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BRCA1 Interacts With Dominant Negative SWI/SNF Enzymes Without Affecting Homologous Recombination or Radiation-Induced Gene Activation of p21 or Mdm2

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Abstract *BRCA1* is a tumor suppressor gene linked to familial breast and ovarian cancer. The BRCA1 protein has been implicated in a diverse set of cellular functions, including activation of gene expression by the p53 tumor suppressor and control of homologous recombination (HR) during DNA repair. Prior reports have demonstrated that BRCA1 can exist in cells in a complex with the BRG1-based SWI/SNF ATP-dependent chromatin remodeling enzymes and that SWI/SNF components contribute to p53-mediated gene activation. To investigate the link between SWI/SNF function and BRCA1 mediated effects on p53-mediated gene activation and on mechanisms of homologous recombination, we have utilized mammalian cells that inducibly express an ATPase-deficient, dominant negative SWI/SNF enzymes. Mutant SWI/SNF ATPases retain the ability to interact with BRCA1 in cells. We report that expression of dominant negative SWI/SNF enzymes does not affect p53-mediated induction of the p21 cyclin dependent kinase inhibitor or the Mdm2 E3 ubiquitin ligase that regulates p53 in cells exposed to UV or gamma irradiation. Similarly, integration of a reporter that monitors homologous recombination by gene conversion into these cells demonstrated no change in the recombination rate in the absence of functional SWI/SNF enzyme. We conclude that the SWI/SNF chromatin remodeling enzymes may contribute to but are not required for these processes. J. Cell. Biochem. 91: 987–998, 2004.

Key words: SWI/SNF; BRCA1; p53; transcription; homologous recombination; DNA repair

Individuals with germline mutations in the *BRCA1* gene are susceptible to breast and ovarian cancer [Miki et al., 1994]. BRCA1 plays a critical role in preventing unregulated cell growth in these tissues in addition to governing genomic stability (reviewed in Jasin [2002]; Moynahan [2002]; and Powell and Kachnic [2003]). The *BRCA1* gene encodes a large protein containing a RING finger motif and an acidic C-terminal domain that has been demonstrated to play a role in transcriptional activation [Chapman et al., 1996; Monteiro et al., 1996]. Of particular interest, BRCA1 exhibits coactivator function in activation of transcription by the p53 tumor suppressor [Somasundaram et al., 1997; Ouchi et al., 1998]. How BRCA1 stimulates p53-mediated and other transcriptional activation events remains under investigation, but biochemical fractionation has shown that BRCA1 can exist in a complex with RNA polymerase II holoenzyme components via interaction with an RNA helicase [Scully et al., 1997a; Anderson et al., 1998; Neish et al., 1998]. Such data may suggest a general role for BRCA1 in transcription. More recently, multiple biochemical approaches definitively indicated that BRCA1 could be found in complexes with SWI/SNF ATP-dependent chromatin remodeling enzymes containing the BRG1 ATPase [Bochar et al., 2000]. Given the numerous links between SWI/SNF chromatin

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remodeling enzymes and transcriptional regulation, this data also suggested a role and a potential mechanism for the involvement of BRCA1 in transcription.

BRCA1 is essential for mouse viability [Gowen et al., 1996; Hakem et al., 1996], and cells deficient for BRCA1 show spontaneous chromosomal abnormalities and defects in some forms of homologous recombination (HR) and in transcription-coupled repair [Moynahan et al., 1999; Xu et al., 1999b; Le Page et al., 2000]. Targeted expression of mutant BRCA1 in mouse mammary epithelium results in mammary tumor formation and aneuploidy and chromosome rearrangements, in addition to defects in p53-mediated gene regulation [Xu et al., 1999a,b]. A cancer cell line deficient for BRCA1 was hypersensitive to ionizing irradiation and only slowly repaired double strand breaks. Expression of wildtype but not clinically identified mutant alleles of BRCA1 restored function [Scully et al., 1999].

BRCA1 can interact with and be purified in association with components of the DNA repair machinery [Scully et al., 1997c, 1999; Cortez et al., 1999; Zhong et al., 1999; Tibbetts et al., 2000; Wang et al., 2000]. BRCA1 protein levels are cell cycle regulated, with highest expression during S phase [Chen et al., 1996; Ruffner and Verma, 1997]. During S phase, BRCA1 is found in discrete nuclear foci [Scully et al., 1997b]. Upon exposure to DNA damaging agents, BRCA1 becomes hyperphosphorylated and redistributes to new foci, some of which coincide with the location of proteins involved in the damage response [Scully et al., 1997b; Zhong et al., 1999; Tibbetts et al., 2000; Wang et al., 2000]. The function of BRCA1 in the DNA damage response remains an area of intense investigation.

