reagent with 0.5 μg of pMMTV-Luc reporter, 0.3 μg of pSV40-β-gal, 25 ng of pSG5-AR, 3 μg of MG-BRCA2, and/or 1 μg of MG-BRCA2L1042stop. Total DNA transfected was the same by adjusting with appropriate empty expression vectors. Because of the large size of the BRCA2 cDNA, we transfected equal moles of MG-BRCA2 (12) and empty expression vector. Transfection efficiency was monitored and corrected with β-galactosidase activity derived from cotransfected pSV40-β-gal. Where indicated, cells were grown in the presence of 10^{-6} M DHT. Luciferase activities represent the mean and SD from three transfections. Results shown are from a single experiment that is representative of three separate experiments. (*B*) Expression levels of wild-type BRCA2 and the tumor-specific mutant L1042stop in 293 cells transfected as described in *A*. Immunoblotting was performed by using anti-BRCA2 sera (3E6).

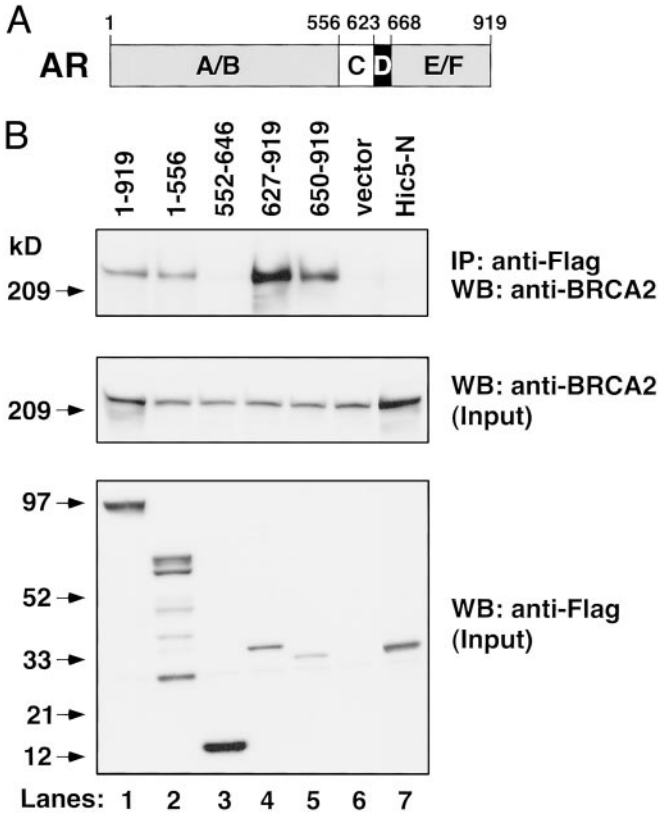

Fig. 2. BRCA2 interacts with AR. (*A*) Schematic representation of AR and its functional domains. (*B*) Mapping of the domain in AR that is required for the interaction with BRCA2 *in vivo*. Flag-tagged full-length AR (amino acids 1–919) and four Flag-tagged AR fragments (amino acids are indicated) were cotransfected with 4.5 μg of MG-BRCA2 expression vector into 293T cells. As a control, FlagHic5.N (amino acids 1–208) expression vector was used. Cells were cultured with DHT (10^{-6} M) for 48 h in media with stripped serum. Total cell lysates were subjected to immunoprecipitation using anti-Flag antibody (M2) in the presence of DHT. Immunoblotting of the precipitates was performed with anti-BRCA2 antibody (BRCA2-A) or anti-Flag antibody (M2).

nonfat dry milk-PBS/0.2% Tween 20. Proteins were detected by chemiluminescence (ECL kit, Amersham Biosciences).

Results

Wild-Type but Not a Tumor-Specific Truncated Mutant BRCA2 Enhances AR Activity. As a first step to test whether BRCA2 is a coactivator for AR, we analyzed the ability of BRCA2 to enhance the transactivation function of AR in a hormone-dependent manner. For this purpose, 293 cells were cotransfected with expression vectors for AR and the MMTV-Luc reporter, which is activated by ligand-bound AR, along with a BRCA2 expression vector. We used 293 cells because they do not express AR and because we were able to express BRCA2 at least 5-fold above the level of endogenous BRCA2. Coexpression of BRCA2 with AR did not elevate reporter gene transcription in the absence of the hormone DHT (Fig. 1*A*, lane 2). However, hormone-dependent AR-mediated reporter gene activity was augmented 3-fold by coexpression of BRCA2 (Fig. 1*A*, lanes 4 and 5). In the absence of coexpressed AR, no effect of BRCA2 was observable with hormone (data not shown).

