THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 276, No. 12, Issue of March 23, pp. 9273–9278, 2001
Printed in U.S.A.

The BRG-1 Subunit of the SWI/SNF Complex Regulates CD44 Expression*

Received for publication, October 25, 2000, and in revised form, December 5, 2000
Published, JBC Papers in Press, December 6, 2000, DOI 10.1074/jbc.M009747200

Matthew W. Strobeck‡§¶, Marc F. DeCristofaro§‖, Fatima Banine‡, Bernard E. Weissman‖**, Larry S. Sherman‡, 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, the ‖Department of Pathology and Laboratory Medicine, University of North Carolina Chapel Hill, North Carolina 27599, and **The Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, North Carolina 27599*

Aberrant regulation of CD44, a transmembrane glycoprotein, has been implicated in the growth and metastasis of numerous tumors. Although both CD44 overexpression and loss have been implicated in tumor progression, the mechanism of CD44 down-regulation in these tumor types is not known. By immunoblot and reverse transcription-polymerase chain reaction analysis we determined that a cervical carcinoma cell line, C33A, lacks CD44 expression. To determine how CD44 is down-regulated in C33A cells, we utilized cell fusions of C33A cells with a CD44-expressing cell line (SAOS-2). We found that SAOS-2 fusion restored CD44 expression in C33A cells, suggesting that a trans-acting factor present in SAOS-2 cells promotes CD44 production. C33A cells are BRG-1-deficient, and we found that CD44 was absent in another BRG-1-deficient tumor cell line, indicating that loss of BRG-1 may be a general mechanism by which cells lose CD44. Reintroduction of BRG-1 into these cells restored CD44 expression. Furthermore, disruption of BRG-1 function through the use of dominant-negative BRG-1 demonstrated the requirement of BRG-1 in CD44 regulation. Finally, we show that Cyclin E overexpression resulted in the attenuation of CD44 stimulation, which is consistent with previous observations that Cyclin E can abrogate BRG-1 action. Taken together, these results suggest that BRG-1 is a critical regulator of CD44 expression, thus implicating SWI/SNF components in the regulation of cellular adhesion and metastasis.

The CD44 family of transmembrane glycoproteins has been implicated in cell-cell and cell-matrix adhesion, cell growth, and metastasis (1–3). A number of different CD44 proteins are produced through alternative RNA splicing, and these proteins are extensively modified. Many tumors express higher than normal levels of total CD44 protein as well as splice variants that do not occur in normal cells (1, 3, 4). How CD44 expression is regulated in normal cells and in tumors is poorly understood.

A role for CD44 in tumor progression has been documented in numerous clinical and experimental studies (1, 2). Ectopic expression of some forms of CD44 can enhance metastasis and tumor growth both *in vitro* and *in vivo* (5–7). It is believed that CD44 expression in some tumors increases as the tumor becomes more proliferative and invasive (1). These findings suggest that CD44 might be regulated by environmental or genetic factors that have been shown to contribute to cancer progression. The expression of activated oncogenes like v-Ras, v-Src, and v-Fos, which promote transformation and invasion, have been reported to induce CD44 expression (8–10). In addition, the epidermal growth factor receptor has also been shown to stimulate CD44 (10, 11).

In contrast to studies that correlate CD44 overexpression with cancer progression, a significant number of reports also indicate that loss of CD44 expression can contribute to tumorigenesis (12). Specifically, it has been shown that loss of CD44 in cervical carcinomas, neuroblastomas, prostate carcinomas, melanomas, and small cell lung carcinomas correlates with increased aggressiveness of these tumors (13–17). It has been hypothesized that loss of CD44 may facilitate the abrogation of epithelial-mesenchymal interactions in some tumors, leading to a more metastatic phenotype (4, 12). Unlike positive regulators of CD44, no activity has been identified that leads to loss of CD44 expression.

In the present study, we have identified the *Brahma-related* gene, BRG-1,[1] as a critical regulator of CD44 expression. BRG-1 is a component of the SWI/SNF complex that regulates gene transcription through ATPase-dependent remodeling of chromatin (18–21). BRG-1 has been shown to inhibit the transcription of a subset of genes involved in cancer such as Cyclin A and c-*fos* (22–25). In addition, BRG-1 can facilitate estrogen receptor, glucocorticoid receptor, c-Myc, and BRCA1-mediated transcription (26–30).