The SWI/SNF enzymes were shown to alter nuclease accessibility to mononucleosome particles and to affect the topology of nucleosomal plasmid templates in an ATP dependent manner, thereby identifying these enzymes as chromatin remodelers [Côté et al., 1994; Imbalzano et al., 1994; Kwon et al., 1994]. A consequence of chromatin alteration by SWI/SNF enzymes was increased ability of transcriptional regulators as well as pol II associated general transcription factors to bind their sequence recognition sites on nucleosomes [Côté et al., 1994; Imbalzano et al., 1994; Kwon et al., 1994], providing biochemical support for earlier hypotheses that alteration of chromatin structure by these enzymes was involved in transcriptional regulation (reviewed in Winston and Carlson [1992] and Carlson and Laurent [1994]). In mammalian cells there are at least three forms of the SWI/SNF enzyme; two contain the BRG1 ATPase and one contains the highly related hBRM ATPase [Wang et al., 1996; Sif et al., 2001]. These enzymes share many of the same subunits. Biochemically, they have similar chromatin remodeling properties, however, in vivo, significant differences in BRG1 and hBRM function exist. Most notably, Brg1 deficient mice are embryonic lethal and the heterozygotes are prone to tumors, implicating Brg1 as a tumor suppressor [Bultman et al., 2000]. Brm deficient mice are viable and show only subtle cell proliferation changes [Reyes et al., 1998].

Multiple reports have demonstrated that the SWI/SNF enzymes do facilitate transcription both in vitro and in vivo. In mammalian cells, SWI/SNF enzymes contribute to or are required for multiple gene activation events, including activation of the hsp70 stress response gene [de la Serna et al., 2000; Sullivan et al., 2001], activation by nuclear hormone receptors [Dilworth et al., 2000; DiRenzo et al., 2000; Nie et al., 2000; Belandia et al., 2002; Fletcher et al., 2002; Inoue et al., 2002], and activation of genes involved in muscle [de la Serna et al., 2001a], myeloid [Kowenz-Leutz and Leutz, 1999], enterocyte [Soutoglou and Talianidis, 2002], erythroid [Armstrong et al., 1998; Lee et al., 1999; O'Neill et al., 1999, 2000; Kadam et al., 2000; Zhang et al., 2001; Brown et al., 2002], and adipocyte differentiation [Lemon et al., 2001; Pedersen et al., 2001]. SWI/SNF enzymes also contribute to and may be required for repression of the *c-fos* gene [Murphy et al., 1999], of neuronal specific gene expression by the REST transcription factor [Battaglioli et al., 2002], and of CD4 silencing during T cell activation [Chi et al., 2002]. Additionally, BRG1 is required for repression of cyclin gene expression during Rb-mediated cell cycle arrest [Strobeck et al., 2000; Zhang et al., 2000], and for repression of E2F by prohibition [Wang et al., 2002]. However, not all genes turned on or up-regulated in response to environmental or differentiation signals require SWI/SNF enzymes [Kowenz-Leutz and Leutz, 1999; de la Serna et al., 2000, 2001b; Roy et al., 2002]. The limited requirement for SWI/

SNF enzymes during constitutive proliferation of cells in culture was further demonstrated by microarray analysis of tumor cells lacking or expressing the BRG1 catalytic subunit of some SWI/SNF enzymes; only 80 genes were activated more than 3-fold by the presence of BRG1 [Liu et al., 2001].

Recently, two reports have implicated SWI/SNF components in p53-mediated gene activation. Lee et al. [2002] demonstrated that p53 associates with the SWI/SNF components BRG1 and INI1 and can be co-immunoprecipitated from high molecular weight fractions following gradient sedimentation. In addition, in experiments where p53 was transfected into cells, activation of a co-transfected p53-dependent reporter was enhanced by co-transfection of BRG1 or INI1 vectors, whereas activation was not enhanced if dominant negative BRG1 or INI1 vectors were co-transfected. Finally, BRG1 and INI1 could be localized both to the p53 reporter plasmid as well as to the endogenous p21 locus by chromatin immunoprecipitation in cells transfected with p53 [Lee et al., 2002]. Other workers demonstrated stable association of SWI/SNF with BRCA1 and showed that the ability of BRCA1 to mediate activation of p53-dependent transfected reporters as well as p53-dependent endogenous genes in cells transfected with p53 required SWI/SNF components [Bochar et al., 2000].

Previously, we generated and described NIH 3T3 cell lines that inducibly express ATPase deficient alleles of BRG1 or hBRM [de la Serna et al., 2000]. These proteins are mutated at the site of ATP binding but interact with other endogenous SWI/SNF subunits and act as dominant negative complexes that interfere with some but not all gene activation events [de la Serna et al., 2000, 2001a,b; Roy et al., 2002]. We noted that mutant SWI/SNF complexes formed in these cells could interact with BRCA1, so we evaluated the potential consequences of this interaction by determining whether the mutant SWI/SNF enzymes affected the activation of radiation induced gene expression or affected the efficiency of homology directed gene conversion events. We found that BRCA1-dependent gene activation and gene conversion events were equivalent regardless of whether the mutant enzyme was expressed. The results suggest that functional SWI/SNF complexes are not absolutely required for these processes.