No *BRCA2* missense mutation has been unequivocally identified as a tumor-specific mutation because of the lack of data on functional domains of BRCA2 (14). Therefore, we tested a tumor-specific truncation mutant of BRCA2, L1042stop, that encodes the N-terminal 1,041 aa (15) for its effect on AR function. In contrast to wild-type BRCA2, the tumor-specific mutant L1042stop did not enhance AR transactivation on DHT stimulation (Fig. 1*A*, lane 6). These results are not caused by a reduced expression of the L1042stop mutant compared with wild-type BRCA2 (Fig. 1*B*).

BRCA2 Interacts with AR in Mammalian Cells. We next asked whether the functional interaction between AR and BRCA2 is based on a physical interaction. Thus, we tested the association between BRCA2 and AR in coimmunoprecipitation assays by using Flag-tagged full-length AR. Indeed, BRCA2 coimmunoprecipitated with full-length AR but not with a control protein, Flag-tagged Hic5-N (Fig. 2*B*, lanes 1 and 7).

AR encompasses several functional domains (Fig. 2*A*). The A/B domain of AR harbors a ligand-independent transcriptional activation function (AF-1). The C and D domains are the conserved DNA binding region and a variable hinge, respectively. The E/F domain is responsible for ligand binding and dimerization and also contains a ligand-dependent transcriptional activation function (AF-2) (16). In addition, the DNA binding and its adjacent region (amino acids 552–644) were shown to interact with the ring finger protein SNURF, which modulates nuclear trafficking of AR and thereby enriches AR in the nuclear matrix (17). To determine which domains of AR interact with BRCA2, several truncations of AR were generated and tested in coimmunoprecipitation assays. Similar to full-length AR, the AB and EF domains interacted with BRCA2 (Fig. 2*B*, lanes 2 and 5), suggesting that BRCA2 might be a