We demonstrate that loss of BRG-1 correlates with a lack of CD44 expression in several cell lines. Reintroduction of BRG-1 restored expression of CD44 in BRG-1-deficient cells, suggesting that BRG-1 regulates basal CD44 levels. Moreover, we show that functional BRG-1 is required for stimulating CD44, because dominant-negative BRG-1 inhibits CD44 production. In addition, we show that Cyclin E can antagonize BRG-1 regulation of CD44. As such, this report provides the first

* This work was supported in part by Grant CA-82525 from the NCI, National Institutes of Health (NIH), by grants from The Ruth Lyons Cancer Foundation and the Sidney Kimmel Cancer Foundation (to E. S. K.), by NIH Grant NS-39550 (to L. S. S.), and by NIH Grants DE-12355 and CA-63176 (to B. E. 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.

¶ To whom correspondence should be addressed: Dept. of Cell Biology, University of Cincinnati College of Medicine, Vontz Center for Molecular Studies, 3125 Eden Ave., Cincinnati, OH 45267-0521. Tel.: 513-558-6849; Fax: 513-558-4454; E-mail: shobecm@email.uc.edu.

[1] The abbreviations used are: BRG-1, *Brahma-related* gene; PBS, phosphate-buffered saline; RT-PCR, reverse transcription-polymerase chain reaction; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; C-S, C33A-SAOS-2 cell fusion line; dnBRG-1, dominant-negative BRG-1; PAGE, polyacrylamide gel electrophoresis.

evidence that the SWI/SNF complex regulates genes involved in cell adhesion and cancer metastasis and suggests that BRG-1 loss in human tumors contributes to the formation of a metastatic phenotype.

MATERIALS AND METHODS

Cells, Cell fusions, Plasmids, and Transfection—C33A, SAOS-2, MCF-7, U87, PC3, U2OS, RD, and SW13 cells were maintained in Dulbecco's modified Eagle's medium 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. 3T3 cells (BO5-1) were grown in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated calf serum (Calbiochem), 100 units/ml penicillin-streptomycin, 2 mM L-glutamine, 350 units of Hygromycin B, and 0.12 mg/ml Geneticin either in the presence or absence of 2 μg/ml tetracycline (28). Plasmids were transfected using the calcium phosphate method (31). Antibiotic-resistant C33A and SAOS-2 cells were generated as previously described (32). The following plasmids: CMV-NEO, pBabe-Puro, Cyclin E, PSM-RB, and BRG-1 have been previously described (33–36). The cell fusions were generated as previously described (32).

Immunoblotting—Approximately 1×10^6 C33A and SW13 cells were plated in 10-cm dishes 24 h before transfection. C33A and SW13 cells were cotransfected with effectors and the puromycin-selectable plasmid (pBabe-Puro). Transfected cells were subsequently selected with 2.5 μg/ml puromycin (Sigma) for 48–72 h and then harvested for immunoblot analysis. To isolate BRG-1, total protein was isolated from subconfluent cultures using an 8 M urea extraction (8 M urea, 0.1 M NaH_2PO_4, 10 mM Tris, pH 8). For immunoblotting, cells were trypsinized and subsequently washed with PBS. C33A and SW13 cell pellets were resuspended in radioimmune precipitation buffer supplemented with the following 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. The lysates were then separated via SDS-polyacrylamide gel electrophoresis and then transferred onto Immobilon-P membranes (Millipore). The membranes were then incubated with either of the following antibodies: anti-CD44 (Hermes 3), anti-Cdk4 (clone H22, Santa Cruz Biotechnology), anti-Cyclin E (clone C19, Santa Cruz), anti-actin (Sigma A-2066), anti-p53 (Dr. K. Fukasawa, University of Cincinnati, College of Medicine, Cincinnati, OH), anti-pRB (Dr. J. Wang, University of California at San Diego, 851 polyclonal antibody), and anti-BRG-1 (Dr. Weidong Wang, National Institutes of Health, Baltimore, MD). The blots were then incubated with either horseradish peroxidase-conjugated anti-rabbit or anti-mouse for 1 h at room temperature. The antibody-antigen complex was detected by enhanced chemiluminescence (ECL, Amersham Pharmacia Biotech). The levels of Cdk4 and CD44 were quantitated using Metamorph software (Universal Imaging Corp.).

