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Research Article

Kinetics of BRCA1 regulation in response to UVC radiation

C. E. K. Clarkin[a], H. Zhang[b] and B. L. Weber[b,c,*]

[a]Department of Biochemistry and Molecular Biophysics, University of Pennsylvania, Philadelphia (Pennsylvania 19104, USA)
[b]Department of Medicine, University of Pennsylvania, Philadelphia (Pennsylvania 19104, USA), Fax + 1 215 573 2486, e-mail: weberb@mail.med.upenn.edu
[c]Department of Genetics, University of Pennsylvania, Philadelphia (Pennsylvania 19104, USA)

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Abstract. To investigate changes in BRCA1 following DNA damage, we exposed MCF-7 cells to increasing doses of ultraviolet C. We observed an increase in BRCA1 protein levels above 78 J/m^2. This increase was observed as early as 5 min after irradiation. BRCA1 levels were then observed to decrease after 2 h, consistent with the previously published data. By pretreating with cycloheximide prior to irradiation, we observed a decrease in the protein half-life, from 3.5 h to 53 min, suggesting that a decrease in protein half-life may cause the lower levels of BRCA1 after irradiation. We also observed an increase in BRCA1 mRNA within 15 min of irradiation, followed by a decrease after 4 h. These data suggest that newly translated protein may contribute to increases in BRCA1 protein levels. The very rapid changes in BRCA1 support its role as a sensor of DNA damage, as opposed to being a repair gene.

Key words. Breast cancer; BRCA1; DNA damage; ultraviolet C; MCF-7.

Introduction

Germline mutations in BRCA1 account for 20–40% of inherited susceptibility to breast cancer, with mutation carriers having as much as an 80–90% lifetime cancer risk [1]. Tumors arise in mutation carriers who lose function of the wild-type allele. This loss is often associated with a region of loss of heterozygosity, and thus BRCA1 is thought to be a classic tumor suppressor gene [2]. The 1863-amino acid BRCA1 protein is regulated in concert with the cell cycle, with protein at its highest levels during late G1/early S [3, 4]. BRCA1 phosphorylation is also coordinated with the G1/S phase transition [5]. BRCA1 is localized to the nucleus in a discrete dot-like pattern [6, 7]. These nuclear foci are also regulated with the cell cycle, forming primarily during S phase while remaining diffuse throughout the nucleus and perhaps the perinuclear region during other cell cycle phases [7, 8]. On initial analysis, the BRCA1 protein appeared to have few motifs suggestive of function. On further inspection, a new motif was uncovered in its carboxy-terminal domain, the BRCT domain [9]. This motif is frequently found in proteins involved in DNA repair or damage-responsive cell cycle checkpoint function [10, 11] suggesting that BRCA1 may be involved in the cellular response to DNA damage.

Several groups have sought to determine whether BRCA1 is required for the response to specific types of DNA damage. Husain et al. [12] first demonstrated that the downregulation of BRCA1 by expression of BRCA1 antisense RNA complementary to exon 11 sequences resulted in a decreased capacity to repair a reporter construct, suggesting that BRCA1 was impor-

* Corresponding author.

tant for global nucleotide excision repair. Gowen and colleagues [13] later used BRCA1-null embryonic stem cells to provide evidence that BRCA1 is required for efficient transcription-coupled repair of ionizing radiation and H_2O_2-induced lesions but suggested that BRCA1 may not play a role in UV-induced damage. Modification of BRCA1 in response to DNA damage was demonstrated by two other groups, which observed phosphorylation following DNA damage induced by H_2O_2, UV, or γ-radiation [14, 15]. These two groups also demonstrated that BRCA1-containing nuclear dots disperse following exposure of cells to several DNA-damaging agents, including UV radiation. Since the time course of the phosphorylation events coincided with that of foci dispersal, the phosphorylation was suggested to be a trigger for the release of BRCA1 from the nuclear foci [15].

These recent studies of specific BRCA1 modifications and global alterations in damage pathways provide evidence that BRCA1 is a component of the cellular response to DNA damage. However, little is known about the specific response of BRCA1 protein levels to DNA damage. Data on the kinetics of BRCA1 changes due to specific damage-inducing agents may provide clues helpful in defining mechanisms by which BRCA1 plays a role in maintaining genome stability. Thus, we performed a detailed analysis of the BRCA1 protein and mRNA following UV irradiation, based on the observation by Scully and colleagues [15] that UV exposure results in dispersal of BRCA1-containing nuclear foci. Our data provide evidence for immediate changes in the BRCA1 protein following UV exposure, in concert with the hypothesis that BRCA1 may play a role in detecting DNA damage and signaling to critical checkpoints, as opposed to actually repairing the damaged DNA.