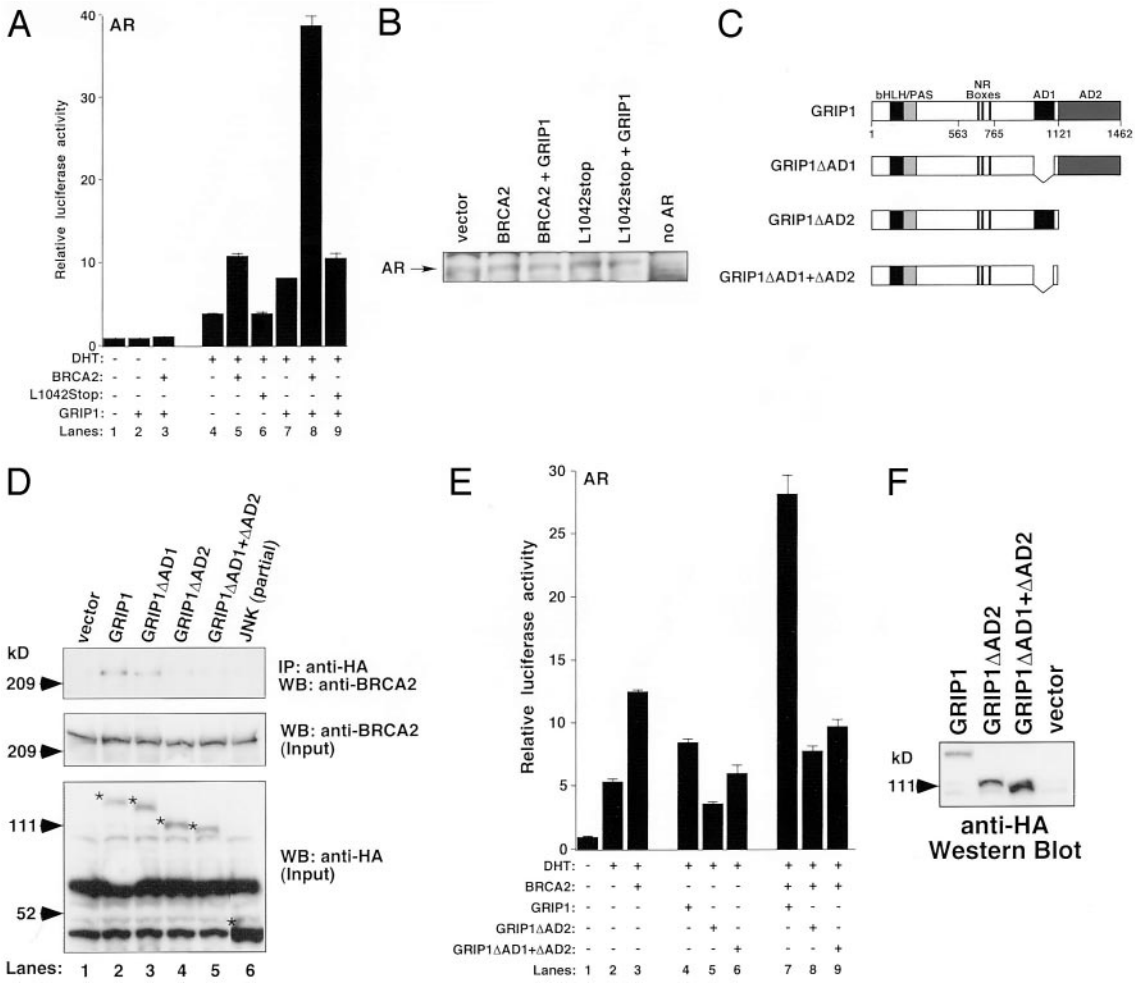

Fig. 3. Both physical and functional interaction with BRCA2 requires the AD2 domain of GRIP1. (*A*) Wild-type but not a tumor-specific mutant BRCA2 synergizes with GRIP1 to enhance AR function. Transient transfection of 293 cells were as described in Fig. 1*A*. Where indicated, 0.4 μg of pCMX-GRIP1 was cotransfected. (*B*) AR protein expression is not affected by coexpression of BRCA2 and/or GRIP1. The same cell extracts used for luciferase assays described in *A* were subjected to immunoblotting using anti-AR antibody (N-20). (*C*) Diagrammatic representation of wild-type and truncated GRIP1 molecules (19). (*D*) The interaction between BRCA2 and GRIP1 requires an intact AD2 domain. 293T cells were transfected with MG-BRCA2 expression vector together with plasmids encoding for the indicated HA-tagged proteins. Total cell lysates were subjected to immunoprecipitation using anti-HA epitope antibody 12CA5. Western blot analysis was performed with anti-BRCA2 (BRCA2-A) or anti-HA (12CA5) antibody. (*E*) Synergistic coactivation mediated by BRCA2 and GRIP1 requires an intact AD2 domain. Transient transfection assays were performed in 293 cells by using FuGENE6 reagent with 0.5 μg of MMTV-Luc reporter, 0.1 μg of pSV40-β-gal, 40 ng of pSG5-AR, 3 μg of MG-BRCA2, 1 μg of pSG5.HA expression vector encoding wild-type or truncated GRIP1 similar as described in Fig. 1. (*F*) Expression levels of wild-type GRIP1 and mutations thereof. Shown is an immunoblot of respective 293 cell lysates by using anti-HA antibody (12CA5).

coactivator for both AF-1 and AF-2 function. On the other hand, amino acids 552–644 of AR failed to interact with BRCA2 (Fig. 2*B*, lane 3).

Both Physical and Functional Interaction with BRCA2 Requires the AD2 Domain of GRIP1. Ligand-bound steroid receptors recruit p160 coactivators, which possess histone acetyltransferase activity and can themselves recruit other coativators with histone acetylating or histone methylating activities to potentiate ligand-dependent steroid receptor signaling. The p160 family of coactivators consists of three related 160-kDa proteins, SRC-1/NcoA-1, GRIP1/TIF2/NcoA-2, and AIB1/pCIP/ACTR (18). Because the *AIB1* gene is implicated as a risk-modifying gene for *BRCA2*-associated breast cancers, we analyzed whether p160 coactivators play a role in BRCA2- and AR-dependent transcription. Among three members of the p160 family tested, SRC-1, GRIP1, and AIB1, p160 GRIP1 was the most efficient coactivator of AR function in 293 cells and subsequently used in our transient transfection assays.

As expected, GRIP1 alone as well as GRIP1 and BRCA2 together did not enhance AR function in the absence of hormone (Fig. 3*A*, lanes 2 and 3). On hormone stimulation, GRIP1 alone enhanced AR transactivation 2-fold and synergized with BRCA2 to further enhance AR function by 10-fold. Importantly, the tumor-specific L1042stop mutant of BRCA2 failed to synergize with GRIP1 (Fig. 3*A*, lane 9). These results are not caused by an alteration of AR protein levels on coexpression of BRCA2 and/or GRIP1 (Fig. 3*B*).