Immunohistochemistry—Cells were fixed in 3.7% formaldehyde for 15 min and then either permeabilized (PBS, 0.3% Triton X-100, 5 mg/ml bovine serum albumin) for p53 staining or directly blocked with 5% normal goat serum for CD44 staining. The p53 antibody (1:100 dilution, ab-6, Calbiochem) was detected with a rhodamine-conjugated anti-mouse antibody. Human CD44 was probed with Hermes 3 (1:25, monoclonal antibody) and detected with a fluorescein-conjugated anti-mouse antibody. Mouse CD44 was identified with IM7 (1:100, Zymed Laboratories Inc.) and visualized with a fluorescein-conjugated anti-rat antibody.

RNA Isolation and RT-PCR—To analyze CD44 mRNA expression levels, reverse transcription-polymerase chain reaction (RT-PCR) was performed. First, $\sim 1 \times 10^6$ C33A cells were seeded into a 10-cm dish and transfected with either Vector-, PSM-RB-, or BRG-1-encoding plasmids along with pBabe. After 48 h of selection with puromycin, total mRNA was isolated using TRIzol (Life Technologies, Inc.) according to the manufacturer's protocol. cDNAs were then synthesized using ~ 1 μg of isolated total mRNA and the ThermoScript RT-PCR system (Life Technologies, Inc.) following the manufacturer's instructions. To amplify human CD44, cDNAs were subjected to PCR using the forward primer: 5′-CAGACCTGCCCAATGCCTTTGATGGACC-3′ and the reverse primer: 5′-CAAAGCCAAGGCCAAGAGGGATGCC-3′. As a control, human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA was amplified using the forward primer: 5′-GGTCATCAATGGGAAACCCATCAC-3′ and the reverse primer: 5′-ACAAACTCTGCTACTTCTGG-3′. PCR was performed with the following conditions: 94 °C, 1 min; 62 °C, 1 min; and 72 °C, 2.5 min, for 30 cycles. Approximately 10 μl of the PCR products were then separated by electrophoresis in a 1% agarose gel. The PCR products were visualized by ethidium bromide staining and then photographed. The levels of GAPDH and CD44 were quantitated using Metamorph software (Universal Imaging Corp.) from two independent PCR reactions.

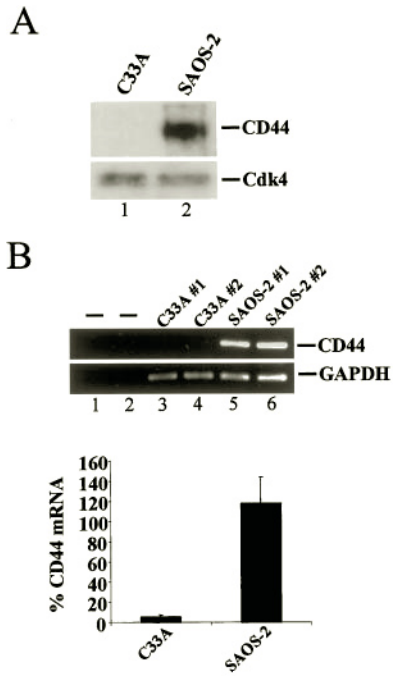

FIG. 1. **C33A cells have reduced CD44 expression.** *A*, C33A (*lane 1*) and SAOS-2 (*lane 2*) cells were harvested and lysed, and equal total protein was resolved by SDS-PAGE and immunoblotted for CD44 and Cdk4. *B*, C33A cells were harvested as in *A*, total RNA was prepared, and RT-PCR was performed utilizing 1 μg of total RNA as described under "Materials and Methods." As a control, PCR analysis of isolated RNA was carried out from C33A (*lane 1*) and SAOS-2 (*lane 2*). The level of CD44 RNA was normalized to that of GAPDH. The results are representative of two independent RT-PCR experiments.