Materials and methods

Cell culture and UV treatment. The human breast adenocarcinoma cell line MCF-7 was obtained from the American Type Culture Collection. Cells were maintained in a humidified atmosphere of 5% CO_2 at 37 °C in DMEM supplemented with 10% (v/v) calf serum, 200 mM glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin. Cells in logarithmic growth were treated with UVC radiation (254 nm) at room temperature using the Fisher UV-crosslinker (FB-UVXL-1000; Fisher Scientific). Sham-treated control cells were subjected to the same medium removal and movements in and out of incubators as irradiated cells, but were not exposed to radiation.

Immunoprecipitation. Cell lysis and immunoprecipitation were performed using the Immunoprecipitation Kit with protein G (Boehringer Mannheim) solutions and protocols. Briefly, cells were lysed in lysis buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, Complete protease inhibitor cocktail tablet) and homogenized using a Dounce homogenizer. Lysate was precleared with protein G agarose beads, then incubated with 1 μg/ml anti-BRCA1 antibody (Ab-1; Oncogene Sciences) for 1 h. Complexes were washed and precipitated by incubation with protein G agarose beads and centrifugation. Beads were then boiled in SDS-lysis buffer and loaded onto acrylamide gels for Western analysis.

Western blot analysis. Cell lysates were harvested by scraping cells into SDS lysis buffer (60 mM Tris, pH 6.8, 100 mM dithiothreitol, 2% SDS) and heating at 100 °C for 5 min. Lysates were pulled through a 28-gauge needle to shear DNA and were clarified by centrifugation. Samples were electrophoresed on a 7.5% polyacrylamide gel and transferred to stabilized nitrocellulose. Blots were blocked with 10% dry milk, 0.1% Tween 20 in PBS for 1 h, then incubated with either 1 μg/ml anti-BRCA1 antibody (Ab-1) for 2 h or a 1:200 dilution of anti-actin antibody (AC-40; Sigma) for 1 h using methods adapted from those described by Scully et al. [6]. Primary antibody incubation was followed by incubation with anti-mouse IgG conjugated to horseradish peroxidase (HRP) secondary antibody (Amersham).

Protein half-life determination. Pilot experiments were performed to determine appropriate cycloheximide concentrations for maximal translation inhibition with minimal cellular toxicity. These experiments determined the minimal effective conditions to be 25 μg/ml cycloheximide incubated with the cells for 1 h. Cells in logarithmic growth phase were treated with cycloheximide (Calbiochem) diluted into complete DMEM at the time of refeeding following irradiation. Cells were lysed and prepared as for standard Western analysis.

Northern blot analysis. Total RNA was prepared using Trizol reagent according to the manufacturer's instructions. RNA (10 μg) was electrophoresed on denaturing formaldehyde gels and transferred by the capillary method overnight to a charged nylon membrane as described previously [16]. Blots were blocked for 6 h with sheared salmon sperm DNA in hybridization solution at 65 °C. BRCA1 mRNA was probed by incubating the blots with a probe mixture corresponding to exon 11 and the amino-terminal RING finger domain of the BRCA1 cDNA overnight at 65 °C. G3PDH was visualized using a commercially available probe (# 9805-1; Clontech).

Flow cytometric analysis. Cells were harvested by trypsin release, washed twice in PBS, and fixed in 70% ethanol overnight at − 20 °C. Fixed cells were pelleted by centrifugation at 2000 rpm and resuspended in Triton-PI stain (0.1% Triton X-100, 20 μg/ml propidium