Next, we tested the possibility that BRCA2 not only interacts with AR, but also with GRIP1 *in vivo*. To this end, we coexpressed HA-tagged GRIP1 with BRCA2, performed an immunoprecipitation with anti-HA antibody, and then analyzed for the presence of BRCA2 in the precipitate by Western blotting with BRCA2 antisera. Indeed, BRCA2 coimmunoprecipitated with GRIP1 but not with a control protein, HA-tagged JNK (Fig. 3*D*, compare lanes 2 and 6).

All members of the p160 coactivator family have two autonomous activation domains, AD1 and AD2, at the C terminus.

The AD1 domain recruits two histone acetyltransferases, p300/CBP and P/CAF, whereas the AD2 domain interacts with two arginine methyltransferases, CARM1 and PRMT1 (see Fig. 3*C* for a sketch of GRIP1). GRIP1 deletion mutants lacking either an AD1 domain (GRIP1ΔAD1) or AD2 domain (GRIP1ΔAD2) are still functional, because GRIP1ΔAD1 and GRIP1ΔAD2 mutants synergistically cooperate with CARM1 and p300, respectively, to enhance nuclear receptor function (19–22). To determine which regions of GRIP1 play a role in the physical and functional interaction with BRCA2, we used GRIP1 deletion mutants lacking an AD1 and/or AD2 domain. When coexpressed with BRCA2, a GRIP1 mutant lacking the AD1 domain interacted with BRCA2 (Fig. 3*D*, lane 3), whereas GRIP1 mutants lacking an AD2 domain (GRIP1ΔAD2 and GRIP1ΔAD1+ΔAD2) showed no interaction (Fig. 3*D*, lanes 4 and 5). Wild-type and all GRIP1 deletion mutants were expressed at similar levels (Fig. 3*D Bottom*). These results indicate that the *in vivo* interaction between BRCA2 and GRIP1 requires the AD2 domain of GRIP1.

To determine whether this physical interaction is required for the synergistic cooperation between GRIP1 and BRCA2 to enhance AR transactivation, transient transfection assays were performed in 293 cells. As shown before, full-length GRIP1 synergistically cooperated with BRCA2 to enhance AR function (Fig. 3*E*, lane 7), whereas the GRIP1ΔAD2 and GRIP1ΔAD1+ΔAD2 mutants lacking an intact AD2 domain failed to enhance the coactivator effect of BRCA2 (Fig. 3*E*, lanes 8 and 9); protein levels of full-length GRIP1 and its mutants were comparable (Fig. 3*F*). GRIP1ΔAD2 even slightly repressed the coactivator function of BRCA2, which may be due to sequestering AR coactivators such as CBP/p300 and/or P/CAF that interact with a functional AD1 domain (Fig. 3*E*; compare lanes 3 and 8). Altogether, we conclude that the synergy between BRCA2 and GRIP1 depends on the AD2 region, which also facilitates an *in vivo* interaction between BRCA2 and GRIP1.

BRCA2 Synergizes with both P/CAF and BRCA1 in a GRIP1-Dependent Manner. The coactivator P/CAF, which possesses histone acetyltransferase activity, has been previously shown to associate with BRCA2 both *in vitro* and *in vivo*, though no function has yet been ascribed to this interaction (23). P/CAF also enhances transcription mediated by ligand-bound nuclear receptors via direct contact with the p160 family of coactivators (24, 25). Our results suggest that BRCA2 can enhance AR signaling through its interaction with p160 GRIP1. These data taken together raise the possibility that P/CAF, GRIP1, and BRCA2 collaborate to enhance AR function.

To determine whether P/CAF and BRCA2 cooperate to potentiate AR function, we used experimental conditions where BRCA2, P/CAF, or GRIP1 alone poorly enhanced reporter gene activity so that any potential collaboration between BRCA2 and P/CAF in the presence or absence of GRIP1 would be revealed. In the absence of GRIP1, BRCA2 and P/CAF did not cooperate to enhance transcription mediated by ligand-bound AR (Fig. 4*A*, lane 4), whereas expectedly either BRCA2 or P/CAF alone cooperated with GRIP1 (Fig. 4*A*, lanes 6 and 7). When both BRCA2 and P/CAF were coexpressed with GRIP1, they synergized to further enhance AR-dependent transcription. Thus, synergistic cooperation between P/CAF and BRCA2 appears to depend on GRIP1.