MATERIALS AND METHODS

Cell Culture and Generation of Cell Lines

The generation and propagation of B22, B05-1, and H17 cells and the induction of dominant negative SWI/SNF enzymes in these cells were previously described [de la Serna et al., 2000]. To generate the recombination clone derivatives of B05-1 cells, cells at 90% confluence in a 10 cm dish were transfected with 20 μg linear p59 × DR-GFP6 [Moynahan et al., 2001]; (generous gift from M.E. Moynahan and M. Jasin) using Lipofectamine 2000 (Invitrogen, Carlsbad, CA). Stable colonies were selected using puromycin (2.5 μg/ml), while maintaining 2 μg/ml tetracyline (Sigma, St. Louis, MO), and either 350 U/ml Hygromycin B (Calbiochem EMD Biosciences, San Diego, CA) or 75 μg/ml G418 (Gibco/BRL) on a rotating basis.

For irradiation treatment, cells were passaged into media containing or lacking tetracycline such that they were at approximately 60% confluence 3 days later. To UV treat cells, the media was removed, the cells were washed with PBS, the PBS was aspirated, and the cells were exposed to UV light at a dosage of 40 J/m^2. Gamma irradiated cells received 8 Gy in a Cesium 137 Irradiation Unit (Atomic Energy of Canada, Ltd.).

Northern and Western Blots and Immunoprecipitations

The protocol followed for Northern blots was described in detail previously [de la Serna et al., 2001b]. The probe for mouse p21 was a 1.7 kb XhoI fragment isolated from the cDNA. The probe for mouse Mdm2 was a 1.7 kb EcoR1 fragment isolated from the cDNA. Immunoprecipitations and Western blots were performed as described [de la Serna et al., 2000], except that the lysis buffer contained 1% triton X-100 instead of NP-40. Antibodies used included anti-FLAG M2 antibody (Sigma) and anti-BRCA1 antibody (Santa Cruz Biotechnology, Santa Cruz, CA no. sc-642).

Analysis of Homologous Recombination Frequency

Experiments with recombination cell lines were conducted by growing cell lines for 3 days in a 6-well plates with or without tetracycline to induce expression of mutant BRG1 protein before transfecting with 2 μg of pCAG NZE (GFP; [Pierce et al., 1999]), pCAGGS (parent vector), or pCAGGS-I-SceI [Richardson et al.,

1998] (all gifts from M.E. Moynahan and M. Jasin). Twelve hours following transfection, the cells were trypsinized and plated onto 10 cm plates and maintained with or without tetracycline for 3 more days. FACS was performed by trypsinizing the cells, washing twice with PBS, and resuspending in RPMI media with no serum. Background fluorescence for each cell line was measured by transfecting cells with pCAGGS. Transfection efficiency was measured by counting the number of fluorescing cells after pCAG NZE transfection, as described by Moynahan et al. [2001]. Recombination events that led to functional GFP were determined by transfecting cell lines with pCAGGS-I-SceI and determining the number of fluorescing cells by FACS.

RESULTS

We previously described cell lines that utilize the original tetracycline-off inducible regulatory system to regulate the induction of FLAG-tagged, ATPase deficient alleles of human BRG1 or BRM, the ATPases present in mammalian SWI/SNF chromatin remodeling enzymes. B22 and B05-1 cells inducibly express mutant BRG1 upon removal of tetracycline from the media, while H17 cells inducibly express mutant hBRM upon removal of tetracycline [de la Serna et al., 2000]. In other experiments, we have demonstrated that induction of the mutant SWI/SNF complexes prevents MyoD-mediated trans-differentiation of these cells along the myogenic lineage [de la Serna et al., 2001a,b]. While attempting to determine whether the mutant SWI/SNF enzyme could interact with MyoD in these differentiated cells, we noted that an antibody against the FLAG epitope could co-immunoprecipitate the mutant ATPase and BRCA1 (data not shown). We repeated the experiments in proliferating cells and demonstrated that ATPase deficient BRG1 produced in B05-1 and B22 cells and the ATPase deficient hBRM produced in H17 cells could co-immunoprecipitate BRCA1 (Fig. 1). These data confirm the previous report indicating an association of BRCA1 and BRG1 in cells [Bochar et al., 2000] and demonstrate that BRCA1 can also interact with the hBRM protein in cells. Additionally, the presence of a mutation at the ATP binding site of BRG1 or hBRM did not prevent the interaction of these proteins.