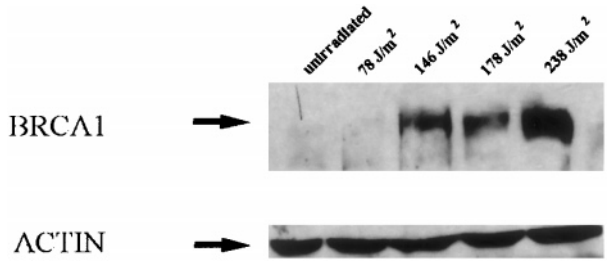

Figure 1. Western blot analysis of BRCA1 following increasing doses of UVC treatment. MCF-7 cells were either irradiated with increasing doses of 254-nm UVC in 100-mm dishes or sham treated and harvested at 2 h. β-Actin was immunolabeled to show equal protein loading.

iodide, DNase-free RNase in PBS). Cells were stained overnight before being analyzed. Data were collected using a FACScan flow cytometer (Becton Dickinson) and modeled for cell cycle phases using ModFitLT (Becton Dickinson).

Results

BRCA1 increases following UV radiation in a time- and dose-dependent manner. When MCF-7 cells are exposed to increasing doses of UVC radiation, BRCA1 protein levels were increased at the 2-h time point measured compared to unirradiated cells (fig. 1). At UVC levels greater than 78 J/m^2, a higher dose correlated with a higher BRCA1 level, indicating that BRCA1 changes in a dose-dependent but apparently not linear manner to UVC radiation, suggestive of a threshold response. We then analyzed BRCA1 at different time points following 178 J/m^2 UVC radiation. BRCA1 increased above baseline levels as early as 5 min postirradiation and remained elevated for at least 30 min (fig. 2). Subsequent experiments demonstrated that BRCA1 levels remained high for approximately 2 h, then decreased below baseline levels by 4–6 h (data not shown). Densitometry analysis of the Western blot shown in figure 2 demonstrated an approximately tenfold increase above baseline levels at 15 min (data not shown). Following UVC radiation, a more slowly migrating form of BRCA1 appeared (fig. 2). Since phosphorylation following UV radiation has been previously described [14, 15], this was likely phosphorylated BRCA1.

BRCA1 increase is not dependent on antibody epitope. The increase in detectable BRCA1 levels was visualized on Western blots using an amino-terminal antibody (Ab-1). We sought to verify that this increase was not due to the unfolding of an amino-terminal epitope following UVC radiation and/or phosphorylation. Therefore, BRCA1 was immunoprecipitated from irradiated cell lysates using a carboxy-terminal antibody (Ab-3). Western analysis was performed on the resulting eluate using Ab-1 (fig. 3). The increase in BRCA1 was also evident using this method, demonstrating that more BRCA1 was physically available for detection.

Cell cycle changes following UVC exposure. MCF-7 cells have previously been demonstrated to arrest in S phase following UVC treatment. Therefore, we considered the possibility that the increase in BRCA1 protein levels following UVC radiation might be due to enrichment of the cell population in S phase, when BRCA1 levels are highest. Flow cytometry analysis demonstrated that at doses up to 238 J/m^2 and at times up to 8 h following UVC exposure, there was a slight increase in S phase cells (table 1). The 78 and 238 J/m^2 S phase distributions were statistically different ($P < 0.04$ and $P < 0.05$, respectively) using Student's t-test, but this increase was consistently less than 15%, suggesting that cell cycle arrest alone likely did not contribute to the observed BRCA1 increase (fig. 4).

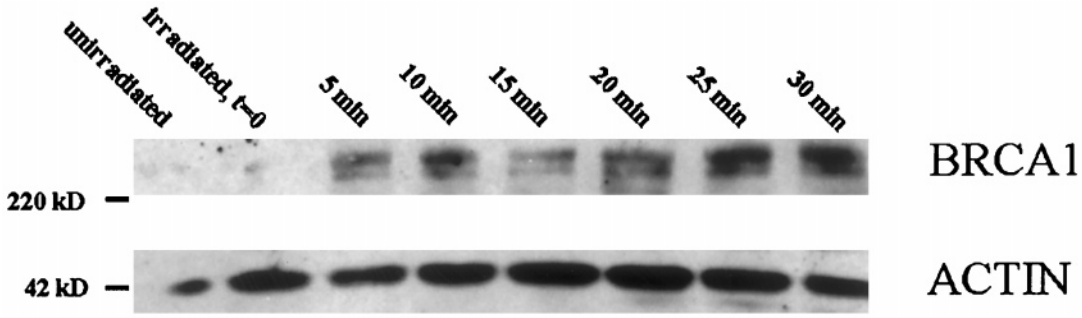

Figure 2. Time course of BRCA1 modification following UVC treatment. MCF-7 cells were irradiated with 178 J/m^2 UVC or sham treated and harvested at the times shown. β-Actin was immunolabeled to demonstrate equal protein loading.