Loss of either BRCA2 or BRCA1 dramatically increases the risk of developing hereditary breast and ovarian cancers, though these two unique tumor suppressor proteins bear no sequence similarity to one another. The phenotypes of cells harboring disrupted BRCA2 or BRCA1 are similar, suggesting that both play a role in DNA repair, albeit BRCA2 and BRCA1 appear to play mechanistically distinct roles (3). In addition, some pools of BRCA2 and BRCA1 were previously shown to coexist (26), yet

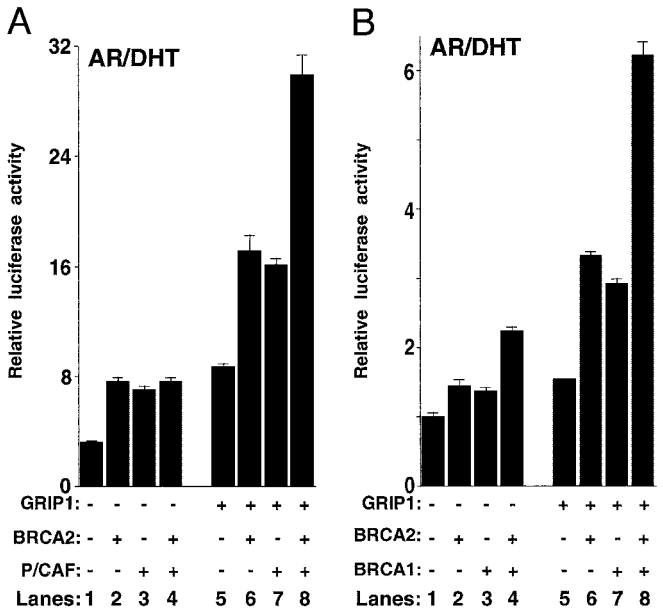

Fig. 4. BRCA2 synergizes with P/CAF or BRCA1 in a GRIP1-dependent manner. (*A*) Synergistic GRIP1-dependent enhancement of AR function by BRCA2 and P/CAF. Transient transfection assays were performed in 293 cells by using FuGENE6 with 0.5 μg of pMMTV-Luc reporter, 50 ng of pSV40-β-gal, 30 ng of pSG5-AR, 2.5 μg of MG-BRCA2, 0.5 μg of pCMX-GRIP1, 1 μg of pCMX-P/CAF, and/or appropriate empty expression vectors. (*B*) Synergistic GRIP1-dependent enhancement of AR function by BRCA2 and BRCA1. Transient transfection assays were performed in 293 cells by using FuGENE6 with 0.5 μg of pMMTV-Luc reporter, 0.3 μg of pSV40-β-gal, 25 ng of pSG5-AR, 2.25 μg of MG-BRCA2, 1.75 μg of CR3-BRCA1, 0.5 μg of pCMX-GRIP1, and/or appropriate empty expression vectors.

the function of this association has not been resolved. We therefore tested whether BRCA2 and BRCA1 together exert additive or synergistic coactivator effects on AR function in the absence and presence of GRIP1. We used experimental conditions where BRCA2, BRCA1, or GRIP1 alone caused a negligible enhancement of reporter gene activity, so that a potential cooperation among coactivators would be detected. In the absence of GRIP1, BRCA2 and BRCA1 together showed little additive coactivator effects on AR activity (Fig. 4*B*, lane 4). Coexpression of GRIP1 with either BRCA2 or BRCA1 caused an ≈2-fold increase of reporter gene activity. However, BRCA2 and BRCA1 together in the presence of GRIP1 synergistically enhanced reporter gene activity when compared with their individual effects (Fig. 4*B*, lane 8). The fact that BRCA2 and BRCA1 acted in a synergistic manner suggests that this coactivator pair enhances AR signaling by independent yet cooperative mechanisms.

Discussion

BRCA2 Functions as a Coactivator for AR. In this report, we have shown that BRCA2 acts as a coactivator for AR in conjunction with p160 GRIP1, thereby pointing at a novel mechanism of how BRCA2 exerts its tumor suppressor function by promoting AR signaling. Indeed, several lines of evidence suggest that AR signaling in the breast protects against cancer development (27). (*i*) AR signaling has inhibitory effects on cell proliferation of some breast cancer cells *in vitro* as well as in nude mice (5–8). (*ii*) Androgen is effective to treat metastatic breast cancer, though its use has been suspended because of its side effects (28). (*iii*) AR signaling inhibits estrogen-induced mammary epithelial proliferation and suppresses estrogen receptor expression in primates (29). (*iv*) Partially inactivating germ-line mutations of

the *AR* gene have been linked with breast cancers in men (9–11). Our results suggest that loss of BRCA2 will result in reduced or impaired AR-mediated transcription, thereby abrogating the antiproliferative effect of AR, which may result in enhanced breast tumorigenesis. Consistently, both germ-line mutations in *BRCA2* and *AR* genes are correlated with an increased risk of male breast cancer (1, 9–11).

