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Repression of BRCA1 through a Feedback Loop Involving p53*

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The BRCA1 and p53 tumor suppressors have been shown to interact and cooperate to activate transcription of p53-responsive genes. In this study, we show that BRCA1 is initially up-regulated, followed by a reduction to below basal levels in response to treatment with the DNA-damaging agents adriamycin and mitomycin C, and that the reduction of BRCA1 expression is dependent on the presence of wild-type p53. Elimination of p53 by expression of human papilloma virus E6 resulted in an inability to down-regulate BRCA1 in response to adriamycin. Ectopic expression of p53 resulted in a rapid decrease in BRCA1 protein and RNA levels and *BRCA1* promoter-driven luciferase activity even in null p21 cells deficient in p53-dependent G_1 arrest. $ATM^{-/-}$ lymphoblastoid cells were deficient in their ability to reduce BRCA1 protein in response to DNA damage, whereas the wild-type counterparts reduced BRCA1 protein levels after exposure to adriamycin. These results, in conjunction with others, suggest a loop wherein BRCA1 initially participates in accumulation of p53 protein, whereas later p53 acts to reduce BRCA1 expression.

The breast and ovarian cancer susceptibility gene *BRCA1* has been suggested to be involved in gene transcription, DNA repair, and transcription-coupled repair (1). Evidence for roles in these processes has come primarily from identification of interacting proteins with BRCA1. For example, binding to such proteins as Rad51 (2) and Rad50, p95, and MRE11 (3) has suggested that BRCA1 may be involved in DNA repair mechanisms, such as double strand break repair, that involve the Rad, p95, and MRE11 proteins. BRCA1 is also bound and phosphorylated by the ATM and human CDS1 DNA damage-activated kinases (3, 4). On the other hand, BRCA1 also complexes with RNA polymerase II (6), RNA helicase A (7), the transcriptional repressor CtIP (8, 9), and histone deacetylase components RbAP46 and RbAP48 (10), and the association of BRCA1 with the polyadenylation factor CstF-50 is bridged by BARD1 (11). These interactions suggest that BRCA1 plays some role in cellular gene transcription. It has been hypothesized that BRCA1 involvement in transcription is a means by which the protein acts in one aspect of DNA repair (12). The findings that $BRCA1^{-/-}$ cells are defective in transcription-coupled repair and that BRCA1 associates with such global transcription components as RNA polymerase II suggest that BRCA1 is not a specific transcriptional activator *per se*. However, BRCA1 has also been shown to bind individual transcription factors such as p53 and c-Myc (13–15). BRCA1 is able to repress c-Myc transcriptional activity, whereas it is able to enhance p53 activity similar to other transcriptional coactivators such as p300 and cAMP-responsive element-binding protein. BRCA1-specific involvement in the p53 response has been enhanced with the finding that $BRCA1^{-/-}$ embryos survive longer if one or both copies of the p53 gene are also missing (16). That activation of specific genes such as $p21^{WAF1}$ and *GADD45* or repression of cyclin B1 (17–19) is also mediated by BRCA1 again raises the possibility that BRCA1 effects on transcription may be specific.

p53 binds to two regions of BRCA1: at the N terminus between amino acids 224 and 500 and also within the BRCT domain at the C terminus (13, 20). Transcriptional coactivation of p53 is dependent on the C terminus of BRCA1, as this region is required for all transcription-related events that BRCA1 performs (14). The N-terminal p53-binding region of BRCA1 has been shown to act as a dominant-negative inhibitor of p53-mediated transcription when expressed without the remainder of the BRCA1 protein, indicating that although this region is able to bind p53, the machinery needed to coactivate p53 either binds to or is present in other areas of the protein (21). Although it is clear that one mechanism by which BRCA1 induces p53 activity is to bind directly and subsequently to coactivate transcription, another mechanism may involve increasing the stability of p53. Overexpression of BRCA1 has been shown to induce accumulation of p53 in wild-type p53-expressing cell lines (21). This accumulation appears to depend on the presence of $p14^{ARF}$, a protein that allows p53 to escape degradation induced by *MDM2*. BRCA1-induced p53 is then transcriptionally active and activates such downstream genes as $p21^{WAF1}$.

The effect of DNA damage on BRCA1 protein has been the subject of recent studies. Treatment of cultured cells with a variety of DNA-damaging agents, including γ-radiation, UV light, mitomycin C, and adriamycin, results in a retardation of BRCA1 protein on denaturing gels that is reversed by the addition of phosphatase, indicating phosphorylation (22). Interestingly, many of these studies report a reduction in BRCA1 protein and RNA levels following extended treatment with DNA-damaging agents (22–24). Here we show that this effect seems to be dependent on the presence of wild-type p53 protein and that ectopic expression of p53 alone is able to down-regulate BRCA1 protein. The reduction appears to occur at the RNA level. Although BRCA1 has been proposed to act in a complementary manner to p53, we propose that this feedback loop is

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not unlike the one that has been described for $p14^{ARF}$ expression: whereas $p14^{ARF}$, like BRCA1, is able to induce p53 (25), p53 is also responsible for negatively regulating $p14^{ARF}$ expression (26).

MATERIALS AND METHODS

Cell Culture—The culture conditions of SW480, PA1, H460, H460-Neo, H460-E6, Saos2, SkOV-3, HCT116, 03189C, and 2184D cells have been previously described (27–29). Adriamycin was obtained from the University of Pennsylvania pharmacy and added to the medium of cells at a concentration of 200 or 400 ng/ml for 18 h. Mitomycin C was obtained from Sigma and added to the medium of cells at a concentration of 20 μg/ml for 8 h.

Adenovirus Infection and Propagation—Ad-LacZ and Ad-p53 (28) were obtained from B. Vogelstein (Johns Hopkins University). Viruses were propagated, titered, and amplified as described (28).