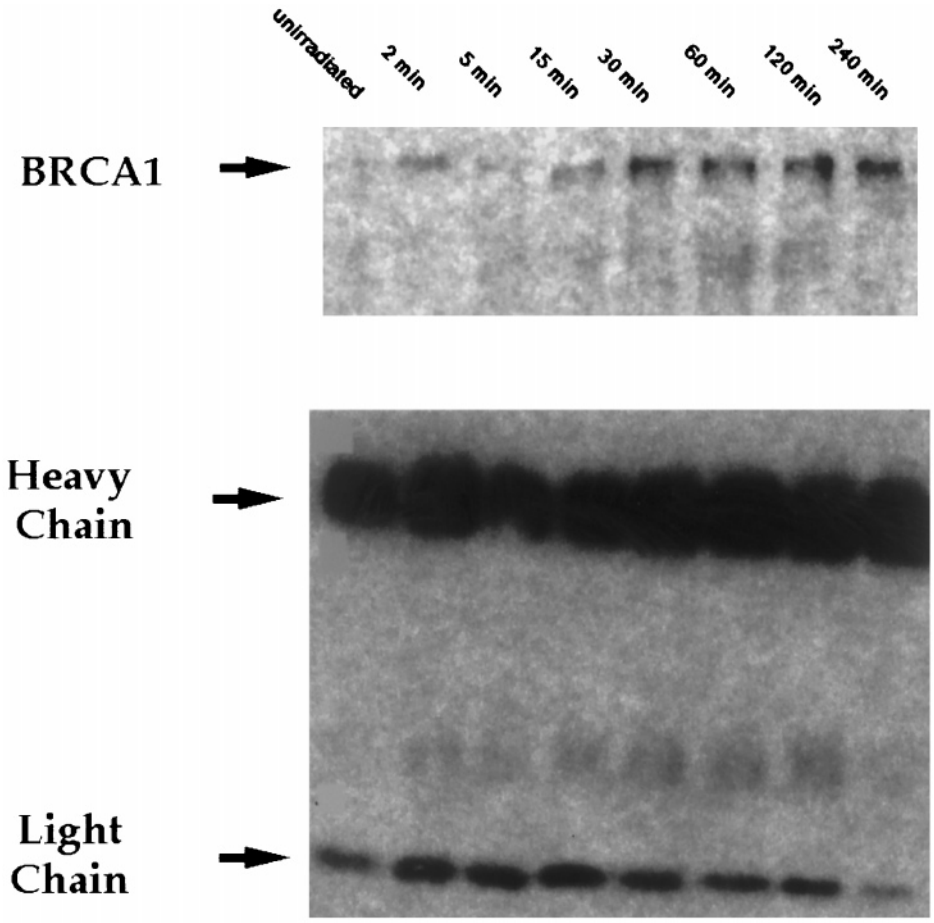

Figure 3. Western blot analysis of immunoprecipitated BRCA1 following UVC treatment. MCF-7 cells were either irradiated with 50 J/m^2 UVC or sham treated and harvested at varying times. BRCA1 protein was isolated by immunoprecipitation with a BRCA1-specific antibody (Ab-3) and precipitated by heating in SDS solution. Western analysis was performed on BRCA1, and IgG heavy chain to demonstrate equal loading.

BRCA1 protein half-life decreases following UVC radiation. Given that BRCA1 levels increase as early as 5 min following UVC radiation, we sought to determine if its stability was altered, as is seen for other proteins including p53 [17, 18]. We treated MCF-7 cells with cycloheximide to inhibit translation of new BRCA1 protein and measured the apparent half-life of the existing BRCA1 protein (fig. 5). Under these conditions, the half-life of BRCA1 decreased from approximately 3.5 h to approximately 53 min following 75 J/m^2 UVC, as determined by densitometry analysis (data not shown). This is consistent with, and provides an explanation for, the previously published decrease in BRCA1 at later time points following UVC [19, 20] and demonstrates that the early increase in BRCA1 is not related to an increase in the stability of existing protein. Interestingly, the phosphorylation observed by us and others is evident for up to 6 h following UV and cycloheximide treatment (fig. 5). These data suggest that neither the phosphorylation of BRCA1 nor the decrease in BRCA1 protein levels require synthesis of new protein.