The mechanisms of inhibitory action of androgens in breast cancer development and growth are presently not known. Interestingly, androgens appear to down-regulate the expression of the antiapoptotic protein Bcl-2 in a human breast cancer cell line. Consequently, survival of breast cancer cells would be impaired, offering a potential explanation for the inhibitory effect of androgens on cancer cell growth (30). The search for and the characterization of novel target genes affected by BRCA2 in collaboration with AR and GRIP1 may further our understanding of how BRCA2 mechanistically safeguards against tumorigenesis.

Role of BRCA2 in Transcriptional Regulation and DNA Repair. In contrast to BRCA1, data supporting a role for BRCA2 in transcriptional regulation are less explored. BRCA2 coimmunoprecipitates with P/CAF, inhibits p53 transactivation, contains a domain of ≈100-aa length that is associated with a putative transactivation activity, and synergizes with Smad3 (23, 31–33). However, how the transcriptional regulation by BRCA2 might contribute to breast cancer development has not been shown. Our study strongly indicates a role of BRCA2 in the regulation of gene transcription by acting as a coactivator for AR.

BRCA2 synergistically cooperated with P/CAF or BRCA1 in a GRIP1-dependent manner to promote AR transactivation. Considering that both P/CAF and BRCA1 are AR coactivators interacting with GRIP1 (34, 35) and also coexist with some pools of BRCA2 (23, 26), our study suggests a complex interplay among AR, BRCA2, p160 GRIP1, BRCA1, and P/CAF. It is possible that BRCA2 participates in the formation and/or stabilization of a macromolecular steroid receptor–coactivator complex with multiple protein–protein interactions.

The interaction of GRIP1 and P/CAF with BRCA2 may also be relevant for the proposed tumor suppressor function of BRCA2 in recombination-mediated DNA repair, because the capacity of the two histone acetyltransferases, GRIP1 and P/CAF, to remodel chromatin structures is not only important for gene transcription but also for DNA repair, recombination, and replication (36). Furthermore, GRIP1 was previously shown to interact and synergize with the CARM1 and PRMT1 arginine methyltransfereases to enhance steroid receptor signaling (19, 21). These methyltrasferases possess histone-methylating activities and are thought to be involved in chromatin remodeling (37, 38). Because of its association with GRIP1, BRCA2 may therefore also coexist with these methyltransferases and functionally communicate with these coactivators during transcriptional regulation and DNA repair.

Risk Modifying Genes for *BRCA2*-Associated Cancers. *BRCA2* mutation carriers show substantial individual variability in the risk for breast cancer development (39, 40), but the reasons for this variability are unclear. Factors affecting hormonal signaling such as oophorectomy, pregnancy, breastfeeding, oral contraceptives, and tamoxifen are implicated as risk-modifying factors for *BRCA2*-associated breast and/or ovarian cancers (41). In addition, genetic polymorphisms of the two genes coding for Rad51 and p160 AIB1 (a breast tissue-specific member the p160 family of coactivators) are linked with modification of *BRCA2*-associated breast cancer risk. Identification of the *Rad51* gene as a risk-modifier for *BRCA2*-associated breast cancers supports an importance of the Rad51 recombinase in the tumor suppressor function of BRCA2 (42, 43).

Based on this study, we speculate that genetic polymorphism of the *AR* gene might be a risk modifier for *BRCA2*-associated breast cancer. Because the p160 GRIP1 coactivator not only synergized with BRCA2 but also was critical for the synergistic cooperation of BRCA2 with both BRCA1 and P/CAF to enhance AR function, we additionally propose that the *GRIP1* gene might be a modifier for *BRCA2*- and *BRCA1*-associated cancer risk. Epidemiological studies linking *AR* and *GRIP1* polymorphisms to *BRCA2* mutations should be undertaken to validate our hypotheses, because this would be clinically important for a better assessment of cancer risk in *BRCA2* mutation carriers.

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