THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 277, No. 7, Issue of February 15, pp. 4782–4789, 2002

Printed in U.S.A.

Compensation of BRG-1 Function by Brm

INSIGHT INTO THE ROLE OF THE CORE SWI·SNF SUBUNITS IN RETINOBLASTOMA TUMOR SUPPRESSOR SIGNALING*

Received for publication, October 2, 2001, and in revised form, November 9, 2001
Published, JBC Papers in Press, November 21, 2001, DOI 10.1074/jbc.M109532200

Matthew W. Strobeck‡§, David N. Reisman¶‖, Ranjaka W. Gunawardena‡, Bryan L. Betz¶‖, Steven P. Angus‡, Karen E. Knudsen‡, Timothy F. Kowalik, Bernard E. Weissman¶‖, and Erik S. Knudsen‡ ‡‡**

*From the ‡Department of Cell Biology, University of Cincinnati College of Medicine, Vontz Center for Molecular Studies, Cincinnati, Ohio 45267-0521, ¶Department of Pathology and Laboratory Medicine and ‖The Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, North Carolina 27599, and **Program in Immunology and Virology, University of Massachusetts Medical School, Worcester, Massachusetts 01655*

The BRG-1 subunit of the SWI·SNF complex is involved in chromatin remodeling and has been implicated in the action of the retinoblastoma tumor suppressor (RB). Given the importance of BRG-1 in RB function, germ line BRG-1 mutations in tumorigenesis may be tantamount to RB inactivation. Therefore, in this study we assessed the behavior of cells harboring discrete BRG-1 alleles for the RB-signaling pathway. Using p16ink4a, an upstream activator of endogenous RB, or a constitutively active RB construct (PSM-RB), we determined that the majority of tumor lines with germ line defects in BRG-1 were sensitive to RB-mediated cell cycle arrest. By contrast, A427 (lung carcinoma) cells were resistant to expression of p16ink4a and PSM-RB. Analysis of the SWI·SNF subunits in the different tumor lines revealed that A427 are deficient for BRG-1 and its homologue, Brm, whereas RB-sensitive cell lines retained Brm expression. Similarly, the RB-resistant SW13 and C33A cell lines were also deficient for both BRG-1/Brm. Reintroduction of either BRG-1 or Brm into A427 or C33A cells restored RB-mediated signaling to cyclin A to cause cell cycle arrest. Consistent with this compensatory role, we observed that Brm could also drive expression of CD44. We also determined that loss of these core SWI·SNF subunits renders SW13 cells resistant to activation of the RB pathway by the chemotherapeutic agent cisplatin, since reintroduction of either BRG-1 or Brm into SW13 cells restored the cisplatin DNA-damage checkpoint. Together, these data demonstrate that Brm can compensate for BRG-1 loss as pertains to RB sensitivity.

The SWI·SNF complex consists of 10–12 proteins that form an ~2-MDa complex. This multisubunit complex regulates transcription through its ability to remodel chromatin (1–3). Specifically, the SWI·SNF complex has been shown to alter nucleosome structure by disrupting histone-DNA interactions in an ATP-dependent manner (4, 5). The precise mechanism through which SWI·SNF regulates gene promoters in mammals is unresolved. However, gene regulation studies in *Saccharomyces cerevisiae* demonstrate that SWI·SNF complexes can localize to specific gene promoters, disrupting nucleosomal DNA to modulate transcription (6–9). Specifically, it has been shown that ISW2, a member of the ISWI class of chromatin remodelers, is recruited to the promoter of meiotic genes by Ume6p (a sequence-specific DNA-binding protein) to repress transcription (6). In addition, it has been shown that the SWI·SNF-related complex RSC (remodels the structure of chromatin) is targeted to the CHA1 promoter to maintain the nucleosomal DNA in a repressed state (8). The mammalian SWI·SNF complex contains either BRG-1 or Brm as its central subunit. In addition, the complex is composed of accessory proteins termed BRG-1-associated factors (Bafs)[1] (10). Both BRG-1 and Brm harbor the ATPase activity required for the complex to function in remodeling (11). BRG-1 has been demonstrated to regulate the transcription of a host of genes involved in disparate cellular processes. For example, BRG-1 has been shown to be required for the basal expression of the membrane glycoprotein, CD44 (12). In addition, BRG-1 has been shown to modulate estrogen receptor, glucocorticoid receptor, c-Myc, BRCA1, and retinoblastoma tumor suppressor protein (RB) transcriptional activity (13–18). Because BRG-1 is involved in regulating the transcription of such a diverse array of genes as well as cooperating with both tumor suppressors and oncogenes, it is a likely candidate for mutation in cancer. Interestingly, it was recently reported that BRG-1 is mutated or deleted in ~10% of tumor cell lines analyzed, bolstering its putative role as a prototypical tumor suppressor (19).

SWI·SNF activity was first linked to RB by the finding that BRG-1 can bind to and cooperate with RB for arrest (20, 21). The retinoblastoma tumor suppressor protein exhibits antitumor activity by regulating the mitotic cell cycle (22–28). RB was initially identified based on bi-allelic loss in retinoblastoma, a rare pediatric eye cancer (22, 23, 27, 28). Further studies revealed that RB is also functionally inactivated in a variety of human tumors via the following mechanisms, which are (i) oncoprotein binding of DNA tumor viruses (*e.g.* E7 and

* This work was supported by NCI, National Institutes of Health (NIH) Grant CA-82525, the Ruth Lyons Cancer Foundation, and the Sidney Kimmel Cancer Foundation (to E. K.), NIH Grants DE-12355 and CA-63176 (to B. W.), CA-86038 (to T. K.), and T32CA09156 and a grant from the Kemper Foundation (to D. R.), and Department of Defense Prostate Cancer Research Award PC010016 (to K. K.). This project is also supported by NIH Grant RO1-CA82525-S1 to promote research collaborations for E. K. and B. W. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

§ Supported by training grants from the National Institutes of Health. A Ryan Fellow.

‡‡ To whom correspondence should be addressed: Tel.: 513-558-8885; Fax: 513-558-4454; E-mail: Erik.Knudsen@UC.edu.

[1] The abbreviations used are: Baf, BRG-1-associated factor; BrdUrd, bromodeoxyuridine; CPPD, cisplatin; GFP, green fluorescent protein; RB, retinoblastoma tumor suppressor.

human papilloma virus (HPV)), (ii) deregulated phosphorylation/inactivation by kinases that specifically phosphorylate RB, and by (iii) mutation of the endogenous RB gene itself (22, 23, 26–29). Although these lesions directly target RB, it was recently demonstrated that RB cell cycle inhibitory activity is also compromised because of the loss of BRG-1, providing evidence for an additional mechanism utilized by tumor cells to disrupt RB signaling (17, 18, 30). Therefore, in the discrete BRG-1 mutant tumor lines identified by Wong *et al.* (19), alteration of endogenous BRG-1 alleles were hypothesized to compromise RB-mediated cell cycle control, providing the first link between germ line mutations of BRG-1 and resistance to the RB pathway (19).

RB regulates the cell cycle by assembling a multi-protein complex that actively represses the transcription of genes responsible for driving DNA synthesis (24, 26, 31, 32). The E2F family of transcription factors, which regulates the expression of genes that drive entry into S-phase, are thought to be the major target of RB (24). In addition, recruitment of histone deacetylases facilitates RB-mediated repression of E2F transcriptional activity (33, 34). Recently it was shown that BRG-1 is also a component of the RB repressor complex, and is required for RB-mediated inhibition of cyclin A expression, a critical component of the Cdk2 kinase complex (17, 18, 30). This regulation is significant as cyclin A/Cdk2 expression is required for cell cycle progression (35, 36).

In this study we analyzed whether tumor cell lines harboring discrete BRG-1 mutations are compromised for RB responsiveness. Of the tumor lines analyzed, only the A427 lung carcinoma cell line was resistant to both p16ink4a and an active phosphorylation site mutant of RB (PSM-RB). We have previously identified C33A and SW13 as lacking in RB function due to the loss of BRG-1 activity (17, 18, 30). Surprisingly, we observed that loss of both BRG-1 and Brm was correlative with RB insensitivity and a lack of CD44 expression in A427, C33A, and SW13 cells. Co-expression of either BRG-1 or Brm with PSM-RB specifically restored RB-mediated cell cycle inhibition in A427 and C33A cells as well as CD44 levels in SW13, C33A, and A427. Using the chemotherapeutic agent cisplatin (CDDP), which has previously been shown to activate RB to inhibit entry into S-phase (37, 38), we show that loss of BRG-1 and Brm also mediates sensitivity to this specific DNA-damage pathway. Thus, this report provides genetic and functional evidence that Brm can substitute for BRG-1 in the regulation of RB tumor suppressor activity.