Of note is that the exposure of MCF-7 cells to cycloheximide in the experiment resulted in a significant amount of BRCA1 detection in unirradiated cells (fig. 5). While this is clearly not consistent with our findings without cycloheximide (figs 1–3), we postulate that this increase could be due to the toxic effects of cycloheximide. Nutt and Lunec [21] previously demonstrated that apoptotic signals may be activated in some cell types, including MCF7 cells, following cycloheximide treatment. These authors suggested that the inhibition of protein synthesis may activate the stress response for tissue breakdown and repair. Considering the evidence that BRCA1 is involved in the apoptotic pathway, cellular damage by cycloheximide could possibly induce BRCA1 as well. For the purposes of this study, we have made the

Table 1. Flow cytometric analysis of MCG-7 cells treated with UVC radiation.

Treatment‡	Percentage distribution		
	G1 phase	S phase	G2M phase
Unirradiated MCF-7	45.12 ± 4.6	42.53 ± 5.3	12.35 ± 0.8
30 J/m^2, 2 h	30.32 ± 9.3	57.57 ± 7.4	12.11 ± 1.9
75 J/m^2, 2 h	26.50 ± 8.1	57.25 ± 5.2	16.25 ± 4.7
78 J/m^2, 2 h	32.35 ± 2.9	58.06 ± 3.9 ($P<0.05$)	9.60 ± 4.7
146 J/m^2, 2 h	37.14 ± 1.5	51.24 ± 4.6	11.61 ± 3.2
178 J/m^2, 2 h	24.33 ± 9.8	58.35 ± 6.1 ($P<0.05$)	17.31 ± 3.7
238 J/m^2, 2 h	27.66 ± 6.9	57.78 ± 4.5	14.56 ± 3.3
75 J/m^2, 1 h	34.99 ± 6.4	50.93 ± 3.2	110.66 ± 1.7
75 J/m^2, 2 h	27.38 ± 6.1	58.25 ± 5.0	14.38 ± 2.5
75 J/m^2, 4 h	32.43 ± 8.0	51.00 ± 4.1	16.57 ± 4.4
75 J/m^2, 6 h	34.48 ± 8.7	53.85 ± 2.2	7.78 ± 1.1
75 J/m^2, 8 h	34.55 ± 4.0	55.05 ± 4.6	10.39 ± 0.9

Results (mean ± SD) are the average of three experiments and 10,000 events/experiment. S phase results were analyzed for significance using Student's t-test.
‡ Times are incubation period following irradiation, at 37 °C.

assumption that the half-life determination, within the limitations of the method, is valid given that the baseline is similarly perturbed in both the irradiated and unirradiated cells.

BRCA1 transcript levels increase following UV treatment. Some proteins which are upregulated at early times following radiation are also transcriptionally upregulated, including c-fos and c-jun [22]. Therefore, we considered the possibility that BRCA1 levels were increased due to transcriptional mechanisms. To test this hypothesis, BRCA1 transcript levels following UVC treatment were measured using Northern blot analysis. BRCA1 transcript levels increased at the first time point measured (15 min), peaking at 30 min following exposure, and remained above baseline levels for up to 1 h (fig. 6). Using densitometry analysis corrected for loading artifacts, peak levels were measured at 60% above baseline BRCA1 transcript levels (data not shown). BRCA1 transcript then decreased as previously observed [19, 20] to approximately 76% below baseline levels by 24 h. These data demonstrate that the early increase in BRCA1 may be at least partially regulated by increased synthesis or stability of BRCA1 mRNA. In addition, the later decrease in BRCA1 protein level following UVC radiation may be due to reduced BRCA1 transcription or transcript stability, as well as the decreases in protein half-life discussed above.