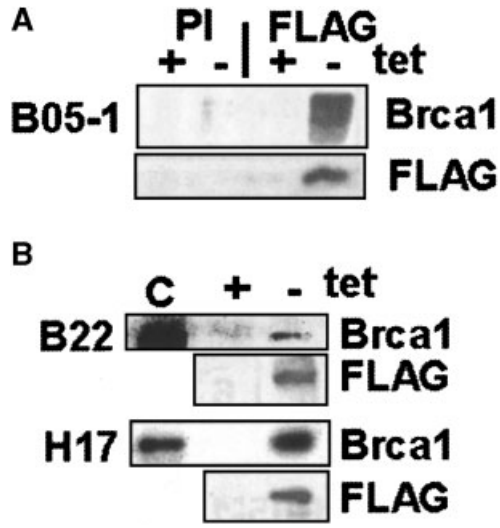

Fig. 1. BRCA1 interacts with BRG1 and hBRM proteins. **A**: Extract from B05-1 cells was immunoprecipitated with anti-FLAG antibody and probed with either BRCA1 or FLAG antibodies showed that BRCA1 interacts with BRG1 while preimmune sera failed to pull down BRCA1. **B**: B22 or H17 cells immunoprecipitated with anti-FLAG and probed for BRCA1 demonstrates the interaction of BRCA1 with both BRG1 and hBRM. FLAG Westerns demonstrate production of mutant BRG1 and hBRM proteins. PI, pre-immune; C, control extract.

Since the mutant ATPases could interact with BRCA1, we sought to investigate the consequences of this interaction by examining the activation of gene expression by radiation induced DNA damage, which is generally mediated by the p53 tumor suppressor. B05-1 and H17 cells were grown in the presence or absence of tetracycline for 3 days and irradiated with either 40 J/m^2 of ultraviolet light or 8 Gy of gamma radiation. Cells were harvested at the times indicated and total cellular RNA was prepared. Levels of the p21 cyclin dependent kinase inhibitor and the Mdm2 tumor suppressor were determined by Northern blot (Fig. 2A). Induction of both p21 and Mdm2 mRNA was similar in cells grown in the presence or absence of tetracycline at 6 h post-irradiation or at 16 h (H17) or 24 h (B05-1) post-irradiation. Other experiments examined induction of p21 and Mdm2 at 4, 8, and 12 h post-irradiation with the same results (data not shown). In no experiment did the amount of p21 or Mdm2 mRNA, at any given timepoint examined differ by more than 1.7-fold, as measured by PhosphorImager analysis. Duplicate plates of cells were used to generate protein extract in order to demonstrate that the cells were expressing the FLAG-tagged BRG1 or hBRM at the time of irradiation

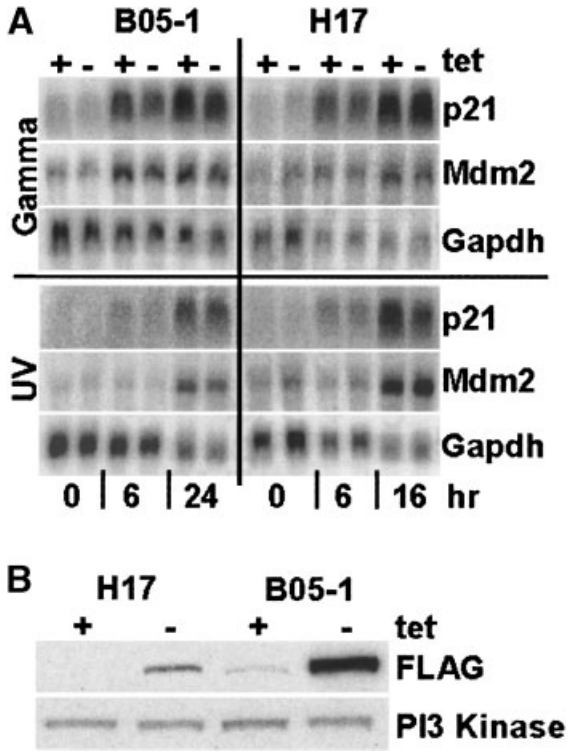

Fig. 2. Gamma and UV irradiation induce both p21 and Mdm2 in B05-1 and H17 cell lines expressing dominant negative BRG1 or hBRM. **A**: B05-1 and H17 cells were exposed to 40 J/m^2 UV or 8 Gy gamma irradiation and harvested at the indicated time points. Northern blots were probed for p21, Mdm2 and, as a control, Gapdh. **B**: FLAG Western of H17 and B05-1 whole cell extracts demonstrates the production of dominant negative hBRM or BRG1 proteins. The blot was stripped and probed with PI3 Kinase as a loading control.

(Fig. 2B). The results indicate that the induction of p21 and Mdm2 by treatment with UV or gamma irradiation occurred in the presence or absence of functional SWI/SNF enzymes. Therefore, in these cell lines, under these conditions, SWI/SNF enzymes are not required for p21 and Mdm2 induction.