Western Blotting—Western blotting was carried out essentially as described (17) using mouse anti-human p53 monoclonal (Ab-2), mouse anti-human BRCA1 monoclonal (Ab-1), mouse anti-human p21 monoclonal (Ab-1) and mouse anti-Rb monoclonal (Ab-5) antibodies (all from Oncogene Science Inc.).

Northern Blotting—Total RNA isolation and Northern blotting were carried out as described previously (28). A *Hin*dIII/*Not*I fragment of 5.6 kilobases was excised from pCR3.1-BRCA1 (30) and used as a probe for *BRCA1* Northern blots.

Luciferase Assays—A total of 1×10^6 Saos2 cells were transfected using Superfect reagent (QIAGEN Inc., Valencia, CA) with 0.5 μg of reporter and 1.5 μg of pCEP4-p53 or vector alone. 24 h after transfection, cells were lysed and analyzed for luciferase activity as described (31).

Fluorescence-activated Cell Sorting Analysis—Adenovirus infections of MCF7 cells were carried out at m.o.i.[1] = 20 in 1% fetal bovine serum and phosphate-buffered saline. Adriamycin treatment was performed as described above. Preparation of cells for fluorescence-activated cell sorting was performed essentially as described (32). Cell sorting was performed on a Coulter Epics Elite counter. DNA content analysis was performed using MacCycle software (Phoenix Flow Systems, San Diego, CA).

Trypan Blue Exclusion—Floating and attached cells were trypsinized from six-well dishes and pelleted by centrifugation. Cells were resuspended in 900 μl of phosphate-buffered saline, to which 100 μl of 0.4% trypan blue dye (Sigma) was added, and incubated at room temperature for 10 min. 10 μl of this mixture was applied to a hemocytometer. Dead cells (those staining blue) were counted and compared with the total number of cells viewed in the hemocytometer.

RESULTS

Repression of BRCA1 in Wild-type p53-expressing Cell Lines—We have previously found that BRCA1 induces p53 protein accumulation (21). Recent evidence on the factors that contribute to such a process has suggested that the effect of p53 on these factors runs contrary to what one might expect. For example, whereas *MDM2* degrades p53, p53 in fact activates *MDM2* transcription. On the other hand, $p14^{ARF}$ is able to potently stabilize p53 protein; however, p53 represses $p14^{ARF}$ expression. Given this evidence, we sought to determine the effect of p53 on BRCA1 expression in light of recent results suggesting that BRCA1 expression may be reduced in some cell lines exposed to DNA-damaging agents.

Adriamycin is a potent DNA-damaging agent that rapidly induces a p53 response in wild-type p53-expressing cell types. We treated one wild-type (PA1), one mutant (SW480) and one null (Saos2) p53 cell line with adriamycin for 24 h and examined BRCA1 protein expression by immunoblotting. Whereas SW480 (colon carcinoma) and Saos2 (osteosarcoma) cells displayed a clear shift in mobility of BRCA1 protein as well as an apparent increase in the protein level, PA1 cells (ovarian carcinoma) appeared to have lost BRCA1 expression entirely (Fig. 1*A*). We also utilized lymphoblastoid cell lines derived from ataxia telangiectasia patients. These cells lack functional activity of ATM, a kinase required for the rapid accumulation of p53 protein and phosphorylation of BRCA1 (4) and NBS1 (33). We tested the 03189C line that is null for ATM activity as well as a control line (2184D) that possesses both functional copies of ATM for their effects on BRCA1 protein expression following 24 h of adriamycin treatment. The $ATM^{-/-}$ cell line displayed a full shift of the BRCA1 protein, which was present in similar amounts as the BRCA1 protein in untreated cells (Fig. 1*B*). In the wild-type ATM-containing lymphoblastoid cell line, BRCA1 also became phosphorylated upon treatment with adriamycin; however, the protein expression level decreased significantly by 24 h post-treatment. As p53 is wild-type in these lines, it is possible that the accumulation of p53 via ATM in the normal cell line led to a reduction of BRCA1 expression. Another DNA-damaging agent (mitomycin C) was also seen to cause a repression of BRCA1 protein expression only in the PA1 and HCT116 (colon carcinoma) wild-type p53-expressing cell lines, whereas no reduction or an induction was observed in the mutant p53-expressing lines SW480 and SkOV-3 (ovarian carcinoma) (Fig. 1*C*).

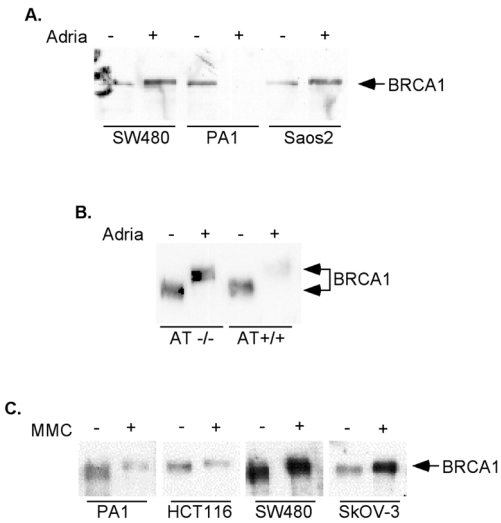

FIG. 1. *A*, BRCA1 protein expression is repressed in cells expressing wild-type p53. SW480, PA1, and Saos2 cells were treated with 0.2 μg/ml adriamycin (*Adria*) for 24 h, harvested for total protein, and electrophoresed on a 10% denaturing gel. Proteins were immunoblotted with anti-BRCA1 monoclonal antibody. *B*, 03189C (*AT* −/−) and 2184D (*AT* +/+) cells were treated with 0.2 μg/ml adriamycin for 24 h, harvested for total protein, and electrophoresed on a 6% denaturing gel. Proteins were immunoblotted with anti-BRCA1 monoclonal antibody. *C*, PA1, HCT116, SW480, and SkOV-3 cells were treated with 20 μg/ml mitomycin C (*MMC*) for 24 h and then harvested for total protein. 50 μg of total protein was electrophoresed on a 6% denaturing gel and subsequently immunoblotted with anti-BRCA1 monoclonal antibody.