Discussion

With these experiments, we demonstrate that immediately after UVC exposure, BRCA1 protein levels increase in a time- and dose-dependent manner. At time points as early as 5 min following UV exposure, BRCA1 levels are quantitatively higher than in unirradiated controls. We could find no physical evidence that BRCA1 accumulates at very early time points due to increased stability of the protein, but at later time points, new BRCA1 protein may contribute to the observed increase. Based on the data we present, this increase is unlikely due to changes in antibody epitope exposure (fig. 3) or the percentage of cells in S phase (table 1, fig. 4). However, 'ready-made' BRCA1 could be added to the protein pool, without increased protein stability or transcription, if protein solubility were increased, making it more readily extracted using standard biochemical methods. We propose that BRCA1 may be tightly bound to the previously described nuclear structures before UV exposure and is released from these complexes very early after damage occurs. Later accumulations of BRCA1 may be due to de novo

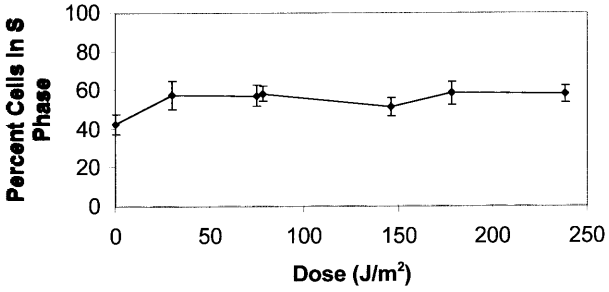

Figure 4. S phase analysis following increasing doses of UVC. Data from table 1 were averaged for each dose point and then graphed as percentage of cells in the S phase of the cell cycle versus dose of UVC. Error bars indicate SD.

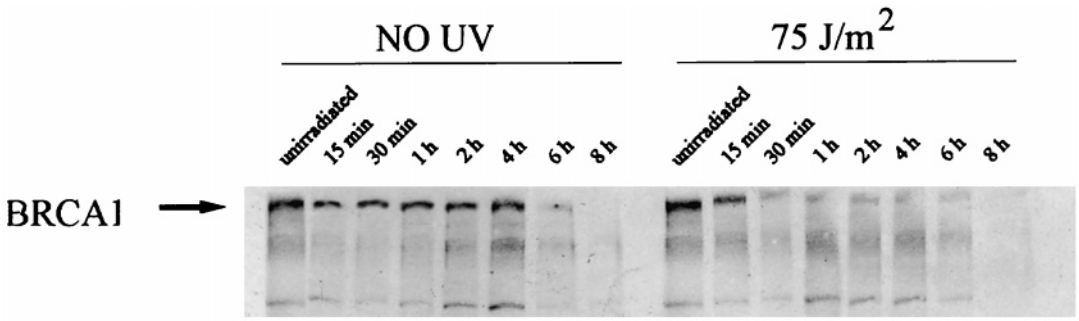

Figure 5. Western blot analysis of BRCA1 following UVC and cycloheximide treatment. MCF-7 cells were fed with medium containing 25 μg/ml cycloheximide and irradiated with 75 J/m^2 or sham treated. Protein was harvested at the times shown following irradiation.

protein produced from increased BRCA1 mRNA levels. Our data suggest that kinetically, BRCA1 accumulation is a very early event in the UV damage response. These data need to be extended and confirmed in other cell lines and under other conditions; however, they provide further evidence for the role of BRCA1 in the cellular response to DNA damage.

The dose-dependent increase in BRCA1 detectability we describe is similar to that found for several proteins involved in the UV response, such as p53, c-fos, and c-jun [18, 22]. The levels of these proteins increase quite rapidly, to either signal to repair pathways or to upregulate the transcription of damage-responsive genes. A very early response of BRCA1 to increasing doses of radiation was noted by Scully et al. [15], who also observed that the number of BRCA1 phosphorylation events increased with higher doses of ionizing radiation. Even though we have demonstrated that the earliest component of this increase was not likely due to new protein, a quantitative change in protein availability is compatible with the idea that BRCA1 is involved in the cellular response to UV-induced damage. The subcellular localization of BRCA1 changes after UV radiation [14, 15], and this change in location may alter the protein solubility to perform a precise cellular function. In fact, this exact phenomenon has been noted for PCNA, a protein that colocalizes with BRCA1 [15]. PCNA becomes bound to the nuclear matrix following DNA damage, causing its solubility to change [23].