The absence of an effect on radiation induced gene activation caused us to ask whether gene conversion events, a form of homology directed repair that has been shown to be mediated by BRCA1 [Moynahan et al., 1999], was affected in the presence of dominant negative SWI/SNF enzyme. We transfected B05-1 cells maintained in the presence of tetracycline with the p59 × DR-GFP6 construct [Moynahan et al., 2001], and selected and expanded drug-resistant colonies. This construct consists of two direct-repeat mutated GFP genes driven by the chicken β-actin promoter separated by PGK-puromycin gene. The first *GFP* gene was mutated by making 11 bp changes to incorporate an I-SceI restriction nuclease site and two in frame stop codons at the Bcl I site. The second GFP gene is an 812 bp internal *GFP* gene sequence that can be used to correct the first GFP through recombination. If an expression plasmid for the I-SceI restriction endonuclease [Richardson et al., 1998] is transfected into the cells, the I-SceI enzyme will cleave at its recognition sequence in the GFP reporter and generate a double-stranded break. If non-crossover gene conversion occurs, the repaired template will create a functional GFP coding sequence, expression of which can be assayed by FACS analysis.

Fourteen stably transfected lines were isolated. We first determined whether the cell lines retained the ability to induce expression of dominant negative BRG1 in the absence of tetracycline. Lysates from cells grown for 3 days in the presence or absence of tetracycline were probed for FLAG immunoreactivity by Western blot. Each of the 14 clones retained the ability to induce dominant negative BRG1 and each of the lines induced similar amounts of the mutant protein (Fig. 3). Thus, integration of the

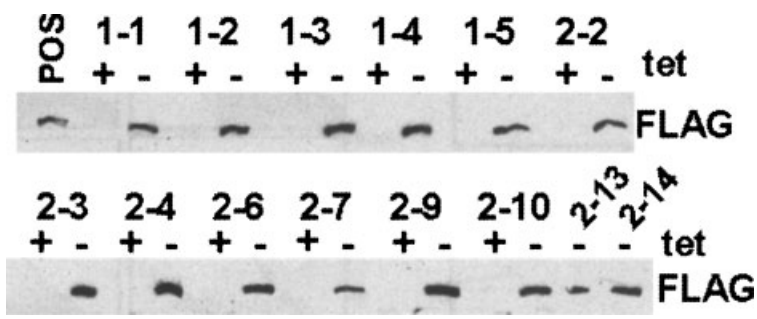

Fig. 3. Mutant BRG1 protein was expressed in the generated recombination (Rec) cell lines harboring the p59 × DR-GFP6 [Moynahan et al., 2001]. FLAG Western of whole cell extracts demonstrates that incorporation of p59 × DR-GFP6 and single cell cloning did not affect induction FLAG-tagged BRG1 expression. The parent cell line, B05-1, was used as a positive control (POS).

recombination reporter and the associated single cell cloning of the cell lines did not deleteriously affect the ability of the cells to induce the dominant negative BRG1.

Each cell line was analyzed in the presence or absence of tetracycline for background levels of GFP expression in the absence of transfected I-SceI enzyme. None of the cell lines showed detectable GFP expression in the absence of the introduced double stranded break (Fig. 4A and data not shown). When the I-SceI expression vector was introduced by transfection, four of the cell lines (Rec2–2, Rec2–9, Rec2–13, Rec2–14) showed no appreciable GFP expression in the presence or absence of tetracycline (Fig. 4B, data not shown), suggesting that the reporter had integrated into chromatin domains that could not be accessed by the restriction enzyme or had integrated such that the integrity of the reporter was compromised. The remaining 10 cell lines were transfected with the I-SceI expression vector while a duplicate plate was