Repression of BRCA1 Is Dependent on the Presence of p53—In addition to the differences in p53 status among the seven cancer cell lines described above, they are likely different genetically in many other ways as well. To more closely draw a link between BRCA1 repression and wild-type p53 presence, we utilized the H460 lung cancer cell line that expresses wild-type p53. Two variants of this line either express the human papilloma virus E6 protein that degrades p53 (H460-E6) or carry only the backbone vector from which human papilloma virus E6 is expressed (H460-Neo). Upon treatment of these lines with either 0.2 or 0.4 μg/ml adriamycin for 24 h, only the H460-Neo line stabilized p53 protein, whereas there was no detectable p53 protein in the H460-E6 line (Fig. 2), consistent with the previously reported E6-mediated p53 degradation in this line (27). Whereas BRCA1 protein shifted in the H460-E6 line treated with adriamycin, the H460-Neo cell line displayed a marked decrease in the BRCA1 protein expression level with

[1] The abbreviation used is: m.o.i., multiplicity of infection.

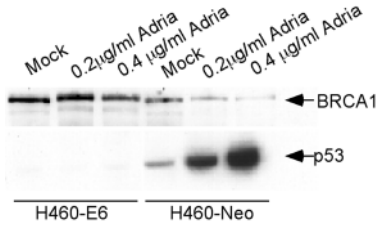

FIG. 2. **p53 dependence on BRCA1 repression.** H460-Neo and H460-E6 cells were treated with either 0.2 or 0.4 μg/ml adriamycin (*Adria*) for 24 h, harvested for total protein, and electrophoresed on a 10% denaturing gel. Proteins were immunoblotted with anti-BRCA1 or anti-p53 monoclonal antibody.

a similar mobility shift in the protein seen in the H460-E6 line. This reduction correlated with accumulation of p53 protein.

Kinetics of BRCA1 Repression by p53—To determine if BRCA1 repression correlated temporally with the onset of p53 protein accumulation post-treatment with adriamycin, we investigated the kinetics of BRCA1 disappearance in the H460 cell line after treatment with 0.2 μg/ml adriamycin. Total cellular RNA and protein were harvested at various time points over the course of 24 h. As early as 8 h after treatment with adriamycin, a reduction in *BRCA1* RNA to below basal levels was observed (Fig. 3*A*). By 12 h post-treatment, BRCA1 protein had completely disappeared, whereas another tumor suppressor, pRb, remained expressed (Fig. 3*B*). The reduction in *BRCA1* RNA correlated with the appearance of p53 protein, and the disappearance of BRCA1 protein correlated with the appearance of the p53 transcriptional target $p21^{WAF1}$. Activation of p53 expression did not affect expression of another tumor suppressor, pRb. Repression of BRCA1 did not appear to be downstream of the apoptotic effects of p53, as no signs of apoptosis in the H460 cell line were noted at 12 h, the time point after adriamycin treatment when repression of BRCA1 protein is most noticeable. We have previously determined the ID_{50} in response to adriamycin for this cell line to be 0.53 μg/ml (27), which is above the concentration used here and was derived from a 7–10-day treatment period. It is interesting to note that immediately upon treatment of the cells with adriamycin, *BRCA1* RNA increased ~2–3-fold, and an increase in BRCA1 protein was noted, indicating a rapid induction response of BRCA1 to DNA damage. This increase may explain why in mutant and null p53 cell lines there is apparently more BRCA1 protein present after DNA damage (Fig. 1).

Ectopic Expression of p53 Reduces BRCA1—To potentially eliminate other pathways activated by adriamycin treatment, we ectopically expressed p53 protein using an adenovirus vector in the mutant p53-expressing cell line SW480. Infection with Ad-p53, but not with Ad-LacZ or Ad-E2F, resulted in a complete disappearance of BRCA1 protein (Fig. 4*A*). Overexpression of p53 also resulted in a significant reduction in *BRCA1* mRNA as well, indicating that the decrease in protein noted by accumulation of p53 by adriamycin or overexpression by p53 adenovirus may be due to lack of *BRCA1* transcription (Fig. 4*B*). As has been suggested in the literature, BRCA1 expression may be cell cycle-regulated, with high levels of protein in cells in S and G_2/M phases and lower expression levels in cells in early G_1 phase (35). The $p21^{WAF1}$ protein has been found to mediate the p53-directed G_1 phase arrest (28). To determine if p53 was causing the repression of BRCA1 through the action of its downstream target p21, HCT116 and DLD1 cells deleted of their respective p21-coding region were infected with p53 adenoviruses and harvested for protein and RNA. As shown in Fig. 4 (*C* and *D*), p53 repressed BRCA1 protein and mRNA equally well in both $p21^{+/+}$ and $p21^{-/-}$ cells, indicating no requirement of p21 for this repression. Interestingly, in