Scully et al. [15] found that the increasing number of phosphorylation events was directly related, temporally, to the observed loss in BRCA1-containing nuclear foci [15]. These data suggest that BRCA1 becomes phosphorylated, changing the protein conformation or charge. This change in BRCA1 could cause its release from dot-like nuclear structures, along with Rad51, BARD1, and BRCA2, allowing the relocalization of all four proteins to a PCNA-containing complex [15, 24]. The transient increase in BRCA1 protein from 5 min to 2 h, following UV radiation, also parallels the previously published data that the number of phosphorylation events increases over time [15]. In fact, the time course of this phosphorylation increase is strikingly similar to the time-dependent protein increase we observed, with the phosphorylation appearing as early as 20 min and increasing out to 1 h after UV exposure. In our experiments, BRCA1 protein increased as early as 5 min postirradiation with protein levels remaining elevated out to 2 h. In addition, we observed a slower migrating band along with the BRCA1 increase, as early as 5 min postirradiation. These findings suggest that phosphorylation may alter the conformation of BRCA1 to a new, relaxed structure which not only releases BRCA1 from the nuclear foci, but also enables it to be more easily solubilized using standard biochemical methods. In support of this hypothesis, Yuan et al. [25] found that after adriamycin treatment, phosphorylated BRCA1 disappeared more rapidly than the unphosphorylated form.

Given the early physical changes to BRCA1, as described here and by others [14, 15], BRCA1 could be involved in the very early events of DNA damage repair, such as sensing or signaling the damage. BRCA1 responds measurably to more than one type of damage, including but not limited to 254-nm UV, γ-radiation, oxidative damage, adriamycin, and hydroxyurea [14, 15, 20, 26]. These agents cause different types of DNA damage requiring repair by multiple pathways. Considering that BRCA1 has not been demonstrated to bind DNA or to exhibit any catalytic activity, it is less likely to play a direct role in the repair of DNA damage.

We considered the possibility that the conformation of BRCA1 was altered by phosphorylation following damage and that, consequently, its antibody reactivity could change [15]. We provide evidence that an alteration in conformation affecting detection by an antibody at the N or the C terminus was not a likely explanation for the

increased protein levels by immunoprecipitating BRCA1 before Western analysis using an antibody that was raised against a different epitope of BRCA1 (Ab-3). While we cannot exclude that a conformational change did not occur, BRCA1 remains reactive to at least two different antibodies. Therefore, the increase in protein levels is not likely to be an artifact of epitope exposure. Our flow cytometry results demonstrated that these MCF-7 cells reacted normally to radiation by demonstrating some accumulation in S phase after UVC exposure, as previously described [14, 15, 27]. However, the increase we observed, on average less than 15%, is not likely to contribute significantly to the approximately tenfold increase in BRCA1 we observed. While we did not perform double-label experiments to observe cell cycle checkpoint responses, we single-labeled with propidium iodide simply to demonstrate that our observation was a specific upregulation of BRCA1, not an artifact of S phase arrest.

Protein stability does not appear to contribute to the accumulation of BRCA1 protein immediately following UVC exposure, as cycloheximide-treated lysates demonstrated a BRCA1 protein half-life that was actually shorter following UV treatment, decreasing from 3.5 h to 53 min. This decrease in protein half-life most likely leads to a lower steady-state level of BRCA1 protein, providing an explanation for the BRCA1 decrease observed 4 h after UV exposure by others [20, 26]. As noted above, we cannot fully explain the increased levels of BRCA1 in the unirradiated cells treated with cycloheximide. Nonetheless, we suggest that the half-life determination is internally consistent, with identical baseline levels in the irradiated and unirradiated cells.

Our Northern analysis results suggest that BRCA1 transcript levels do increase following UV treatment. Since we are looking at the total levels of BRCA1 transcript and not specifically at mRNA stability or transcription rates, whether this early increase is due to new transcript formation or increased transcript stability cannot be determined. Considering a BRCA1 protein of approximately 1863 amino acids and the fact that, in general, cells have an average translation rate of approximately 2 amino acids/s [28], the increased BRCA1 protein could possibly be new protein after approximately 15 min. This increased transcript probably does not account for the increase in detectable BRCA1 5 min after UVC exposure, but translation of new protein is likely to contribute to the increase observed from 15 min out to 2 h, consistent with published results [20, 26]. By looking at earlier time points than were previously published, we demonstrated that this decrease begins as early as 2 h following UV treatment. We also observed with Western analysis that BRCA1 protein levels decreased below baseline levels at 4–6 h following UVC insult, also confirming previously published results by many groups [20, 26].