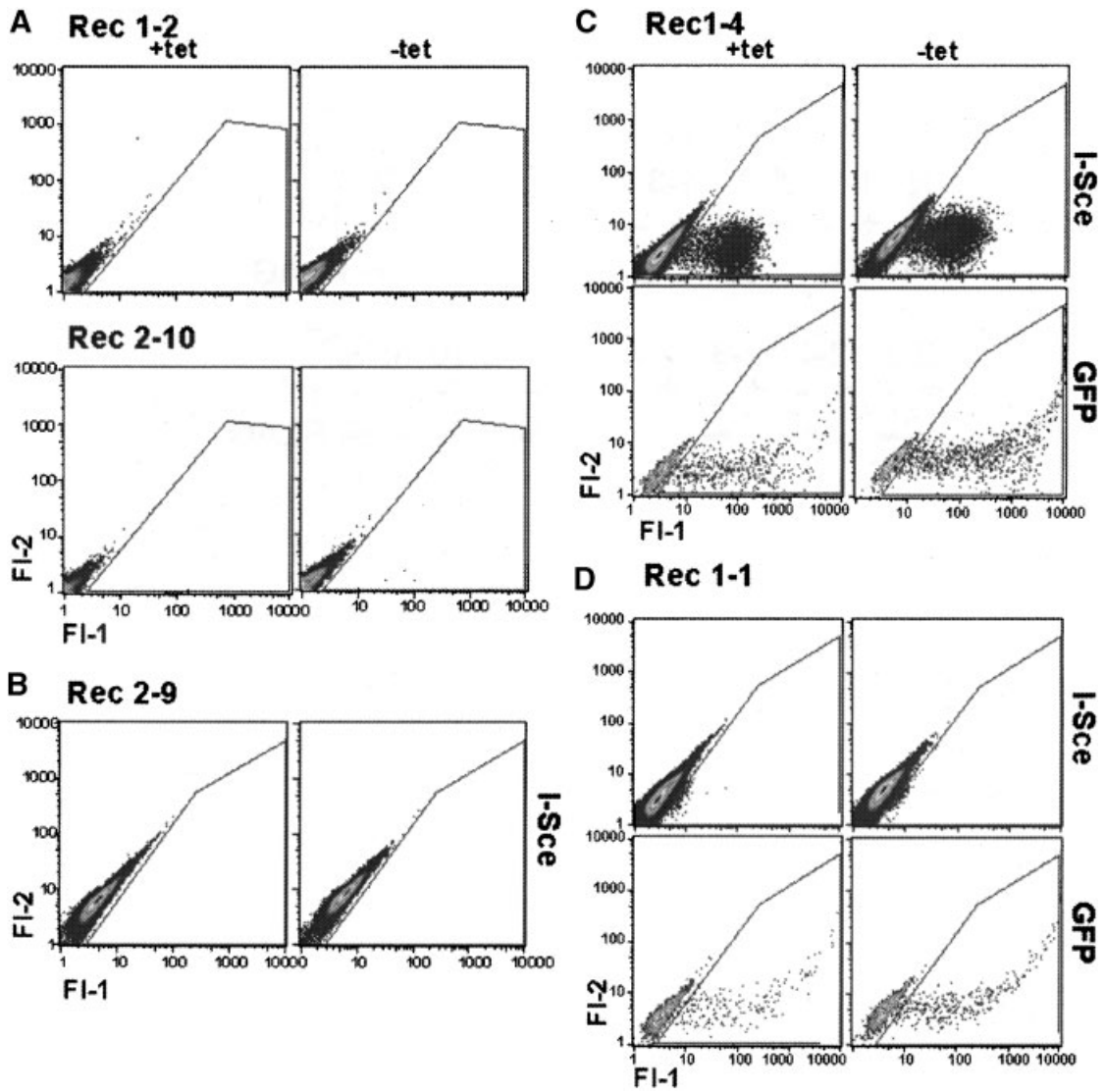

Fig. 4. FACS analysis of recombination cell lines. **A**: All cell lines either with or without tetracycline showed no background GFP expression. Data from Rec1–2 and Rec2–10 cell lines are presented. **B**: Selected cell lines failed to produce any GFP following I-SceI expression. Data from the Rec2–9 cell line is presented. **C** and **D**, **top panels**: Cell lines exhibiting GFP expression following transfection with the I-Sce expression vector were examined in the presence or absence of tetracycline to determine whether there was a difference in the rate of recombination between cells containing dominant negative BRG1-based SWI/SNF complexes. **Bottom panels**: Duplicate plates were transfected with a GFP expression plasmid to normalize for transfection efficiency as described by Moynahan et al. [2001]. Fl-1, green fluorescence; Fl-2, orange fluorescence.

transfected with a GFP expression plasmid [Pierce et al., 1999] to determine transfection efficiency (Fig. 4C,D lower panels). Different lines maintained in tetracycline gave variable levels of GFP expression. For example, the Rec1–4 line generated relatively high levels of GFP expression (Fig. 4C, top left), whereas line Rec1–1 generated relatively low levels of GFP expression following I-SceI introduction (Fig. 4D, top left). However, regardless of the percentage of cells expressing GFP, no significant differences in the number of GFP expressing cells were observed when the cell lines were grown in the absence of tetracycline and were expressing dominant negative BRG1. The lack of functional BRG1-based SWI/SNF complexes, therefore, did not impact upon the ability of the cell to repair double-stranded breaks via noncrossover gene conversion. Quantification of the results from all 10 of the cell lines is presented in Figure 5. Individual experiments generally showed less than a 2-fold difference in recombination efficiency, and the frequency of small increases in recombination efficiency was equivalent to the frequency of small decreases in recombination efficiency. In contrast, Moynahan et al. [1999] reported that BRCA1 deficient ES cells were reduced 5- to 6-fold in the level of homologous repair of double-stranded breaks using this reporter system. It remains possible that recombination efficiency might be BRG1 dependent in a locus dependent manner, however, the data from the 10 independently derived clones tested did not support this hypothesis. We conclude that the observed changes in our individual experiments are not significant and that interaction of BRCA1 with dominant negative BRG1 did not significantly affect gene conversion events.