RESULTS

CD44 Expression Is Down-regulated in the C33A Cervical Carcinoma Cell Line—CD44 is lost some in cervical carcinomas, and this loss correlates with increased metastatic potential (13, 14). We identified a human cervical carcinoma cell line, C33A, which demonstrates loss of CD44 expression as determined by immunoblot analysis with a CD44-specific antibody (Fig. 1*A*, *lane 1*). By contrast, the osteogenic sarcoma cell line, SAOS-2, readily expresses detectable CD44 (Fig. 1*A*, *lane 2*). Blotting for Cdk4 showed that equal total protein was loaded (Fig. 1*A*). To examine whether loss of CD44 expression in C33A occurred at the RNA level, RT-PCR analysis was used to examine the levels of CD44 mRNA in C33A cells *versus* the CD44-positive SAOS-2 cells. In C33A cells, the levels of CD44 mRNA were ~20-fold less than that found in SAOS-2 (Fig. 1*B*, *lanes 3*, *4*, *5*, and *6*). Thus, CD44 expression in C33A is lost at the level of RNA, suggesting that C33A cells either possess a factor important for repressing CD44 expression or alternatively lack a factor required for stimulating CD44 expression.

Factors Expressed by SAOS-2 Cells Act in-trans to Restore CD44 Signaling in C33A Cells—To examine the underlying difference in CD44 signaling between C33A and SAOS-2, we utilized C33A-SAOS-2 (C-S) cell fusion lines that have been previously characterized (32). Four independent C-S fusion clones (#*1–4*) exhibited robust expression of CD44 comparable to that observed in parental SAOS-2 cells (Fig. 2*A*, *lanes 1–6*). Immunohistochemical analysis confirmed that CD44 expression was restored and appropriately localized in the cell fusions (Fig. 2*B*, *right panel*). As a control, p53 staining confirmed that

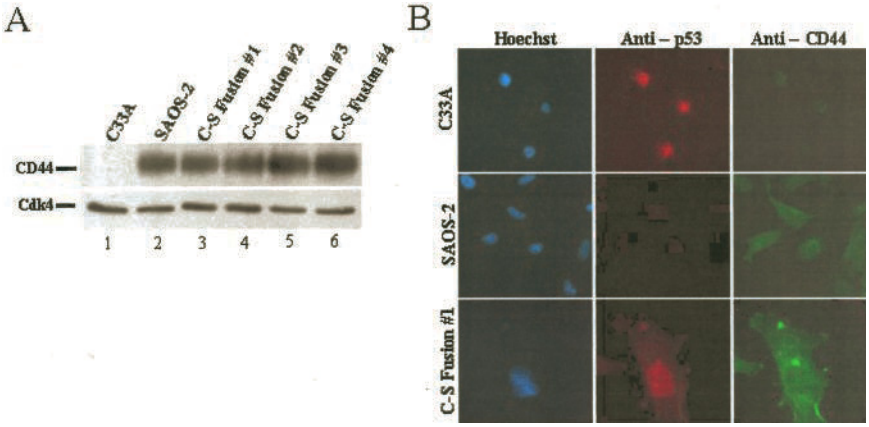

FIG. 2. **Genetic determinants in SAOS-2 restore CD44 signaling in C33A.** *A*, parental C33A and SAOS-2 cells along with four independent C-S fusion clones were harvested and lysed, and equal total protein was resolved by SDS-PAGE and immunoblotted for CD44 and Cdk4. *B*, immunohistochemical staining of both parental lines and a C-S fusion clone for p53 and CD44 expression. The results of this fusion clone are representative of all other fusion clones tested.

the fusion expressed p53 protein (contributed from C33A nuclei), because SAOS-2 are p53-negative (Fig. 2*B*, *left* and *middle panel*). These findings suggest that C33A cells lack a factor(s) required for CD44 signaling, which is restored after nuclear fusion with SAOS-2.