MATERIALS AND METHODS

Cells, Plasmids, and Transfection—SW13, TSU-Pr-1, Hs578t, Hs683, and HCT-116 cells were maintained in Dulbecco's modified Eagle's medium, A427, WiDr, NCI-H1299, and SU86.86 cells were maintained in RPMI. Both Dulbecco's modified Eagle's medium and RPMI were supplemented with 10% heat-inactivated fetal bovine serum, 100 units/ml penicillin-streptomycin, and 2 mM L-glutamine at 37 °C in 5% CO_2. Plasmids were transfected using either calcium phosphate or the lipid-based transfection reagent FuGENE (Roche Molecular Biochemicals) (39). The plasmids CMV-NEO, pBabe-Puro, PSM-RB, Brm, and BRG-1 have been previously described (20, 40–42). All of the cell lines except TSU-Pr1 were obtained from ATCC. TSU-Pr1 was obtained from J. Isaacs (Johns Hopkins).

Adenoviral Infections—For adenoviral infections $\sim 2 \times 10^5$ cells were seeded on coverslips in six-well dishes. The green fluorescent protein (GFP) adenovirus was provided by Dr. Gustavo Leone (Ohio State University), and the p16ink4a adenovirus was supplied by Dr. Timothy Kowalik (University of Massachusetts) and prepared as previously described (43). The infections were performed at a calculated multiplicity of infection of 50–100 for ~95–100% infection efficiency after 16 h, as judged by GFP expression.

Immunoblotting—Approximately 1×10^6 A427, C33A, or SW13 cells were plated in 10-cm dishes 24 h before transfection. A427, C33A, and SW13 cells were co-transfected with effectors and the puromycin-selectable plasmid (pBabe-Puro). Transfected A427, C33A, and SW13 cells were subsequently selected with 1.0, 2.5, or 2.5 μg/ml puromycin (Sigma), respectively, for 24–48 h and then harvested for immunoblot analysis. Before CDDP treatment or adenovirus infection, $\sim 2 \times 10^5$ cells were seeded into each well of a 6-well dish. After 24 h of CDDP treatment or infection, the cells were harvested. To isolate protein for Western blotting, cells were trypsinized and subsequently washed with PBS. The cell pellets were resuspended in radioimmune precipitation buffer supplemented with protease inhibitor mixture (10 mg/ml, 1,10 phenanthroline, 10 mg/ml aprotinin, 10 mg/ml leupeptin, and 1 mM phenylmethylsulfonyl fluoride) and incubated for 15 min on ice. The lysates were briefly sonicated and then centrifuged for 10 min at $20{,}000 \times g$ at 4 °C. To isolate BRG-1 or Brm, total protein was isolated from sub-confluent cultures using an 8 M urea extraction (8 M urea, 0.1 M NaH_2PO_4, 10 mM Tris, pH 8). The lysates were then separated via SDS-polyacrylamide gel electrophoresis and then transferred onto Immobilon-P (Millipore). Membranes were then incubated with either of the following antibodies: anti-CD44 (Hermes 3), anti-Cdk4 (clone H22, Santa Cruz), anti-cyclin E (clone C19 Santa Cruz), anti-cyclin A (clone H432, Santa Cruz), anti-β-tubulin (Sigma), anti-p53 (Dr. K. Fukasawa, University of Cincinnati, College of Medicine, Cincinnati, OH), anti-pRB (Dr. J. Wang, University of California San Diego, 851 polyclonal antibody), anti-phospho-RB (Ser-780) and anti-BRG-1 or anti-Brm (Dr. Weidong Wang, National Institute of Health, Baltimore, MD; Dr. Moshe Yaniv, Pasteur Institute). The blots were then incubated with either horseradish peroxidase-conjugated anti-rabbit or anti-mouse anti-sera for 1 h at room temperature. The antibody-antigen complex was detected by enhanced chemiluminescence (ECL, Amersham Biosciences, Inc.). The levels of β-tubulin and cyclin A were quantitated using Metamorph software (Universal Imaging Corporation).

Bromodeoxyuridine (BrdUrd) Incorporation—Approximately 2×10^5 cells were seeded onto coverslips in each well of a 6-well dish. Twenty-four hours later the cells were either transfected or infected with adenovirus and then treated with CDDP. Forty-eight hours post-transfection, 24 h post-infection, or 24 h post-CDDP treatment the cells were labeled with BrdUrd to detect DNA synthesis. After 16 h of labeling, the cells were fixed in 3.7% formaldehyde and processed to detect BrdUrd via indirect immunofluorescence as previously described (30).

TABLE I
Disparate mutant BRG-1 alleles

As reported by Wong *et al.* (19), the following BRG-1 mutations were discovered using radiation hybrid mapping and PCR sequence-based mutation screens as previously described.

Tumor cell line	Tissue	Genotype	Mutation
TSU-Pr1	Prostate	Hemizygous	Deletion/truncated
A-427	Lung	Hemizygous	Deletion/truncated
NCI-H1299	Lung	Hemizygous	69-Base deletion
Hs 700t	Pancreas	Hemizygous	729C/frameshift
Hs 578t	Breast	Hemizygous	C589T/Pro → Ser
HCT-116	Colon	Hemizygous	T348C/Leu → Pro
SW13	Adrenal		
C33A	Cervix		
SU86.86	Pancreas	Heterozygous	A479G/Gln → Arg
WiDr	Colon	Heterozygous	G3850A/Asp → Asn
Hs 683	Brain	Heterozygous	T4826C/Leu → Pro

RESULTS

Genetic analysis performed by Wong *et al.* (19) demonstrated that BRG-1 is deleted or mutated in ~10% of the tumor cell lines examined. Several types of mutations were reported, as summarized in Table I. For example, it was determined that the COOH-terminal region of BRG-1 is homozygously deleted in the prostate carcinoma cell line TSU-Pr1 and the A427 lung carcinoma cell line. In addition, the tumor lines NCI-H1299 (lung), Hs 700t (pancreatic), Hs 578t (breast) and the HCT-116 (colorectal) cell line contained hemizygous mutations that resulted in either a point mutation, frameshift, or a truncated product (19). Last, heterozygous mutations were also observed in the SU86.86 (pancreatic), WiDr (colorectal), and the Hs 683 (brain) tumor cell lines. We have previously shown that BRG-1 is required for RB activity and predicted that some of these