DISCUSSION

The SWI/SNF chromatin remodeling enzymes have been implicated in the regulation of gene expression in numerous systems. Though only a subset of these reports actually document that the chromatin remodeling properties of these enzymes correlate with or directly mediate changes in gene expression, it is widely presumed that the function of these enzymes in modulating transcription is entirely dependent upon their chromatin remodeling activities. Thus, if it is the chromatin remodeling function that is critical, it is possible that the SWI/SNF enzymes can also contribute toward other cellular functions that require changes in chromatin structure, such as DNA replication, DNA repair, and/or DNA recombination [Kwon et al., 2000; Hara and Sancar, 2002].

A previous study documented the interaction and co-purification of BRCA1 and BRG1

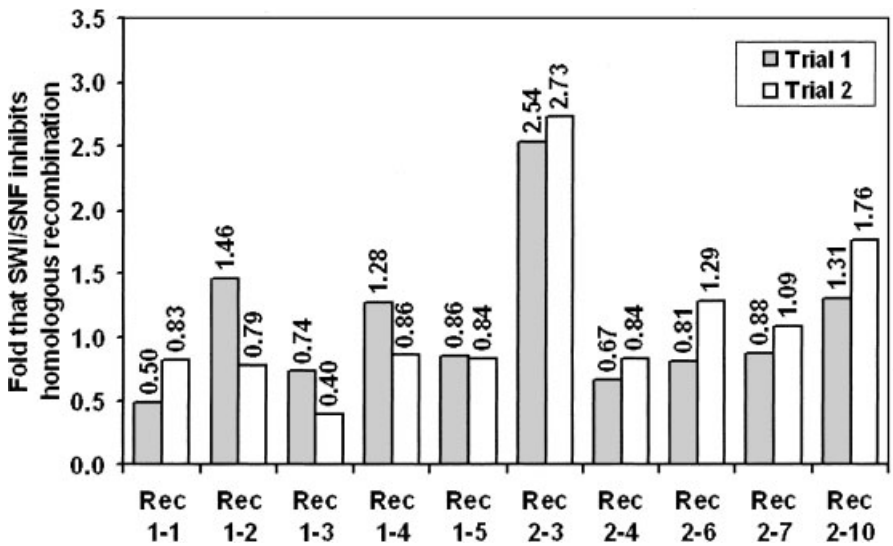

Fig. 5. Lack of requirement for SWI/SNF complexes toward repair of double strand breaks by gene conversion. Two independent trials were performed. The graph shows the ratio of the number of recombination events in cells expressing the mutant BRG1 (−tet) to the number of recombination events in wildtype cells (+tet). Ratios less than 1.0 indicate the more GFP expressing cells were observed in wildtype cells than in cells expressing the mutant BRG1.

[Bochar et al., 2000]. Here we confirm and extend these findings by demonstrating that ATPase deficient BRG1 and hBRM proteins could be isolated in complex with BRCA1. Since BRG1 and hBRM are only known to exist in cells as part of SWI/SNF complexes, we draw two conclusions from these data. First, BRCA1 is capable of interacting with different SWI/SNF enzymes containing either Brg1 or hBRM, suggesting that the interaction is mediated by a domain(s) that is common to BRG1 and hBRM. Second, the mutation at the ATP binding site did not prevent interaction, indicating that the interaction does not require the presence of ATP or a functional ATP binding site.

The observed interaction and co-purification of BRCA1 and BRG1 implicate SWI/SNF enzymes in mediating one or more of BRCA1 functions. Two studies have implicated SWI/SNF components in activation of transcription by the p53 tumor suppressor. Both studies examined p53 dependent reporters and endogenous p53 responsive genes in cells transfected with p53 and showed that co-transfection of wildtype but not mutant SWI/SNF components enhanced activation by p53 [Bochar et al., 2000; Lee et al., 2002]. One study extended the findings to demonstrate that wildtype but not ATPase deficient BRG1 contributed to the ability of BRCA1 to enhance p53 mediated gene expression [Bochar et al., 2000]. These data support the idea that SWI/SNF contributes to p53 mediated gene activation. In our study, we induced p53 dependent gene activation not by transfecting a p53 expression vector but by exposing the cells to UV and gamma irradiation, both of which are physiologically relevant inducers of DNA damage. We examined endogenous mRNA levels of the p21 CDK inhibitor and the Mdm2 tumor suppressor, both of which are induced by DNA damage. Both genes were turned on in response to the radiation treatments, however, the presence of the non-functional SWI/SNF enzymes made no difference in the extent of the induction for either gene. In contrast, the dominant negative BRG1 and hBRM alleles, when transiently transfected and when expressed inducibly in cells, have been shown to deleteriously affect multiple gene regulation events [Khavari et al., 1993; Muchardt and Yaniv, 1993; Bochar et al., 2000; de la Serna et al., 2000, 2001a; Strobeck et al., 2000; Barker et al., 2001; Lee et al., 2002; Wang et al., 2002]. The results indicate that SWI/SNF enzymes are not required for activation of the *p21* or *Mdm2* genes.