Loss of BRG-1 Correlates with Reduced CD44 Expression—A known difference between SAOS-2 cells and C33A cells is that SAOS-2 cells express BRG-1, a component of the SWI/SNF complex involved in both gene regulation and chromatin remodeling, whereas C33A lack BRG-1 (36). To examine whether loss of BRG-1 correlated with lack of CD44 expression, we analyzed several tumor lines that were either positive or negative for BRG-1 expression. Immunoblot analysis revealed that those tumor lines that were positive for BRG-1 were also positive for CD44 expression (Fig. 3*A*, *upper* and *middle panel*, *lanes 2*, *3*, *5*, *6*, and *7*). However, the SW13 adenocarcinoma cell line, which is BRG-1-negative, was deficient in CD44 protein expression, similar to that observed in C33A cells (Fig. 3*A*, *upper* and *middle panel*, *lanes 1* and *4*) (36). As a control, the detection of actin shows the relative amount of protein loaded per lane (Fig. 3*A*, *lower panel*, *lanes 1–7*). These data demonstrate that loss of BRG-1 correlates with a lack of CD44 protein, suggesting that BRG-1 is required for CD44 expression.

BRG-1 Restores CD44 Expression in C33A and SW13 Cells—To determine whether BRG-1 is sufficient to induce CD44 expression, BRG-1 was restored in BRG-1-negative (C33A and SW13) cells. C33A and SW13 cells were cotransfected with vector, a phosphorylation site mutant of RB (PSM-RB) as controls, or BRG-1 along with a puromycin resistance plasmid (pBabe-Puro). After selecting the transfected cells with puromycin, cells were harvested and subjected to immunoblot analysis. In C33A and SW13 cells, transfection of either vector or PSM-RB did not alter CD44 levels. However, ectopic expression of BRG-1 caused a significant increase in CD44 protein levels (Fig. 4*A*, *upper panel*, *lanes 1–6*). Immunoblotting for BRG-1 and PSM-RB confirmed their expression in C33A and SW13 cells, whereas Cdk4 levels indicate that equal total protein was loaded per lane (Fig. 4*A*, *middle* and *lower panels*, *lanes 1–6*). Because RB has been shown to recruit BRG-1 for transcriptional repression, we also examined whether expression of PSM-RB could alter BRG-1 stimulation of CD44. To test this, we cotransfected PSM-RB with BRG-1 into C33A cells and found that PSM-RB did not alter the ability of BRG-1 to modulate CD44 production (data not shown).

Because BRG-1 is known to mediate its function through transcriptional regulation, we investigated whether BRG-1 stimulates CD44 mRNA levels (37). C33A cells were transfected with either vector or PSM-RB along with pBabe-Puro and selected in puromycin. RT-PCR analysis revealed that the level of CD44 mRNA quantitated from vector-transfected cells was equal to that of PSM-RB-transfected cells after normalization to GAPDH (Fig. 4*B*, *upper* and *lower panels*, *lanes 4*, *5*, *8*, and *9*). In contrast, C33A cells transfected with BRG-1 showed an ~40-fold induction in CD44 mRNA when compared with that of vector- or PSM-RB-transfected cells and normalized against GAPDH (Fig. 4*B*, *upper* and *lower panels*, *lanes 4–9*). PCR analysis of the RNA prior to reverse transcribing revealed no amplification, thus eliminating the possibility of DNA contamination (Fig. 4*B*, *upper* and *lower panels*, *lanes 1–3*). The effect of ectopic BRG-1 expression on the CD44 promoter was assessed by reporter analysis in transient transfection assays. Ectopic expression of BRG-1 did not stimulate CD44 reporter activity, and high basal activity of the reporter was observed in cells either expressing or deficient in BRG-1 (data not shown). This finding is consistent with the idea that BRG-1 stimulates transcription through the remodeling of chromatin, because we show that BRG-1 clearly stimulates the expression of endogenous CD44 RNA and protein.

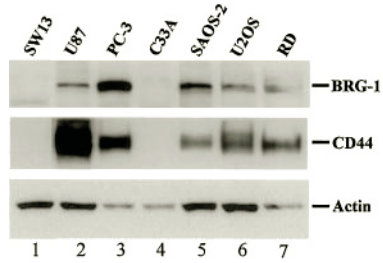

FIG. 3. **Loss of BRG-1 correlates with a lack of CD44 expression.** Thirty micrograms of lysate was isolated from subconfluent SW13, U87, PC-3, C33A, SAOS-2, U2OS, and RD cells using an 8 M urea extraction (8 M urea, 0.1 M NaH_2PO_4, 10 mM Tris, pH 8). The lysates were then separated via SDS-PAGE and blotted for BRG-1, CD44, and actin.