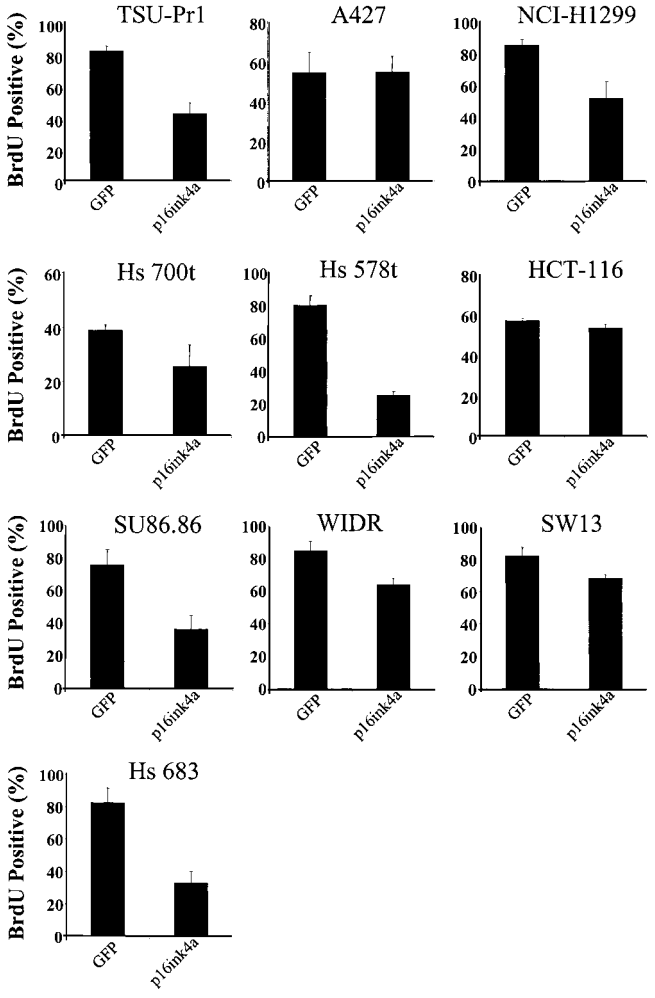

FIG. 1. **Analysis of p16ink4a signaling in mutant BRG-1 tumor lines.** TSU-Pr1, A427, NCI-H1299, Hs 700t, Hs 578t, HCT-116, SU86.86, SW13, Hs 683, and WiDr were infected with adenoviruses harboring either GFP (vector) or p16ink4a. The GFP encoding adenoviruses infected ~95–100% of the cells. The percentage of BrdUrd-positive cells was determined from at least two independent experiments with at least 150 infected cells scored per experiment.

BRG-1-derived tumor alleles may confer resistance to the antiproliferative activity of RB.

Analysis of p16ink4a Signaling in Discrete Mutant BRG-1 Tumor Cell Lines—To test this hypothesis, p16ink4a was first employed. Numerous studies show that p16ink4a inhibits cell cycle progression by inactivating the cyclin D-Cdk4 kinase complex, ultimately resulting in the dephosphorylation/activation of RB (44, 45). Because BRG-1 is required for RB to mediate cell cycle arrest in response to p16ink4a, we examined whether this RB signaling pathway is intact in the disparate BRG-1 mutant tumor lines (17). Each indicated BRG-1 mutant line was infected with adenovirus encoding either GFP or p16ink4a and subsequently monitored for their ability to incorporate BrdUrd (43). As shown in Fig. 1, several BRG-1 mutant cell lines were inhibited for BrdUrd incorporation in response to p16ink4a infection. TSUPr1 demonstrated an ~50% reduction in BrdUrd incorporation as compared with GFP infection. Likewise, NCI-H1299, Hs 578t, SU86.86, and Hs 683 demonstrated a 40, 68, 52, and 60% decrease in BrdUrd, respectively (Fig. 1.). These results indicate that the p16ink4a/RB axis is intact in these cells. By contrast, A427 and HCT-116 were relatively resistant to p16ink4a infection, showing only a 0 and 6.4% reduction in BrdUrd incorporation, respectively. Hs 700t and WiDr demonstrated intermediate effects, showing only a 34 and 25% reduction in BrdUrd incorporation, respectively. These results provided the impetus to further study the BRG-1-RB link in A427, Hs 700t, WiDr, and HCT-116 cells.

Active/Dephosphorylated RB Is Defective in Signaling to Critical Cell Cycle Targets in the A427 Cell Line—The role of BRG-1 in transducing RB inhibitory signals has been shown to be due to its ability to facilitate RB-mediated repression of cyclin A (18, 30). To determine whether activation of RB in either the p16ink4a-resistant cell lines A427, Hs 700t, and HCT-116 or p16ink4a-sensitive cell lines TSU-Pr1 and NCI-H1299 can signal to endogenous Cdk/cyclins, we utilized either GFP- or p16ink4a-encoding recombinant adenoviruses. In p16ink4a-sensitive cells (TSU-Pr1 and NCI-H1299), p16ink4a introduction resulted in RB dephosphorylation/activation concomitant with a decrease in cyclin A levels by ~98 and 92%, respectively. p16ink4a expression in TSU-Pr1 and NCI-H1299 had no significant effect on cyclin E or Cdk2 protein levels after comparison to GFP-infected cells and normalized to β-tubulin (Fig. 2*A*, *lanes 3* and *4* and *lanes 5* and *6*). In Hs 700t cells, overexpression of p16ink4a was inconsequential, as no detectable RB protein was observed (Fig. 2A, *lanes 1* and *2*). In HCT-116 cells, p16ink4a expression resulted in only minimal RB dephosphorylation (Fig. 2*A*, *lanes 7* and *8*). As would be expected based on these results, RB activity was also lacking, as evidenced by the retention of cyclin A protein (Fig. 2*A*, *lanes 7* and *8*). These data support the hypothesis that RB is largely refractory to the effects of p16ink4a in HCT-116 cells. Similar results were observed in WiDr cells (data not shown). Thus, the inability of p16ink4a to completely activate RB in HCT-116 and WiDr provides an explanation as to why these cell lines were not arrested by p16ink4a. Interestingly, ectopic expression of p16ink4a in A427 cells resulted in the efficient dephosphorylation of RB; however, activation of RB in this cell line was defective in attenuating cyclin A levels when compared with GFP-infected cells (Fig. 2*A*, *lanes 9 versus 10*). To confirm that p16ink4a was efficiently dephosphorylating RB in A427 cells, immunoblots were performed using a phospho-RB-specific antibody (Ser-780). This antibody has been previously documented to detect only the phosphorylated form of RB (46). Using HCT-116 as a positive control, we observed as expected that ectopic expression of p16ink4a does not efficiently dephosphorylate RB (Fig. 2*B*, *lanes 1 versus 2*). Therefore, with the Ser-780 antibody, we detected phosphorylated RB in both the GFP- and p16ink4a-infected lanes (Fig. 2*B*, *lanes 1 versus 2*). Next we probed A427 cells and determined that the Ser-780 antibody was able to detect the phosphorylated form of RB in the GFP-infected cells. However, we were unable to detect phosphorylated RB in A427 cells infected with p16ink4a (Fig. 2*B*, *lanes 3 versus 4*). Thus, these data indicate that of the lines tested, RB signaling to critical downstream effectors is abrogated specifically in the A427 cell line.

The A427 Tumor Line Is Resistant to an Active Form of RB—Because p16ink4a failed to activate endogenous RB in WiDr and HCT-116 cells and since endogenous active RB failed to signal in A427 cells, we tested the effect of activated RB on cell cycle progression in these cells. For these experiments we used a constitutively active allele of RB, PSM-RB, which cannot be phosphorylated/inactivated. Specifically, PSM-RB was co-transfected with an H2B-GFP expression construct, and the ability of the transfected cells (GFP-positive) to incorporate BrdUrd was monitored. As expected, ectopic expression of PSM-RB in Hs 683 resulted in a 57% decrease in BrdUrd incorporation when compared with vector-transfected cells (Fig. 3). Interestingly, overexpression of PSM-RB in HCT-116, Hs 700t, and WiDr was sufficient to inhibit cell cycle progression by ~70, 65, and 83%, respec-