The nature of our system, inducible expression of a dominant negative enzyme, leaves open the possibility that the wildtype BRG1 or hBRM expressed from the endogenous loci is sufficient to mediate BRG or hBRM dependent activities despite the presence of the dominant negative enzymes. Were this the case, the amount of functional BRG1/hBRM required to facilitate radiation induced activation of p21 and Mdm2 would be considerably lower than that required to mediate other gene activation events that are deleteriously affected by dominant negative SWI/SNF enzymes in this experimental system. Our conclusions may not be in conflict with previously published results, as the previous studies indicated a contribution of SWI/SNF components to p53 mediated gene activation but not a requirement for SWI/SNF enzymes. Given that in our studies, gene activation was induced by physiological agents that activate the entire DNA damage response pathway, it is possible that even if SWI/SNF enzymes contribute to these gene activation events, SWI/SNF independent mechanism exist and function redundantly. In summary, our study does not exclude the possibility that SWI/SNF enzymes contribute to the activation of the p21 and Mdm2 loci during the DNA damage response. However, it strongly suggests that there is not an absolute requirement for SWI/SNF enzymes.

In the absence of a notable effect of the dominant negative SWI/SNF enzymes on these gene activation events, we investigated the requirement for SWI/SNF in specific forms of homologous HR, which is a distinct BRCA1 dependent function that nevertheless could involve an enzyme that can alter chromatin structure. Moynahan et al. [1999] have elegantly shown that BRCA1 deficient ES cells are impaired in their ability to repair double stranded chromosomal breaks by gene conversion. Thus one could hypothesize that the ability of BRCA1 to promote this form of HR might involve chromatin remodelers such as SWI/SNF enzymes. Our results, however, did not support such a hypothesis. Since we only tested recombination frequency in one of the dominant negative BRG1 cells lines, we can rigorously exclude only a requirement for BRG1-based SWI/SNF enzymes. However, in previous analyses of the role of SWI/SNF enzymes in the regulation of

different genes, results obtained with our cells expressing dominant negative BRG1 have always been similar if not identical to those obtained with the cells expressing dominant negative hBRM [de la Serna et al., 2000, 2001a,b; Roy et al., 2002; Wang et al., 2002]. Since the two SWI/SNF complexes share many of the same subunits, it is possible, perhaps likely, that expression of either mutant ATPase affects the other endogenous complex by competing with it for shared subunits. Thus we anticipate that the results would not have been different in cells expressing dominant negative hBRM.

To our knowledge, this is the first evaluation of the requirement of SWI/SNF enzymes in homology directed repair of double strand breaks via gene conversion. Repair of double strand breaks can also occur via a single strand annealing (SSA) mechanism if there is a direct repeat of the damaged DNA [Jackson, 2002]. Since GFP signal will result only if the repair is completed by non-crossover gene conversion, it is possible that SSA occurs and that SWI/SNF enzymes contribute to SSA mediated repair. However, if SWI/SNF enzymes are facilitating the access of the repair machinery to the DNA via the remodeling of chromatin, we believe it is likely that SWI/SNF dependency would be independent of the repair mechanism utilized.

As with the activation of p21 and Mdm2 in response to DNA damage, it remains possible that SWI/SNF enzymes contribute to, but are not required for repair of double strand breaks by gene conversion. It also remains possible that a chromatin remodeling activity other than SWI/SNF is involved in this and other forms of HR. Additionally, BRCA1 has been implicated in other recombination events such as transcription coupled repair [Le Page et al., 2000]. To date, we have not assessed our cells expressing dominant negative SWI/SNF enzymes for their competency to perform this repair event.

Among the other proteins previously demonstrated to interact with BRCA1 is BARD1, which forms a heterodimeric complex with BRCA1 [Wu et al., 1996; Meza et al., 1999; Brzovic et al., 2001]. Both BRCA1 and BARD1 possess a RING-finger domain; these domains can independently act as E3 ubiquitin ligases in vitro [Lorick et al., 1999]. Recently, the BRCA1/BARD1 complex was shown to act as an E3 mono-ubiquitin ligase [Mallery et al., 2002]. The four histones that comprise the nucleosome, but not linker histone H1, were good in vitro substrates for mono-ubiquitylation. Intriguingly, the histone variant H2AX, which co-localizes with BRCA1 at sites of DNA damage, was also mono-ubiquitlyated in vitro. An alternative hypothesis for the function of the BRCA1:SWI/SNF interaction is that chromatin remodeling by SWI/SNF makes one or more histone proteins accessible for ubiquitylation by BRCA1. If H2AX were demonstrated to be an in vivo substrate for BRCA1, multiple findings regarding the subcellular localization of BRCA1, the interaction between BRCA1 and SWI/SNF enzymes, and the interaction of BRCA1 with proteins involved in DNA repair could be linked to support this hypothesis.

To date, functional studies of BRCA1 and studies of physical interaction with proteins involved in transcription, DNA repair, and cell cycle progression indicate an important and essential role for BRCA1. The exact nature of BRCA1 function and its relation to BRCA1s role as a tumor suppressor clearly requires continued study.

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