Functional BRG-1 Is Required for Stimulating CD44 Production—Because the data presented imply that BRG-1 may have a role in maintaining the production of CD44, we investigated whether BRG-1 is required for this process. To test this we utilized an NIH3T3 cell line, called BO5-1, that inducibly expresses dominant-negative BRG-1 (dnBRG-1) (28). The mutant BRG-1 protein that is induced in BO5-1 cells contains a mutation in the ATP-binding site that renders it catalytically inactive, and in the presence of tetracycline, BO5-1 cells fail to demonstrate detectable levels of dnBRG-1 (Fig. 5*A*, *lanes 1* and

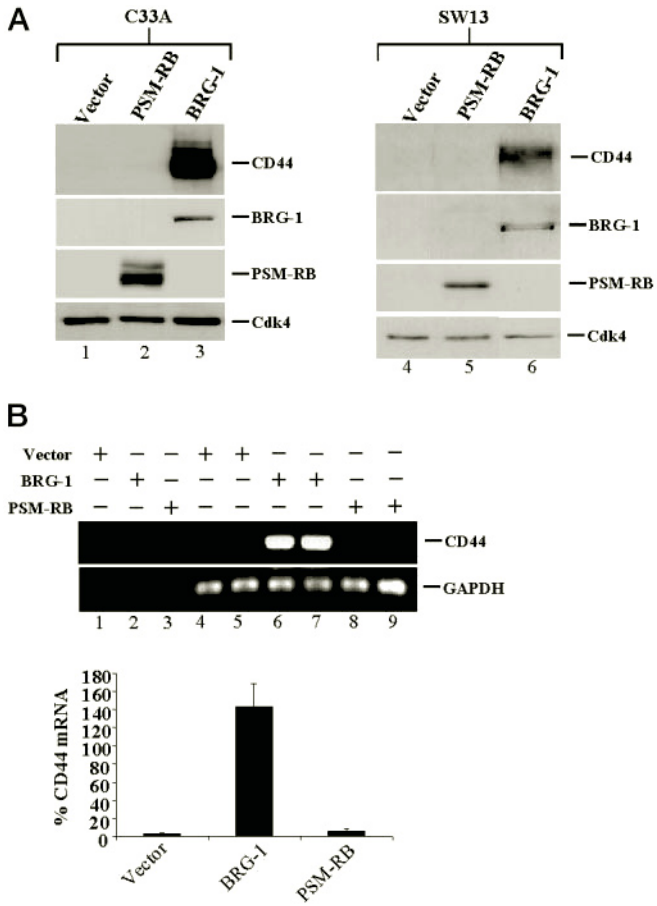

FIG. 4. **BRG-1 stimulates CD44 expression in C33A and SW13 cells.** *A*, C33A and SW13 cells were transfected with (1 μg) pBabe-Puro and (15 μg) of vector (*lane 1*), PSM-RB (*lane 2*), or BRG-1 (*lane 3*). Following puromycin selection, equal total protein was resolved by SDS-PAGE and then immunoblotted for CD44, BRG-1, PSM-RB, and Cdk4. *B*, C33A cells were transfected with vector (*lanes 4*, *5*), BRG-1 (*lanes 6* and *7*), and PSM-RB (*lanes 8* and *9*) and then selected as performed in *A*. Total RNA was prepared, and RT-PCR was performed utilizing 1 μg of total RNA as described under "Materials and Methods." As a control, PCR analysis of isolated RNA was carried out from the vector (*lanes 1*)-, BRG-1 (*lane 2*)-, and PSM-RB (*lane 3*)-transfected cells. The level of CD44 RNA was normalized to that of GAPDH. The results are representative of two independent RT-PCR experiments.