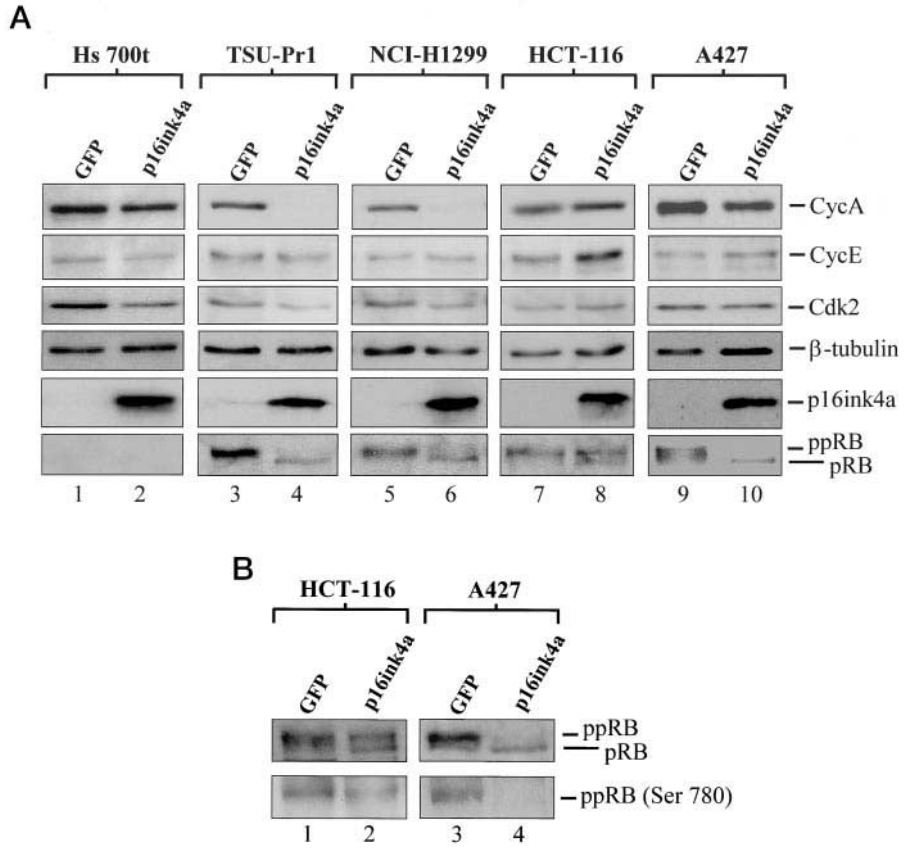

FIG. 2. **In A427 cells active/hypophosphorylated RB is abrogated in signaling to cyclin A.** *A*, Hs 700t, TSU-Pr1, NCI-H1299, HCT-116, and A427 cells were treated with either GFP or p16ink4a adenoviruses. After incubating for 24 h, equal total protein was resolved by SDS-PAGE and then immunoblotted for cyclin A, cyclin E, Cdk2, β-tubulin, pRB, and p16ink4a. *B*, HCT-116 and A427 cells were infected with either GFP or p16ink4a adenoviruses. After incubating for 24 h, equal total protein was resolved by SDS-PAGE and then immunoblotted for RB using an antibody that detects both the hypo- and hyperphosphorylated forms of RB (851) and also a phospho-specific RB (*ppRB*) antibody (Ser-780).

tively, when compared with vector (Fig. 3). These results indicate that although p16ink4a does not initiate RB-mediated cell cycle arrest, the RB pathway is still intact. By contrast, in A427 cells, the percent of PSM-RB-transfected cells incorporating BrdUrd was similar to that of vector transfected cells (Fig. 3). Because A427 cells are resistant to cell cycle arrest initiated by both ectopic RB and by activation of endogenous RB, these results indicate that the BRG-1-RB axis is specifically compromised in this cell line.

Loss of Both BRG-1 and Brm Expression Correlates with RB Resistance—The mutant BRG-1 alleles present in several RB/p16ink4a-responsive lines were reported to result in a lack of detectable BRG-1 protein (19). Because we have previously shown that BRG-1 function is required for an RB-mediated cell cycle arrest, it was therefore surprising that so many cell lines arrested in response to RB. One possible explanation could be that Brm was still expressed in the disparate tumor lines. Therefore, we initially probed the status of Brm as well as the Bafs, Baf 250, Baf 180, Baf 155, Baf 57, and Baf 53, in the various mutant BRG-1 tumor lines. Immunoblot analysis revealed that the RB-sensitive SU86.86, HCT-116, Hs 683, WiDr, and Hs 578t cell lines express BRG-1 and Brm as well as the accessory SWI·SNF subunits Baf 250, Baf 180, Baf 155, Baf 57, and Baf 53 after comparison to the HeLa-positive control (Fig. 4*A*, *lanes 1*, *4*, *5*, *7*, *9*, and *12*) (19). Interestingly, the RB-sensitive Hs 700t, TSU-PR1, and NCI-H1299 cell lines had low levels of BRG-1 (19), comparable with that observed in C33A, but Brm was still detectable, suggesting that in these cell lines Brm may compensate for BRG-1 in mediating RB action (Fig. 4*A*, *lanes 3*, *6*, *8*, and *11*). In the Hs 700t, TSU-PR1, and NCI-H1299 cell lines, the expression of Baf 250, Baf 180, Baf 155, Baf 57, and Baf 53 proteins was also detected(Fig. 4*A*, *lanes 6*, *8*, and *11*). Analysis of the RB-resistant A427 cell line revealed the absence of both BRG-1 and Brm protein (Fig. 4*A*, *lane 10*). The loss of both BRG-1 and Brm observed in the A427 cell line was similar to that found in the RB-resistant C33A and SW13 cell lines (Fig. 4*A*, *lanes 2*, *3*, and *10*). Therefore, these data suggest that loss of both BRG-1 and Brm may be a general mechanism through which cells bypass RB inhibitory signals and that Brm may compensate for BRG-1 in tumor cells.

We have previously shown that BRG-1 regulates the expression of CD44 (12). Therefore, we wanted to determine whether these BRG-1 mutant tumor lines were compromised for this signaling pathway. Immunoblot analysis revealed that the BRG-1/Brm-positive SU86.86, HCT-116, Hs 683, WiDr, and Hs 578t cell lines express CD44 when compared with HeLa cells (Fig. 4*B*, *lanes 1*, *4*, *5*, *7*, *9*, and *12*). The Hs 700t, TSU-PR1, and NCI-H1299 cell lines, which express Brm and limiting amounts of BRG-1, were positive for CD44 (Fig. 4*B*, *lanes 6*, *8*, and *11*), suggesting that Brm may also compensate for the lack of BRG-1. However, A427 cells, like SW13 and C33A, were negative for CD44 expression, consistent with the notion that BRG-1 and Brm function are compromised in this cell line (Fig. 4*B*, *lanes 2*, *3*, and *10*). Thus, CD44 loss is correlative with disruption of SWI·SNF activity and could potentially be used as a marker for RB resistance.

Restoration of Core SWI·SNF Subunits Restores RB Inhibitory Activity—Because these data suggest that Brm can compensate for BRG-1 mutation or loss, we investigated whether reintroduction of Brm could restore RB signaling in C33A and A427 cells. To test this idea, A427 and C33A cells were co-transfected with H2B-GFP and either vector, BRG-1, Brm, PSM-RB, BRG-1 + PSM-RB, or Brm + PSM-RB and monitored for BrdUrd incorporation. A427 and C33A cells transfected with vector, BRG-1, and PSM-RB alone incorporated BrdUrd at approximately equal rates (Fig. 5*A*, *left* and *right panels*). Interestingly, co-transfection of PSM-RB + BRG-1 or PSM-RB + Brm caused a decrease in BrdUrd incorporation by ~63 and 62%, respectively, in A427 cells and by ~70 and 68% in C33A cells (Fig. 5*A*, *left* and *right panels*). We have previously shown

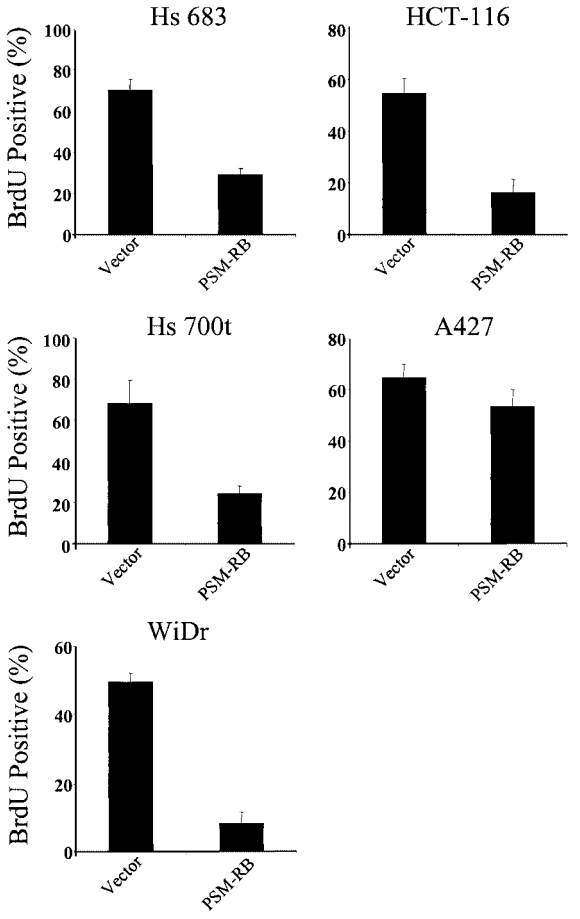

FIG. 3. **A427 cells are resistant to active RB.** HCT-116, Hs 700t, and Hs 683 cells were co-transfected with an H2B-GFP expression plasmid (0.125 μg) and vector (3.875 μg) or PSM-RB (3.875 μg) using calcium phosphate. A427 and WiDr cells were co-transfected with an H2B-GFP expression plasmid (0.062 μg) and vector (1.94 μg) or PSM-RB (1.94 μg) using the FuGENE transfection reagent. The percentage of BrdUrd-positive cells was determined from at least two independent experiments with at least 150 transfected cells (GFP-positive) scored per experiment.