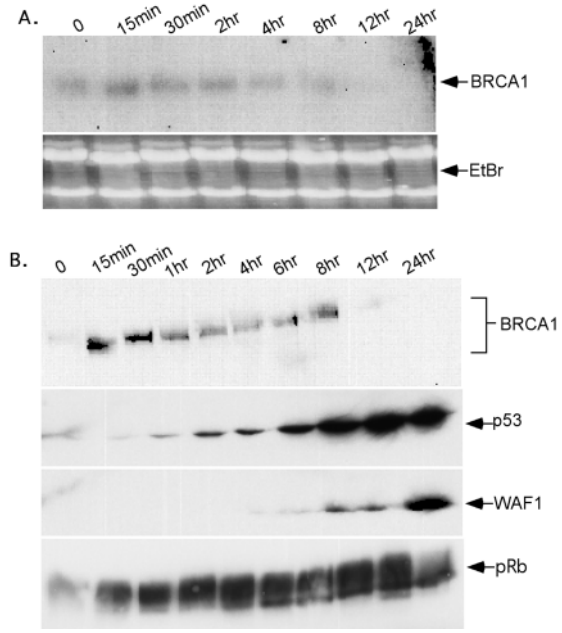

FIG. 3. **Time dependence of induction and repression of BRCA1.** H460 cells were treated with 0.2 μg/ml adriamycin and harvested for total RNA or protein at the indicated time points after treatment. *A*, RNA was Northern-blotted for BRCA1 expression using full-length BRCA1 as probe; *B*, total protein was immunoblotted for BRCA1, p53, p21, and pRb using the antibodies described under "Materials and Methods."

several experiments, we found BRCA1 protein and RNA to be expressed at much lower levels in both null p21 cells used here. p21 may in fact exert positive effects on the expression level or stability of the *BRCA1* transcript or protein unrelated to the cell cycle in asynchronously growing cells. To determine if p21 overexpression may be able to repress BRCA1 protein to levels seen in cases of p53 overexpression, we infected HCT116 cells with p53- and p21-expressing adenoviruses, harvested for total protein, and immunoblotted for BRCA1. Whereas some decrease in BRCA1 protein levels was noted in Ad-p21-infected cells (as expected due to a presumed G_1 phase arrest), the decrease in Ad-p53-infected cells was much greater, indicating that the effect that p53 has on BRCA1 expression levels goes beyond that of halting the cell cycle and may be a direct effect on transcriptional levels.

p53 Represses the BRCA1 Promoter—Luciferase reporter constructs with 1500, 800, or 200 bases upstream of the *BRCA1* transcriptional start site (34) were cotransfected into Saos2 cells with a p53 mammalian expression vector, and luciferase activity was determined. Compared with vector-alone transfections, p53 repressed all *BRCA1* promoter-luciferase constructs ~10-fold, whereas p53 expressed from the same vector was able to activate a construct containing 13 copies of the consensus p53-binding site (pG13) (Fig. 5).

Reintroduction of BRCA1 into Adriamycin-treated Wild-type p53-expressing Cells—To determine if re-expression of BRCA1 under conditions where it is normally repressed after DNA damage has any effect on cellular division, we treated MCF7 breast cancer cells with adriamycin and subsequently infected them with a BRCA1-expressing adenovirus. Whereas adriamycin treatment resulted in a G_1 and G_2 phase arrest, reintroduction of BRCA1 caused a reduction in G_2 phase cells and an increase in cells containing greater than 4N DNA content (Fig. 6*A*). This is opposed to ectopic expression of BRCA1 alone, which causes a G_1 and (to a lesser extent) G_2 phase arrest in MCF7 cells, with virtually no cells progressing beyond mitosis. Whereas adriamycin caused cell cycle arrest in MCF7 cells,

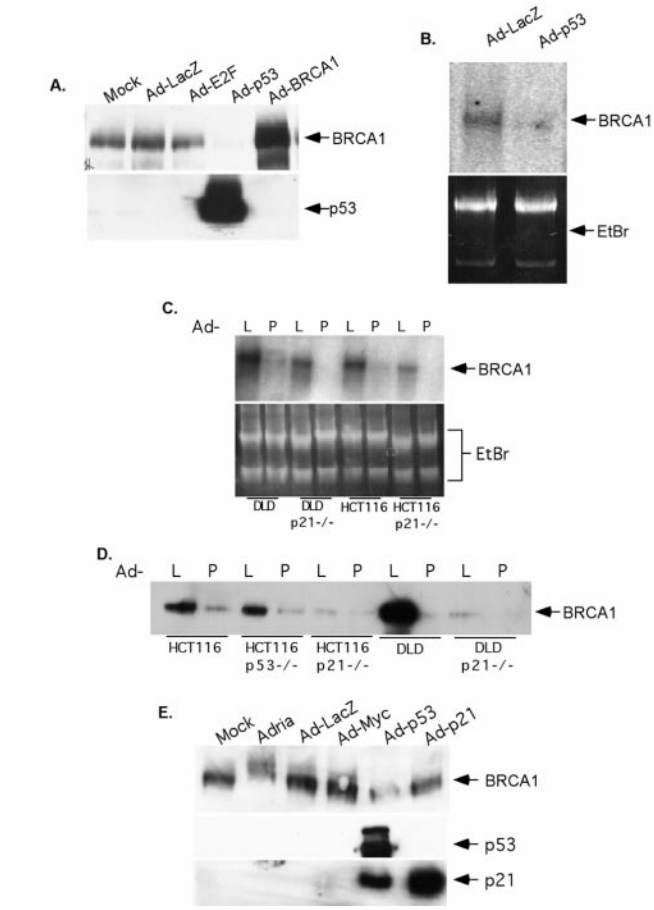

FIG. 4. **Ectopic expression of p53 represses BRCA1 protein.** SW480 cells were infected with the indicated adenoviruses at m.o.i. = 50. At 24 h post-infection, cells were harvested for total protein (*A*) or RNA (*B*). HCT116 and DLD1 cells and their $p21^{-/-}$ variants were infected with the indicated adenoviruses at m.o.i. = 40. 24 h post-infection, cells were harvested for total RNA (*C*) or protein (*D*). HCT116 cells were infected with the indicated adenoviruses at m.o.i. = 40 (*E*). 24 h post-infection, cells were harvested for total protein, electrophoresed on a 6% SDS-polyacrylamide gel, and immunoblotted for the indicated proteins. *Adria*, adriamycin. *L*, LacZ; *P*, p53.