While the general paradigm is an increase in gene products associated with response to DNA damage, the decrease seen here suggests that BRCA1 may be performing some activity, such as cell cycle control or apoptotic induction, that is not necessary or becomes detrimental to the cell at later points in the repair process. Evidence that BRCA1, with p53, activates the transcription of p21 and bax supports this hypothesis [29]. Upregulation of p21 and bax has been demonstrated to cause cell cycle arrest and apoptosis, respec-

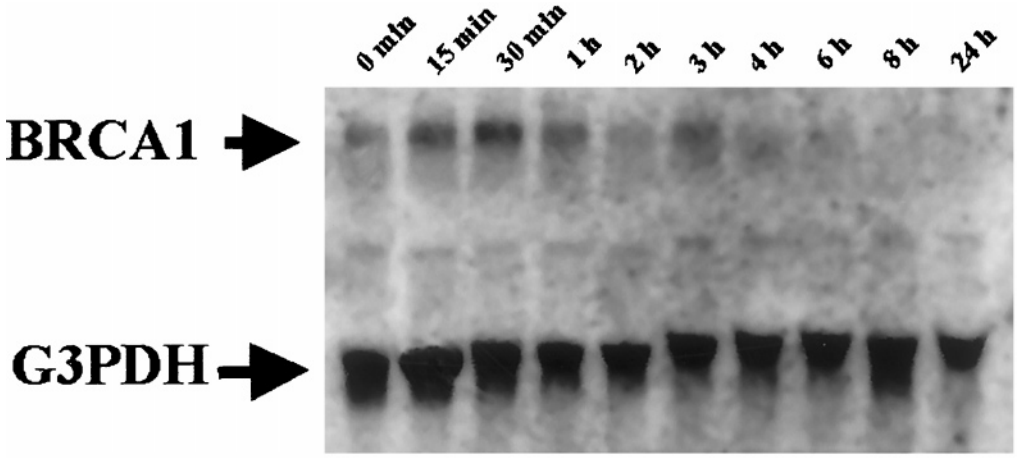

Figure 6. Northern blot analysis of BRCA1 following UVC treatment. MCF-7 cells were either irradiated or sham treated and harvested for total RNA. G3PDH was probed to show equal loading.

tively [30, 31]. A decrease in BRCA1 may prevent the upregulation of these transcripts and allow the cell cycle to progress normally. This hypothesis is further supported by the evidence that the retinoblastoma protein, Rb, decreases approximately 2 h following UV treatment, a time course similar to our observations with BRCA1. By decreasing the levels of Rb, cell cycling can continue, which may allow for later stages of DNA repair or for the continuance of the cellular life cycle [32].

The time course for the alterations to BRCA1 and the ability of multiple damaging agents to induce modifications of BRCA1 suggest that BRCA1 plays a role in sensing damage or in signaling for the appropriate response to the damage. This hypothesis is supported by a multitude of suggestive protein-protein interactions between BRCA1 and known damage-responsive proteins. p53, which is known to bind BRCA1 [29, 33] and is hypothesized to be a sensor itself, is activated by the ataxia-telangiectasia gene product, ATM [34], that transduces the damage signal to different pathways. In fact, evidence is growing evidence that BRCA1 itself is phosphorylated by ATM [35, 36]. This implies that BRCA1 could be a sensor itself, or that upstream damage-responsive proteins activate BRCA1 to transduce the damage signal to the appropriate pathway. Additionally, BRCA1 has been shown to associate with proteins in almost every major pathway of DNA repair. BRCA1 binds and/or colocalizes with the PCNA protein involved in mismatch repair, TFIIH and RPA associated with excision repair, Rad51 and BRCA2 involved with double-strand break repair, and p53, which is thought to be important for many repair pathways.

A unifying hypothesis for all these data is that BRCA1 is stored in pools localized to the nuclear foci before damage. Following DNA damage, BRCA1 is phosphorylated and released. Therefore, BRCA1 may act as a cellular mediator, integrating signals and/or protein 'switching' between different pathways as well as regulating other cellular functions, while the damage is being repaired.

This work provides clues and a proposed mechanism by which BRCA1 may be important for the cellular response to UV-induced DNA damage, despite a report to the contrary [13]. Future work should provide insight into the interaction of BRCA1 with specific pathways, and the role of BRCA1 in DNA damage induced by UV radiation and other damaging agents.

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