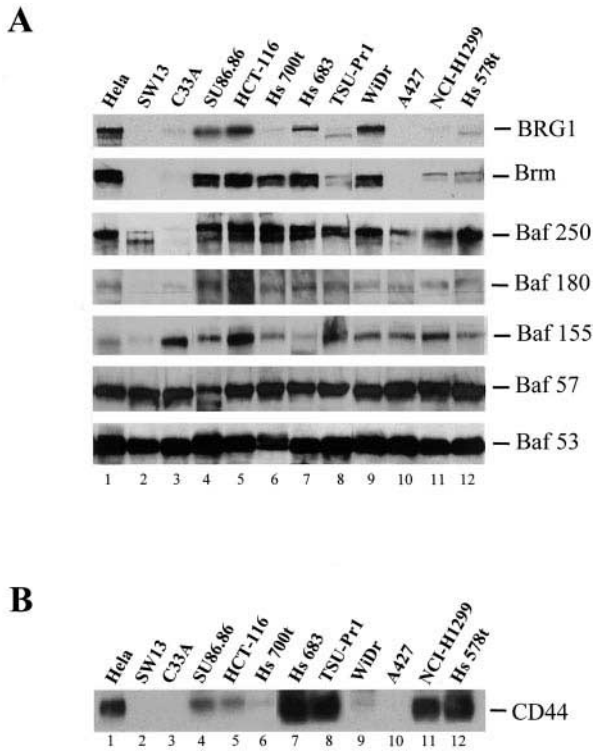

FIG. 4. **Loss of BRG-1 and Brm correlates with RB resistance.** Thirty micrograms of lysate was isolated from subconfluent HeLa, SW13, C33A, SU86.86, HCT-116, Hs 700t, Hs 683, TSU-Pr1, SNU-C2B, WiDr, A427, NCI-H1299, and Hs 578t cells using an 8 M urea extraction (8 M urea, 0.1 M NaH_2PO_4, 10 mM Tris, pH 8). The lysates were then separated via SDS-PAGE and blotted for BRG-1, Brm, Baf 250, Baf 180, Baf 155, Baf 53, and Baf 47 in *A* and CD44 in *B*.

that the ability of BRG-1 to cooperate with RB for cell cycle arrest is dependent on its ability to facilitate RB-mediated inhibition of cyclin A protein. Therefore, to confirm that Brm can functionally substitute for BRG-1 in regard to mediating RB inhibitory signals to its critical effector cyclin A, PSM-RB was co-transfected with Brm in A427 and C33A cells. Ectopic expression of vector, Brm, or PSM-RB alone did not alter endogenous cyclin A levels in either A427 or C33A cells (Fig. 5*B*, *lanes 1–3*, *left* and *right panels*). However, co-expression of Brm with PSM-RB restored RB signaling to cyclin A, as evidenced by a significant decrease in cyclin A protein (Fig. 5*B*, *lane 4*, *left* and *right panels*). Thus, Brm can substitute for BRG-1 in restoring RB-mediated signaling to cyclin A to inhibit cell cycle progression.

Because reintroduction of either BRG-1 or Brm into C33A and A427 cells restored RB inhibitory action, we investigated whether this was sufficient to restore CD44 signaling. To test this, SW13, A427, and C33A cells were co-transfected with either vector, BRG-1, or Brm along with a puromycin resistance plasmid. As expected, expression of vector had no effect on CD44 protein. However, co-expression of BRG-1 or Brm in SW13, A427, or C33A cells restored CD44 expression to similar levels, providing additional evidence that Brm can functionally substitute for BRG-1 (Fig. 5*C*, *lanes 1–3*, *left*, *middle*, and *right panels*).

BRG-1/Brm Mediates the RB-dependent CDDP Checkpoint in A427 Cells—The chemotherapeutic agent CDDP is commonly prescribed for the treatment of multiple tumor types (47). CDDP treatment is known to generate inter- and intramolecular DNA cross-links. This damage elicits an RB-dependent cell cycle arrest in mouse embryonic fibroblasts as well as a host of tumor lines (37, 38). To examine whether loss of BRG-1 or Brm can mediate resistance to CDDP, SW13 cells were treated with 32 μM CDDP, a dose previously described to cause a RB-dependent cell cycle arrest (37). Treatment of the RB-sensitive TSU-Pr1 cell line and the RB-resistant SW13 cell line with CDDP resulted in efficient dephosphorylation of RB (Fig. 6*A*, *lanes 2* and *4*). Next, we monitored signaling to critical cell cycle targets and determined that treatment of TSU-Pr1 with CDDP resulted in the attenuation of cyclin A protein expression by ~74.2% when compared with untreated cells. However, cyclin E and Cdk2 levels were not significantly altered by CDDP treatment, after comparison to untreated cells and normalization to β-tubulin (Fig. 6*B*, *lanes 1* and *2*). Also, treatment of TSU-Pr1 did not alter p53 levels, supporting the observation that p53 is functionally inactivated in this cell line. Analyses of SW13 cells treated with and without CDDP revealed no alteration in cyclin A, cyclin E, or Cdk2 protein expression after normalization to β-tubulin (Fig. 6*B*, *lanes 3 versus 4*). In addition, CDDP signaling to p53 was also compromised in SW13 cells. These data suggest that although RB is dephosphorylated in response to CDDP in SW13 cells, RB activity is compromised.

To confirm this hypothesis, we evaluated the effect of CDDP on cell cycle progression. We observed that treatment of TSU-Pr1 with CDDP resulted in the significant inhibition of BrdUrd incorporation when compared with untreated cells (Fig. 6*C*, *left panel*). This finding is consistent with CDDP activating RB,