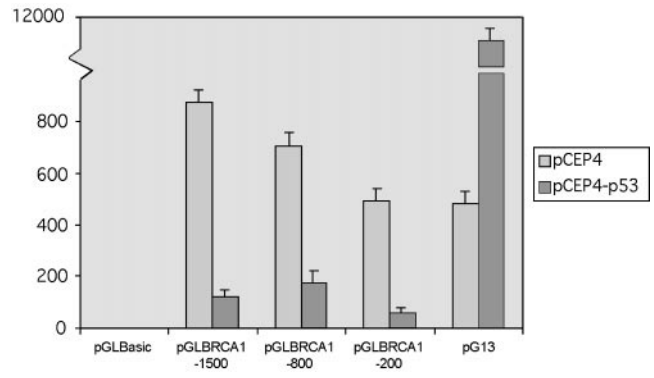

FIG. 5. **p53 represses the *BRCA1* promoter.** Saos2 cells were transfected with the *BRCA1* promoter reporter (−200BRCA, −800BRCA, and −1500BRCA) or pG13 along with pCEP4 or pCEP4-p53 as well as pCMVβ as an internal control. The luciferase activities of the lysates were measured and normalized to β-galactosidase activity.

p53-mediated apoptosis was the predominant result from this treatment in H460 cells. To determine if BRCA1 had any effect on the induction of cell death by adriamycin, H460 cells were infected with LacZ-, p53-, or BRCA1-expressing adenoviruses, treated with 0.2 μg/ml adriamycin, and monitored for cell death over the course of 36 h by trypan blue exclusion (Fig. 6*B*). Consistent with previously published results (43), Ad-p53 infection was strongly synergistic with adriamycin in apoptosis induction. On the other hand, Ad-BRCA1 in fact slightly delayed cell death compared with uninfected adriamycin-treated cells up until 36 h after infection/treatment. Ad-BRCA1 infection also had a dampening effect on the Ad-p53/adriamycin synergy, suggesting that p53 may in part provide this synergy by rapidly down-regulating endogenous BRCA1 expression. Therefore, the repression of BRCA1 in wild-type p53-expressing cells that undergo programmed cell death in response to adriamycin may be a means by which cells prepare for apoptosis, and untimed expression of BRCA1 during this process (via adenovirus) may delay this induction.

DISCUSSION

The effect on BRCA1 in response to cellular stresses has been the topic of much recent interest. BRCA1 was initially found to become rapidly phosphorylated after treatment with DNA-damaging agents such as γ-radiation, UV light, adriamycin, and mitomycin C (22). This phosphorylation has been hypothesized to result in the release of CtIP (8) and subsequent activation of the transcriptional activity of BRCA1, resulting in up-regulation of such genes as *GADD45* that are induced upon cellular stresses (18, 19).

We have shown previously that BRCA1 is able to induce the accumulation of p53, another event that occurs when wild-type p53-expressing cells are stressed (21). This result was achieved by overexpression of BRCA1 in wild-type p53-expressing cells. Here it is shown that BRCA1 expression in fact slightly increases at both the RNA and protein levels immediately after exposure to adriamycin. It is possible that this up-regulation sets off a process that ultimately leads to p53 stabilization, a process that is simulated by hyperexpression of BRCA1. A similar event that leads to p53 stabilization is the induction of $p14^{ARF}$. However, recent evidence suggests that $p14^{ARF}$ is quickly repressed by p53 itself, acting in a negative feedback loop (25). Here we describe that regulation of BRCA1 expression works in much the same way, seems to be dependent on ATM and p53, and can be simulated by overexpression of p53.

Whereas wild-type p53-expressing cells such as H460 and PA1 did not display significant BRCA1 expression after adriamycin treatment, mutant or null p53-expressing cells possessed abundant BRCA1 protein that was often increased above levels found in untreated cells. Since BRCA1 protein and RNA were found to be expressed at higher levels immediately following treatment, we hypothesize that this increased level of BRCA1 expression in mutant or null p53-expressing cells is due to the fact that p53 may be responsible for reduction of BRCA1 to or below basal levels after the initial treatment. In the absence of functional p53, BRCA1 levels remained high throughout the treatment. Similar observations have been made regarding rapid up-regulation of BRCA1 expression following exposure of human cells to ultraviolet radiation.[2]

p53 appears to act on the region closest to the transcriptional start site of *BRCA1* for its repressive effects. Similar results have been found with respect to p53 repression of other promoters such as *Map4*, *stathmin*, and *bcl-2* (36, 37), and in the case of *Map4* and *stathmin*, p53 has been shown to induce repression to this region of the promoter by recruitment of mSin3a and associated histone deacetylases (37). There has been a p53-negative regulatory element described for the *bcl-2* promoter that is able to confer repression of heterologous promoters by p53 and that resides within 200 bases upstream of the transcriptional start site of *bcl-2* (36). Therefore, p53 may be acting on this region of the *BRCA1* promoter for its repres-

[2] B. L. Weber, personal communication.