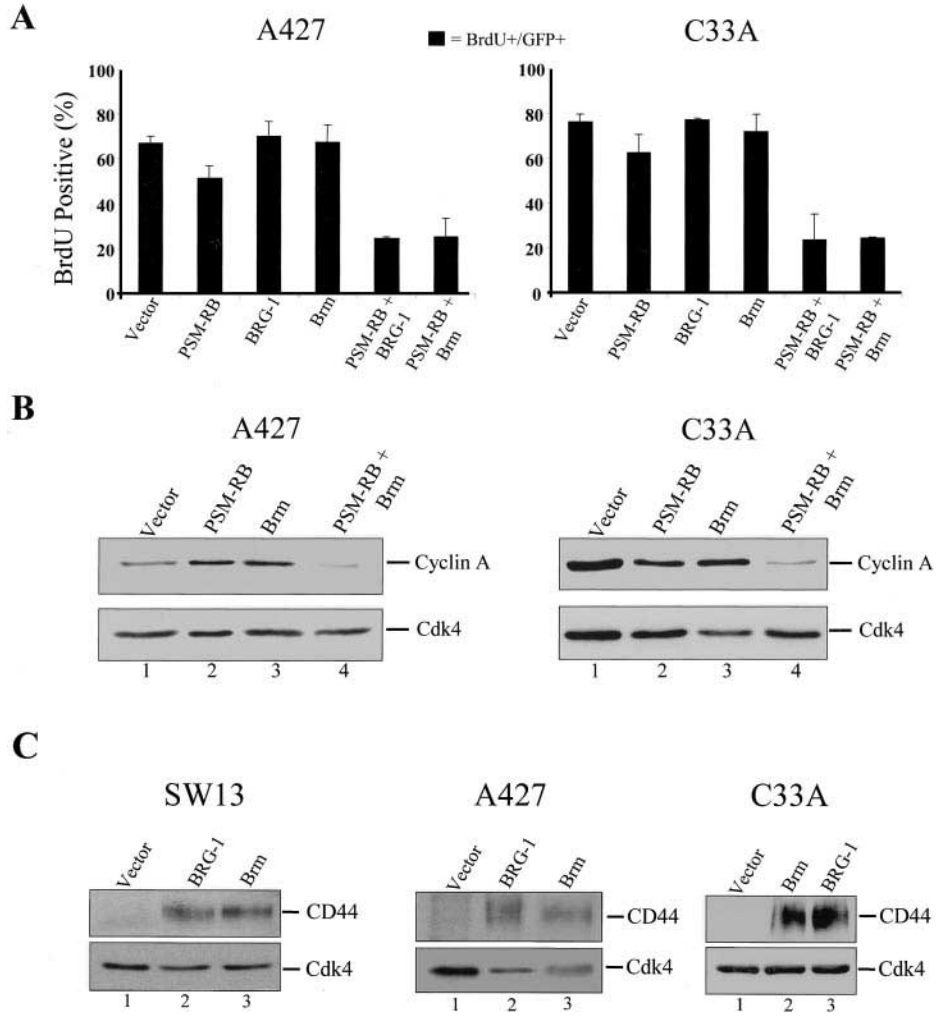

FIG. 5. **Reintroduction of core SWI·SNF subunits restores RB inhibitory signals.** *A*, A427 cells (*left panel*) were co-transfected with an H2B-GFP-expression plasmid (0.062 μg) and either vector (1.94 μg), PSM-RB (1.94 μg), BRG-1 (1.94 μg), Brm (1.94 μg), BRG-1 (1.0 μg) + PSM-RB (1.0 μg), or Brm (1.0 μg) + PSM-RB (1.0 μg) using the FuGENE transfection reagent. In addition, C33A cells (*right panel*) were co-transfected with an H2B-GFP expression plasmid (0.125 μg) and either vector (3.875 μg), PSM-RB (3.875 μg), BRG-1 (3.875 μg), Brm (3.875 μg), BRG-1 (1.9 μg) + PSM-RB (1.90 μg), or Brm (1.9 μg) + PSM-RB (1.9 μg) using calcium phosphate. The percentage of BrdUrd-positive cells was determined from at least two independent experiments with at least 150 transfected (GFP-positive) scored per experiment. *B*, using the aforementioned transfection methods, A427 cells (*left panel*) were co-transfected with 0.5 μg of pBabe-Puro and either 7.5 μg of vector (*lane 1*), 7.5 μg of PSM-RB (*lane 2*), 7.5 μg of Brm (*lane 3*), and 3.75 μg of Brm + 3.75 μg of PSM-RB (*lane 4*). In addition, C33A cells (*right panel*) were co-transfected with 1 μg of pBabe-Puro and either 15 μg of vector (*lane 1*), 15 μg of PSM-RB (*lane 2*), 15 μg of Brm (*lane 3*), and 7.5 μg of Brm + 7.5 μg of PSM-RB (*lane 4*) as indicated. After puromycin selection, equal total protein was resolved by SDS-PAGE and then immunoblotted for cyclin A and Cdk4. *C*, SW13 cells (*left panel*) were co-transfected with 1 μg of pBabe-Puro and either 15 μg of vector (*lane 1*), 15 μg of BRG-1 (*lane 2*), or 15 μg of Brm (*lane 3*). A427 cells (*middle panel*) were co-transfected with 0.5 μg of pBabe-Puro and either 7.5 μg of vector (*lane 1*), 7.5 μg of BRG-1 (*lane 2*), or 7.5 μg of Brm (*lane 3*). C33A cells (*right panel*) were co-transfected with 1 μg of pBabe-Puro and either 15 μg of vector (*lane 1*), 15 μg of Brm (*lane 2*) or 15 μg of BRG-1 (*lane 3*). After puromycin selection, equal total protein was resolved by SDS-PAGE and then immunoblotted for CD44 and Cdk4.

resulting in the down-regulation of cyclin A (Fig. 6*B*, *lane 2*). Strikingly, treatment of the SW13 cell line with CDDP had no effect on the ability of these cells to undergo DNA synthesis when compared with the untreated SW13 cells (Fig. 6*C*, *right panel*). To determine whether loss of BRG-1 and Brm confers a defect in the CDDP checkpoint in SW13 cells, either BRG-1 or Brm was co-transfected with H2B-GFP, and the transfected SW13 cells were monitored for their ability to incorporate BrdUrd in the presence and absence of CDDP. Interestingly, reintroduction of either BRG-1 or Brm into SW13 cells restored the ability of CDDP to cause a G_1 arrest, as evidenced by a respective 85 and 73.4% decrease in BrdUrd incorporation when compared with vector-treated cells (Fig. 6*D*). Therefore, reintroduction of either BRG-1 or Brm restores the RB-dependent CDDP checkpoint in SW13 cells.

DISCUSSION

The SWI·SNF complex mediates both transcriptional activation as well as repression (11). Specifically, it has been shown that both BRG-1 and Brm subunits use the energy of ATP to remodel chromatin structure as well as facilitate the transfer of nucleosomes from one DNA template to another *in vitro* (4, 5). The BRG-1 and Brm subunits of the SWI·SNF complex share 75% homology at the amino acid level; however, whether these two subunits carry out similar functions in a promoter or cell type-specific fashion remains equivocal (11). Because BRG-1 is involved in regulating cellular proliferation through its ability to cooperate with RB, the finding that it is lost or mutated in human tumors suggested a role in tumor suppression (19). Therefore, we hypothesized that loss or mutation of BRG-1 in the discrete tumor cell lines compromise RB inhibitory action.

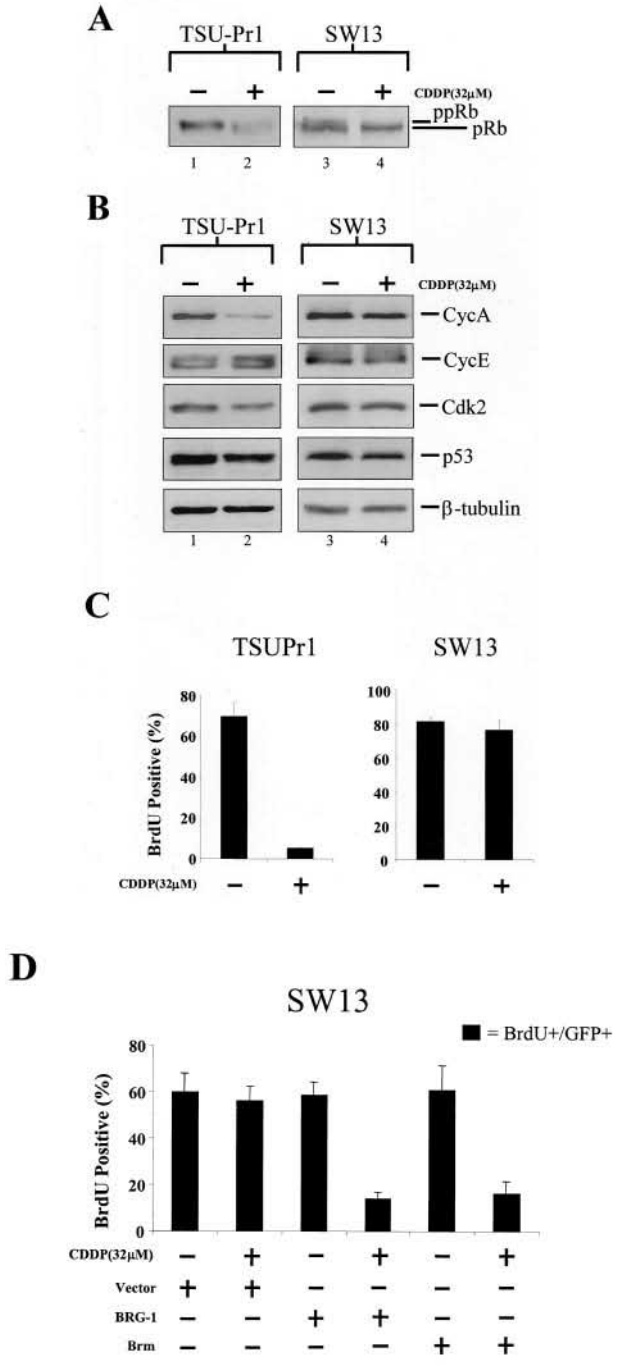

FIG. 6. **Loss of BRG-1 mediates CDDP resistance.** *A*, TSU-Pr1 and SW13 cells were treated with or without 32 μM CDDP and then harvested for immunoblot analysis. Next, equal total protein was resolved by SDS-PAGE and then immunoblotted for RB. *B*, in addition, after TSU-Pr1 and SW13 cells were treated with or without CDDP, we immunoblotted for cyclin A, cyclin E, Cdk2, β-tubulin, and p53. The level of cyclin A protein was normalized to that of β-tubulin. *C*, TSUPr1 (*left panel*) and SW13 cells (*right panel*) were seeded onto coverslips, treated with and without CDDP, and monitored for BrdUrd incorporation. *D*, SW13 cells were co-transfected with an H2B-GFP expression plasmid (0.125 μg) and either vector (3.875 μg), BRG-1 (3.875 μg), or Brm (3.875 μg). After the cells were transfected, they were treated either with 0 or 32 μM CDDP for 24 h. The percentage of BrdUrd-positive cells was determined from at least two independent experiments with at least 150 transfected (GFP-positive) scored per experiment.