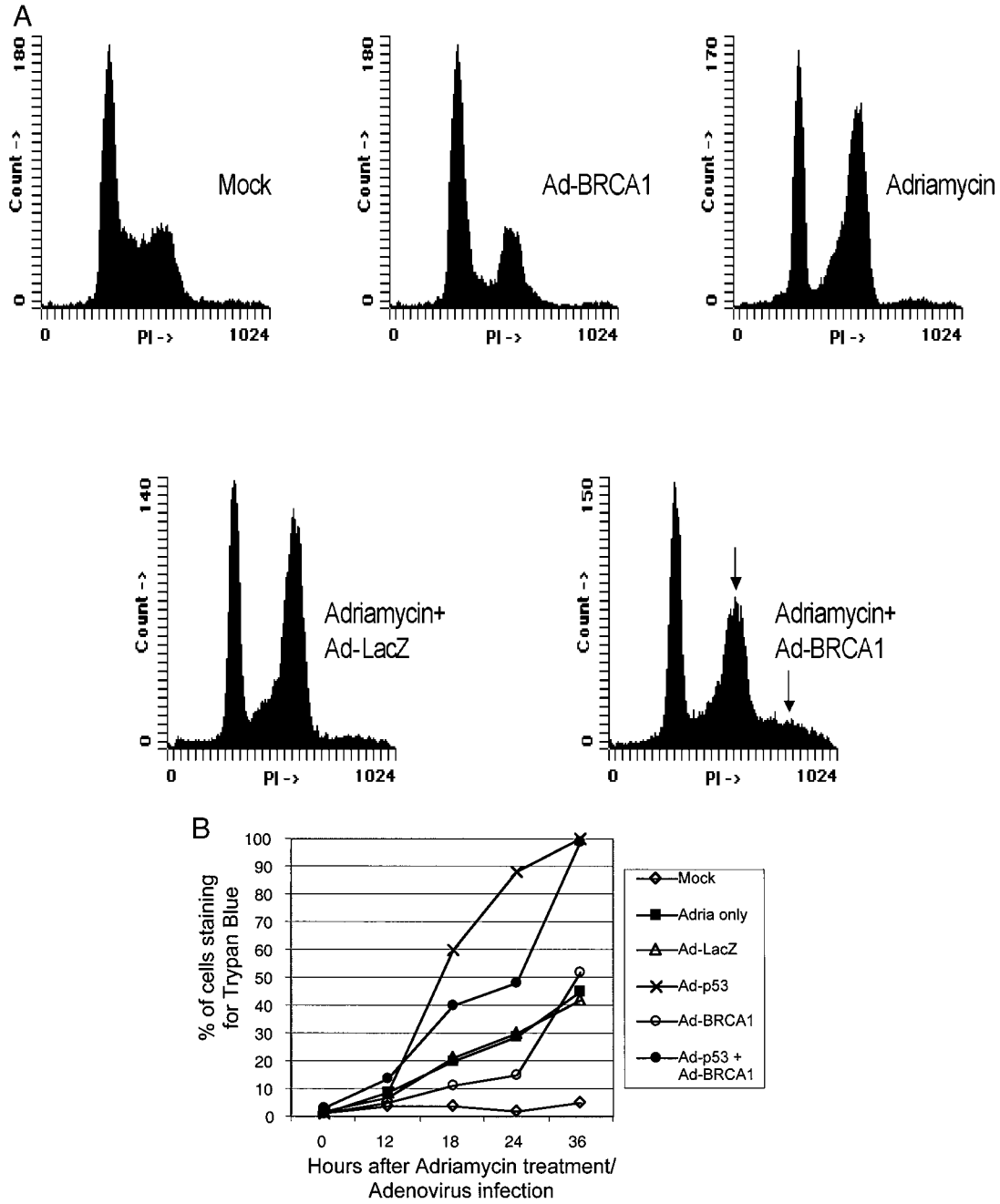

FIG. 6. **Effect of Ad-BRCA1 infection on wild-type p53-expressing cells after adriamycin treatment.** *A*, MCF7 cells were either treated with 0.2 μg/ml adriamycin or mock-treated for 24 h. Cells were then left in adriamycin for an additional 18 h and infected with LacZ or BRCA1 adenoviruses at m.o.i. = 20 or mock-infected. Cells were harvested, fixed, and analyzed for DNA content on a Coulter flow cytometer. *PI*, propidium iodide. *B*, H460 cells were infected with the indicated adenoviruses at m.o.i. = 10 and treated with 0.2 μg/ml adriamycin (*Adria*). Cells were harvested 12, 18, 24, and 36 h post-infection/treatment and measured for their ability to absorb trypan blue dye. The amount of cells taking up this dye is proportional to the number of cells undergoing cell death.

sive effects, although additional work is required to precisely map the negative regulatory elements. We have not ruled out, however, that p53 may be additionally contributing to a decreased stability of the BRCA1 protein and mRNA, noting that BRCA1 protein has recently been shown to be degraded through the ubiquitin-proteasome pathway (38).

Some data have shown that BRCA1 expression is regulated throughout the cell cycle (35). Cells in G_1 phase possess a reduced level of BRCA1 protein, whereas those cells transitioning through S and G_2 express much higher levels of BRCA1. An argument can be made that BRCA1 levels are reduced after adriamycin treatment and p53 overexpression by virtue of cell cycle arrest in G_1 phase. Indeed, p53 protein is induced by adriamycin as well as its downstream target $p21^{WAF1}$ (a potent inhibitor of the G_1/S phase transition) after treatment with DNA-damaging agents in wild-type p53-expressing cell lines. However, we have found that whereas $p21^{WAF1}$ expression is able to down-regulate BRCA1 to approximately basal levels, p53 causes BRCA1 protein to disappear altogether. In addition, cells lacking the p53 target $p21^{WAF1}$ that are not able to arrest in G_1 in response to p53 stabilization by DNA damage (39) also display repression of BRCA1 by p53. Nonetheless, down-regulation of BRCA1 by p53 may be due in part to a halt in cell cycle progression past the G_1/S border, but is repressed to levels beyond those seen in cell cycle arrest, indicating additional mechanisms to repress BRCA1 expression.