Through our analysis, we demonstrate that the majority of BRG-1 mutant tumor lines were sensitive to the RB pathway. However, we identified the A427 lung carcinoma cell line to be resistant to both the inhibitory action of p16ink4a, which activates endogenous RB, and PSM-RB. To investigate how these cells bypass RB signaling, we analyzed the status of BRG-1 and Brm as well as the other Baf subunits in these tumor lines. Our findings revealed that A427, like the previously characterized RB-resistant C33A and SW13 cell lines, were compromised for both BRG-1 and Brm expression. In addition, CD44, an RB-independent marker for BRG-1 activity, was also absent in A427, similar to C33A and SW13 cells. Reintroduction of either BRG-1 or Brm restored both RB inhibitory action and CD44 expression in the resistant SW13, A427, and C33A cells. Using the commonly prescribed chemotherapeutic agent cisplatin, which has been shown to cause a physiological stress to activate RB, we demonstrate that SW13 cells are compromised in their response to CDDP (37, 38, 47, 48). Furthermore, ectopic expression of BRG-1 or Brm alone in SW13 restored their sensitivity to activation of the RB pathway by CDDP. Thus, these studies demonstrate that Brm can compensate for BRG-1 in mediating RB inhibitory signals.

RB is lost or functionally inactivated in the majority of human tumors (22, 23, 27, 28). The tumor suppressor activity of RB is manifested through its ability to inhibit cell cycle progression. This inhibitory action of RB requires the recruitment of accessory proteins to generate an RB-dependent transcriptional repressor complex (22–24, 26–28, 31). One critical component of the RB repressor complex is the SWI·SNF subunit BRG-1 (17, 18, 20, 30). In this study we show that in the Hs 700t, TSU-PR1, and NCI-H1299 tumor cell lines, Brm is present, but BRG-1 expression is attenuated. Interestingly, these cell lines are sensitive to RB, suggesting that Brm may compensate for the lack of BRG-1 expression in the context of these signaling pathways. This hypothesis was confirmed after restoration of Brm restored RB-mediated transcriptional repression to cause cell cycle arrest. Interestingly, not only RB, but the expression of CD44 appears to adhere to the presence of BRG-1 and Brm.

The mechanism through which BRG-1 and Brm expression is attenuated in the different tumor lines is currently unclear. Presumably germ line mutations in BRG-1 alter its stability/expression. Surprisingly, no mutations have been identified in SW13 cells despite low levels of BRG-1/Brm mRNA.[2] To date, no germ line mutations of Brm have been described. However, it has been shown that ectopic expression of Ras in 3T3 cells results in the specific attenuation Brm message, suggesting that alternative mechanisms exist for reducing Brm mRNA levels (49). Therefore, different tumor lines may utilize disparate genetic mechanisms for attenuating BRG-1 and Brm expression. Other gene products have been previously shown to be altered by epigenetic pathways in tumor cells. Specifically, the tumor suppressors p16ink4a and p53 as well as RB are inactivated in cancer via methylation of their promoter (50–52). Therefore, BRG-1 or Brm loss might be caused through similar epigenetic pathways. Because we observed reduced BRG-1 protein expression and not Brm in the Hs 700t, TSU-PR1, and NCI-H1299 cell lines, this finding suggests that multiple hits may occur in these tumor lines that result in the attenuation of BRG-1. The biological significance of selectively targeting BRG-1 or Brm is still unclear, although knockout studies have revealed distinct roles for these subunits. Specifically, mice homozygous for BRG-1 die during the periimplantation stage, whereas Brm −/− mice develop normally (53, 54). Whether the disparate phenotypes observed is reflective of developmental timing remains to be determined.

[2] M. W. Strobeck, D. N. Reisman, R. W. Gunawardena, B. L. Betz, S. P. Angus, K. E. Knudsen, T. F. Kowalik, B. E. Weissman, and E. S. Knudsen, unpublished information.

SWI·SNF subunits have been reported to be targeted in cancer (55). Specifically, it was shown that the SWI·SNF subunit hSNF5/Ini1 is lost in rhabdomyosarcoma, a rare pediatric cancer (56, 57). Loss of this subunit is thought to contribute to tumorigenesis because mice heterozygous for hSNF5/Ini1 develop malignant rhabdoid tumors (58–60). Interestingly, BRG-1 is lost in ~10% of tumor lines, and mice heterozygous for BRG-1 develop tumors of apocrine origin (54). In this study, loss of some of the other SWI·SNF subunits were observed in the discrete tumor lines, providing additional evidence that alternative accessory subunits are lost. Specifically, C33A was compromised for Baf 250, whereas SW13 lost Baf 180 (61). However, these accessory Baf subunits do not appear to be required for cooperating with RB or signaling to CD44, because reintroduction of either BRG-1 or Brm alone was sufficient to restore these discrete events.

The RB pathway is compromised in a large number of human tumors. Here, we show that dual loss of BRG-1 and Brm is tantamount to RB loss. In addition, loss of both of these central subunits renders cells resistant to activation of the RB pathway by CDDP. Thus, these results provide evidence that Brm can compensate for BRG-1 in cooperating with RB.

Acknowledgments—We thank Dr. Kenji Fukasawa, Christin Petre and Yelena Wetherill for critical reading of the manuscript. We are also grateful to Dr. S. Goff for supplying us with the BRG-1 plasmid and Dr. E. Lees for helpful scientific discussion.