The purpose of down-regulation of a protein supposedly involved in DNA repair and sensing of DNA damage when a cell is stressed is somewhat of an enigma. However, given that the phosphorylation of BRCA1 and up-regulation of the RNA and protein are rapid immediate events following DNA damage, it is possible that the utility of BRCA1 after the initial sensing of damage is no longer present. We have reintroduced BRCA1 into MCF7 cells treated with adriamycin for 24 h by way of adenovirus infection, and we have observed subtle differences in cell cycle phase distribution that included reduction in G_2 phase-arrested cells as well as an increase in cells containing greater than 4N DNA content. Perhaps in this situation, the repression of BRCA1 is a mechanism by which p53 ensures that a cell does not progress into another replicative phase. BRCA1 protein has been shown to be expressed at high levels in S phase (35), to be present in a nuclear dot pattern during replication (22), and to be required for embryonic cell proliferation (40). Therefore, untimed expression of BRCA1 after DNA damage, once endogenous BRCA1 has been fully repressed, may allow the cell into a DNA replicative phase uncoupled from cell division that is inhibited by the DNA damage through other mechanisms. Alternatively, exogenous expression of BRCA1 in adriamycin-treated wild-type p53-expressing cells that undergo apoptosis appears to delay apoptosis induction by ~12 h. Combined with recent data showing resistance of BRCA1 mutant HCC1937 cells to γ-irradiation once wild-type BRCA1 is reintroduced to the cells via transfection or retrovirus infection (41, 42), these results suggest that, under these conditions, BRCA1 repression (or functional absence) may allow the cell to initiate apoptosis earlier or more effectively, whereas the presence of wild-type BRCA1 may provide sufficient DNA repair to sustain cell viability at least for a short period.

It is also possible that p53 may regulate BRCA1-mediated DNA repair mechanisms. Transcription-coupled repair, double strand break repair, and reversion of UV-induced DNA adducts have been suggested as functionalities of BRCA1 (2, 12). p53 may attempt to down-regulate these functions after they are completed by repression of BRCA1. This may point to a control p53 exerts over DNA repair: how much, what form, and when certain repair functions can take place following damage.

The results presented here are similar to those published by Andres *et al.* (23) and Fan *et al.* (24). In those reports, *BRCA1* mRNA expression was predominantly described by reverse transcription-polymerase chain reaction methods. We also found that the protein was reduced 12 h after treatment as well in all wild-type p53-expressing cells examined. This runs contrary to the findings of Andres *et al.*, who reported that decreases in BRCA1 protein were not observed until several days after treatment with adriamycin. Therefore, our study differs from previous reports on this issue in that a definitive link between p53 status and expression and BRCA1 repression was obtained. We also observed an induction of BRCA1 protein and mRNA immediately following DNA damage. This result, together with the findings of p53 regulation of BRCA1, helps explain why there appear to be higher levels of BRCA1 protein after exposure to adriamycin in mutant and null p53-expressing cells. These results also lend credence to previous findings of BRCA1 overexpression causing p53 accumulation and activation of gene expression in that an increase in BRCA1 expression is indeed a physiological event that may truly cause these downstream effects *in vivo*.

In conclusion, we have found that p53 may be responsible for the reduction in BRCA1 protein and RNA levels following the onset of DNA damage. We also observed an increase in both protein and RNA immediately after treatment of H460 cells with the DNA-damaging agent adriamycin, an induction that does not regress in cells that express mutant p53 or are null for the gene. Although we have shown previously that BRCA1 is able to induce p53 protein accumulation upon overexpression, our hypothesis is that p53 acts in a negative feedback loop with BRCA1, as it does with other proteins that induce its stabilization.

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REFERENCES

1. Irminger-Finger, I., Siegel, B. D., and Leung, W. C. (1999) *Biol. Chem.* **380,** 117–128
2. Scully, R., Chen, J., Plug, A., Xiao, Y., Weaver, D., Feunteun, J., Ashley, T., and Livingston, D. M. (1997) *Cell* **88,** 265–275
3. Zhong, Q., Chen, C. F., Li, S., Chen, Y., Wang, C. C., Xiao, J., Chen, P. L., Sharp, Z. D., and Lee, W. H. (1999) *Science* **285,** 747–750
4. Cortez, D., Wang, Y., Qin, J., and Elledge, S. J. (1999) *Science* **286,** 1162–1166
5. Lee, J.-S., Collins, K. M., Brown, A. L., Lee, C.-H., Chung, J. H. (2000) *Nature* **404,** 201–204
6. Scully, R., Anderson, S. F., Chao, D. M., Wei, W., Ye, L., Young, R. A., Livingston, D. M., and Parvin, J. D. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94,** 5605–5610
7. Anderson, S. F., Schlegel, B. P., Nakajima, T., Wolpin, E. S., and Parvin, J. D. (1998) *Nat. Genet.* **19,** 254–256
8. Li, S., Chen, P. L., Subramanian, T., Chinnadurai, G., Tomlinson, G., Osborne, C. K., Sharp, Z. D., and Lee, W. H. (1999) *J. Biol. Chem.* **274,** 11334–11338
9. Yu, X., Wu, L. C., Bowcock, A. M., Aronheim, A., and Baer, R. (1998) *J. Biol. Chem.* **273,** 25388–25392
10. Yarden, R. I., and Brody, L. C. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96,** 4983–4988
11. Kleiman, F. E., and Manley, J. L. (1999) *Science* **285,** 1576–1579
12. Gowen, L. C., Avrutskaya, A. V., Latour, A. M., Koller, B. H., and Leadon, S. A. (1998) *Science* **281,** 1009–1012
13. Zhang, H., Somasundaram, K., Peng, Y., Tian, H., Zhang, H., Bi, D., Weber, B. L., and El-Deiry, W. S. (1998) *Oncogene* **16,** 1713–1721
14. Ouchi, T., Monteiro, A. N., August, A., Aaronson, S. A., and Hanafusa, H. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95,** 2302–2306
15. Wang, Q., Zhang, H., Kajino, K., and Greene, M. I. (1998) *Oncogene* **17,** 1939–1948
16. Ludwig, T., Chapman, D. L., Papaioannou, V. E., and Efstratiadis, A. (1997) *Genes Dev.* **11,** 1226–1241
17. Somasundaram, K., Zhang, H., Zeng, Y. X., Houvras, Y., Peng, Y., Zhang, H., Wu, G. S., Licht, J. D., Weber, B. L., and El-Deiry, W. S. (1997) *Nature* **389,** 187–190
18. Harkin, D. P., Bean, J. M., Miklos, D., Song, Y. H., Truong, V. B., Englert, C., Christians, F. C., Ellisen, L. W., Maheswaran, S., Oliner, J. D., and Haber, D. A. (1999) *Cell* **97,** 575–586
19. MacLachlan, T. K., Somasundaram, K., Sgagias, M., Shifman, Y., Muschel, R. J., Cowan, K. H., and El-Deiry, W. S. (2000) *J. Biol. Chem.* **275,** 2777–2785
20. Chai, Y. L., Cui, J., Shao, N., Reddy, E. S. P., and Rao, V. N. (1999) *Oncogene* **18,** 63–68
21. Somasundaram, K., MacLachlan, T. K., Burns, T. F., Sgagias, M., Cowan, K. H., Weber, B. L., and El-Deiry, W. S. (1999) *Oncogene* **18,** 6605–6614
22. Scully, R., Chen, J., Ochs, R. L., Keegan, K., Hoekstra, M., Feunteun, J., and Livingston, D. M. (1997c) *Cell* **90,** 425–435
23. Andres, J. L., Fan, S., Turkel, G. J., Wang, J. A., Twu, N. F., Yuan, R. Q., Lamszus, K., Goldberg, I. D., and Rosen, E. M. (1998) **16,** 2229–2241
24. Fan, S., Twu, N. F., Wang, J. A., Yuan, R. Q., Andres, J., Goldberg, I. D., Rosen, E. M. (1998) *Int. J. Cancer* **77,** 600–609
25. Stott, F. J., Bates, S., James, M. C., McConnell, B. B., Starborg, M., Brookes, S., Palmero, I., Ryan, K., Hara, E., Vousden, K. H., and Peters, G. (1998) *EMBO J.* **17,** 5001–5014
26. Robertson, K. D., and Jones, P. A. (1998) *Mol. Cell. Biol.* **18,** 6457–6473
27. Wu, G. S., and El-Deiry, W. S. (1996) *Clin. Can. Res.* **2,** 623–633
28. El-Deiry, W. S., Tokino, T., Velculescu, V. E., Levy, D. B., Parsons, R., Trent, J. M., Lin, D., Mercer, W. E., Kinzler, K. W., and Vogelstein, B. (1993) *Cell* **75,** 817–825
29. Wu, G. S., Burns, T. F., McDonald, E. R., Meng, R., Kao, G., Muschel, R., Yen, T., and El-Deiry, W. S. (1999) *Oncogene* **18,** 6411–6418
30. Thakur, S., Zhang, H. B., Peng, Y., Le, H., Carroll, B., Ward, T., Yao, J., Farid, L. M., Couch, F. J., Wilson, R. B., and Weber, B. L. (1997) *Mol. Cell. Biol.* **17,** 444–452
31. Zeng, Y. X., Somasundaram, K., and El-Deiry, W. S. (1997) *Nat. Genet.* **15,** 78–82
32. van den Heuvel, S., and Harlow, E. (1993) *Science* **262,** 2050–2054
33. Lim, D. S., Kim, S.-T., Xu, B., Maser, R. S., Lim, J., Petrini, J. H. J., and Kastan, M. B. (2000) *Nature* **404,** 613–617
34. Thakur, S., and Croce, C. M. (1999) *J. Biol. Chem.* **274,** 8837–8843
35. Ruffner, H., and Verma, I. M. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94,** 7138–7143
36. Miyashita, T., Harigai, M., Hanada, M., and Reed, J. C. (1994) *Cancer Res.* **54,** 3131–3135
37. Murphy, M., Ahn, J., Walker, K. K., Hoffman, W. H., Evans, R. M., Levine, A. J., and George, D. L. (1999) *Genes Dev.* **13,** 2490–2501
38. Blagosklonny, M. V., An, W. G., Melillo, G., Nguyen, P., Trepel, J. B., and Neckers, L. M. (1999) *Oncogene* **18,** 6460–6468

39. Waldman, T., Lengauer, C., Kinzler, K. W., and Vogelstein, B. (1996) *Nature* **381,** 713–716
40. Hakem, R., de la Pompa, J. L., Sirard, C., Mo, R., Woo, M., Hakem, A., Wakeham, A., Potter, J., Reitmair, A., Billia, F., Firpo, E., Hui, C. C., Roberts, J., Rossant, J., and Mak, T. W. (1996) *Cell* **85,** 1009–1023
41. Abbott, D. W., Thompson, M. E., Robinson-Benion, C., Tomlinson, G., Jensen, R. A., and Holt, J. T. (1999) *J. Biol. Chem.* **274,** 18808–18812
42. Scully, R., Ganesan, S., Vlasakova, K., Chen, J., Socolovsky, M., and Livingston, D. M. (1999) *Mol. Cell* **4,** 1093–1099
43. Blagosklonny, M. V., and El-Deiry, W. S. (1998) *Int. J. Cancer* **75,** 933–940