REFERENCES

1. Imbalzano, A. N., Kwon, H., Green, M. R., and Kingston, R. E. (1994) *Nature* **370,** 481–485
2. Khavari, P. A., Peterson, C. L., Tamkun, J. W., Mendel, D. B., and Crabtree, G. R. (1993) *Nature* **366,** 170–174
3. Kwon, H., Imbalzano, A. N., Khavari, P. A., Kingston, R. E., and Green, M. R. (1994) *Nature* **370,** 477–481
4. Schnitzler, G., Sif, S., and Kingston, R. E. (1998) *Cell* **94,** 17–27
5. Lorch, Y., Zhang, M., and Kornberg, R. D. (1999) *Cell* **96,** 389–392
6. Goldmark, J. P., Fazzio, T. G., Estep, P. W., Church, G. M., and Tsukiyama, T. (2000) *Cell* **103,** 423–433
7. Larschan, E., and Winston, F. (2001) *Genes Dev.* **15,** 1946–1956
8. Moreira, J. M., and Holmberg, S. (1999) *EMBO J.* **18,** 2836–2844
9. Wilson, C. J., Chao, D. M., Imbalzano, A. N., Schnitzler, G. R., Kingston, R. E., and Young, R. A. (1996) *Cell* **84,** 235–244
10. Wang, W., Xue, Y., Zhou, S., Kuo, A., Cairns, B. R., and Crabtree, G. R. (1996) *Genes Dev.* **10,** 2117–2130
11. Kingston, R. E., and Narlikar, G. J. (1999) *Genes Dev.* **13,** 2339–2352
12. Strobeck, M. W., DeCristofaro, M. F., Banine, F., Weissman, B. E., Sherman, L. S., and Knudsen, E. S. (2001) *J. Biol. Chem.* **276,** 9273–9278
13. DiRenzo, J., Shang, Y., Phelan, M., Sif, S., Myers, M., Kingston, R., and Brown, M. (2000) *Mol. Cell. Biol.* **20,** 7541–7549
14. Fryer, C. J., and Archer, T. K. (1998) *Nature* **393,** 88–91
15. Cheng, S. W., Davies, K. P., Yung, E., Beltran, R. J., Yu, J., and Kalpana, G. V. (1999) *Nat. Genet.* **22,** 102–105
16. Bochar, D. A., Wang, L., Beniya, H., Kinev, A., Xue, Y., Lane, W. S., Wang, W., Kashanchi, F., and Shiekhattar, R. (2000) *Cell* **102,** 257–265
17. Strobeck, M. W., Knudsen, K. E., Fribourg, A. F., DeCristofaro, M. F., Weissman, B. E., Imbalzano, A. N., and Knudsen, E. S. (2000) *Proc. Natl. Acad. Sci. U. S. A.* **97,** 7748–7753
18. Zhang, H. S., Gavin, M., Dahiya, A., Postigo, A. A., Ma, D., Luo, R. X., Harbour, J. W., and Dean, D. C. (2000) *Cell* **101,** 79–89
19. Wong, A. K., Shanahan, F., Chen, Y., Lian, L., Ha, P., Hendricks, K., Ghaffari, S., Iliev, D., Penn, B., Woodland, A. M., Smith, R., Salada, G., Carillo, A., Laity, K., Gupte, J., Swedlund, B., Tavtigian, S. V., Teng, D. H., and Lees, E. (2000) *Cancer Res.* **60,** 6171–6177
20. Dunaief, J. L., Strober, B. E., Guha, S., Khavari, P. A., Alin, K., Luban, J., Begemann, M., Crabtree, G. R., and Goff, S. P. (1994) *Cell* **79,** 119–130
21. Strober, B. E., Dunaief, J. L., Guha, and Goff, S. P. (1996) *Mol. Cell. Biol.* **16,** 1576–1583
22. Bartkova, J., Lukas, J., and Bartek, J. (1997) *Prog. Cell Cycle Res.* **3,** 211–220
23. Bartek, J., Bartkova, J., and Lukas, J. (1997) *Exp. Cell Res.* **237,** 1–6
24. Dyson, N. (1998) *Genes Dev.* **12,** 2245–2262
25. Harbour, J. W., and Dean, D. C. (2000) *Nat. Cell Biol.* **2,** 65–67
26. Reed, S. I. (1997) *Cancer Surv.* **29,** 7–23
27. Sherr, C. J. (1996) *Science* **274,** 1672–1677
28. Wang, J. Y., Knudsen, E. S., and Welch, P. J. (1994) *Adv. Cancer Res.* **64,** 25–85
29. Sellers, W. R., and Kaelin, W. G., Jr. (1997) *J. Clin. Oncol.* **15,** 3301–3312
30. Strobeck, M. W., Fribourg, A. F., Puga, A., and Knudsen, E. S. (2000) *Oncogene* **19,** 1857–1867
31. Harbour, J. W., and Dean, D. C. (2000) *Genes Dev.* **14,** 2393–2409
32. Harbour, J. W., and Dean, D. C. (2001) *Curr. Top. Microbiol. Immunol.* **254,** 137–144
33. Brehm, A., Miska, E. A., McCance, D. J., Reid, J. L., Bannister, A. J., and Kouzarides, T. (1998) *Nature* **391,** 597–601
34. Luo, R. X., Postigo, A. A., and Dean, D. C. (1998) *Cell* **92,** 463–473
35. Knudsen, K. E., Fribourg, A. F., Strobeck, M. W., Blanchard, J. M., and Knudsen, E. S. (1999) *J. Biol. Chem.* **274,** 27632–27641
36. Meraldi, P., Lukas, J., Fry, A. M., Bartek, J., and Nigg, E. A. (1999) *Nat. Cell Biol.* **1,** 88–93
37. Knudsen, K. E., Booth, D., Naderi, S., Sever-Chroneos, Z., Fribourg, A. F., Hunton, I. C., Feramisco, J. R., Wang, J. Y., and Knudsen, E. S. (2000) *Mol. Cell. Biol.* **20,** 7751–7763
38. Harrington, E. A., Bruce, J. L., Harlow, E., and Dyson, N. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95,** 11945–11950
39. Chen, C., and Okayama, H. (1987) *Mol. Cell. Biol.* **7,** 2745–2752
40. Knudsen, K. E., Arden, K. C., and Cavenee, W. K. (1998) *J. Biol. Chem.* **273,** 20213–20222
41. Knudsen, K. E., Weber, E., Arden, K. C., Cavenee, W. K., Feramisco, J. R., and Knudsen, E. S. (1999) *Oncogene* **18,** 5239–5245
42. Muchardt, C., and Yaniv, M. (1993) *EMBO J.* **12,** 4279–4290
43. Castillo, J. P., Yurochko, A. D., and Kowalik, T. F. (2000) *J. Virol.* **74,** 8028–8037
44. Lukas, J., Sorensen, C. S., Lukas, C., Santoni-Rugiu, E., and Bartek, J. (1999) *Oncogene* **18,** 3930–3935
45. Koh, J., Enders, G. H., Dynlacht, B. D., and Harlow, E. (1995) *Nature* **375,** 506–510
46. Boylan, J. F., Sharp, D. M., Leffet, L., Bowers, A., and Pan, W. (1999) *Exp. Cell Res.* **248,** 110–114
47. Cohen, S. M., and Lippard, S. J. (2001) *Prog. Nucleic Acid Res. Mol. Biol.* **67,** 93–130
48. Kartalou, M., and Essigmann, J. M. (2001) *Mutat Res* **478,** 23–43
49. Muchardt, C., Bourachot, B., Reyes, J. C., and Yaniv, M. (1998) *EMBO J.* **17,** 223–231
50. Foster, S. A., Wong, D. J., Barrett, M. T., and Galloway, D. A. (1998) *Mol. Cell. Biol.* **18,** 1793–1801
51. Stirzaker, C., Millar, D. S., Paul, C. L., Warnecke, P. M., Harrison, J., Vincent, P. C., Frommer, M., and Clark, S. J. (1997) *Cancer Res.* **57,** 2229–2237
52. Schroeder, M., and Mass, M. J. (1997) *Biochem. Biophys. Res. Commun.* **235,** 403–406
53. Reyes, J. C., Barra, J., Muchardt, C., Camus, A., Babinet, C., and Yaniv, M. (1998) *EMBO J.* **17,** 6979–6991
54. Bultman, S., Gebuhr, T., Yee, D., La Mantia, C., Nicholson, J., Gilliam, A., Randazzo, F., Metzger, D., Chambon, P., Crabtree, G., and Magnuson, T. (2000) *Mol. Cell* **6,** 1287–1295
55. Muchardt, C., and Yaniv, M. (2001) *Oncogene* **20,** 3067–3075
56. Versteege, I., Sevenet, N., Lange, J., Rousseau-Merck, M. F., Ambros, P., Handgretinger, R., Aurias, A., and Delattre, O. (1998) *Nature* **394,** 203–206
57. DeCristofaro, M. F., Betz, B. L., Wang, W., and Weissman, B. E. (1999) *Oncogene* **18,** 7559–7565
58. Roberts, C. W., Galusha, S. A., McMenamin, M. E., Fletcher, C. D., and Orkin, S. H. (2000) *Proc. Natl. Acad. Sci. U. S. A.* **97,** 13796–13800
59. Guidi, C. J., Sands, A. T., Zambrowicz, B. P., Turner, T. K., Demers, D. A., Webster, W., Smith, T. W., Imbalzano, A. N., and Jones, S. N. (2001) *Mol. Cell. Biol.* **21,** 3598–3603
60. Klochendler-Yeivin, A., Fiette, L., Barra, J., Muchardt, C., Babinet, C., and Yaniv, M. (2000) *EMBO Rep.* **1,** 500–506
61. Decristofaro, M. F., Betz, B. L., Rorie, C. J., Reisman, D. N., Wang, W., and Weissman, B. E. (2001) *J. Cell. Physiol.* **186,** 136–145