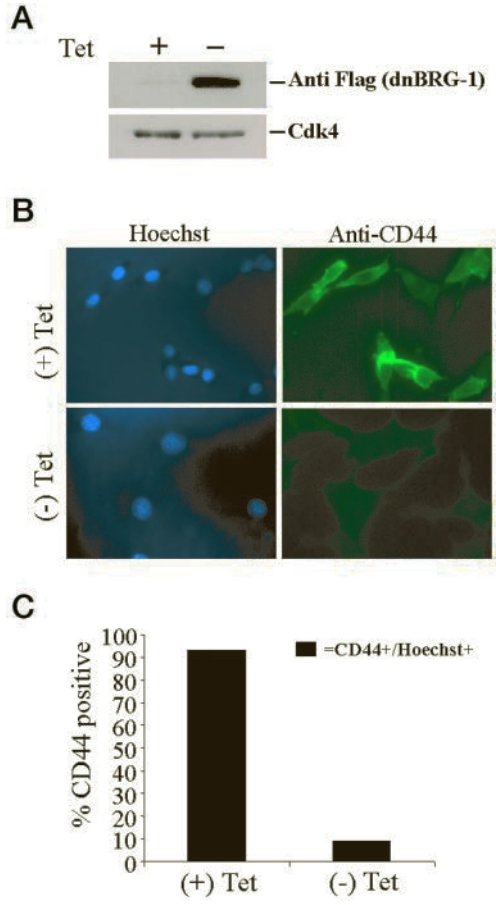

FIG. 5. **BRG-1 is required for CD44 production.** *A*, BO5-1 cells were washed three times with PBS and then split into plates containing media supplemented with and with out tetracycline (2 μg/ml). After 3 days, the cells were harvested and resolved by SDS-PAGE, and the expression of dnBRG-1 was detected with an anti-Flag antibody. *B*, immunohistochemical staining for CD44 in BO5-1 cells cultured either in the presence or absence of tetracycline. *C*, quantitation of the immunohistochemical staining in *B*.

2). However, after incubation in the absence of tetracycline for 72 h, a strong induction of dnBRG-1 was observed. Immunostaining of the BO5-1 cells grown in the absence of tetracycline revealed that expression of dnBRG-1 results in the reduction of CD44 expression when compared with uninduced BO5-1 cells (Fig. 5*B*). Microscopic analysis revealed that expression of dnBRG-1 significantly inhibits CD44 staining in ~90% of induced cells when compared with BO5-1 cells grown in the presence of tetracycline (Fig. 5*C*). These data imply that functional BRG-1 is required for stimulating CD44 expression.

Cyclin E Antagonizes BRG-1 Transcriptional Activity—It has been previously reported that Cyclin E can bind to and antagonize the ability of BRG-1 to induce growth arrest (38). Based on this, and the findings that Cyclin E is also overexpressed in tumors, we examined whether expression of Cyclin E could disrupt the ability of BRG-1 to signal to CD44 (39). To test this, C33A cells were cotransfected with vector, BRG-1, or BRG-1 and Cyclin E together with pBabe-Puro. After rapid puromycin selection the cells were harvested and analyzed for CD44 expression via immunoblotting. As expected, ectopic expression of BRG-1 in C33A stimulated CD44 expression when compared with vector (Fig. 6, *upper panel*, *lanes 1* and *2*). However, when Cyclin E was cotransfected with BRG-1 in two independent transfections, CD44 expression was inhibited by ~62% as determined after normalization to Cdk4 (Fig. 6, *upper* and *lower panels*, *lanes 1*, *3*, and *4*). Immunoblotting for BRG-1 and Cyclin E confirms their expression while Cdk4 levels were monitored as a loading control (Fig. 6, *middle* and *lower panels*, *lanes 1–4*). These findings show that Cyclin E can antagonize the ability of BRG-1 to stimulate CD44 production.

DISCUSSION

Aberrant expression of CD44 is associated with advanced tumor progression and poor clinical outcome (1–3). However, little is known about what factors are required for maintaining CD44 expression. Here we show that BRG-1 plays a critical role in regulating CD44 transcription. Reintroduction of BRG-1 restored CD44 expression in BRG-1-negative (C33A and SW13) cells, whereas expression of dominant-negative BRG-1 in NIH3T3 cells inhibited CD44 production. Furthermore, ectopic expression of Cyclin E inhibited the ability of BRG-1 to stimulate CD44, consistent with the notion that at least some components of the cell cycle machinery could also mediate CD44 expression. These results indicate that BRG-1 is required for regulating CD44 expression, suggesting that loss of BRG-1 may contribute to the invasive and metastatic potential of certain cancers.

BRG-1 plays a critical role in transcriptional regulation and chromatin remodeling and can both stimulate and repress the

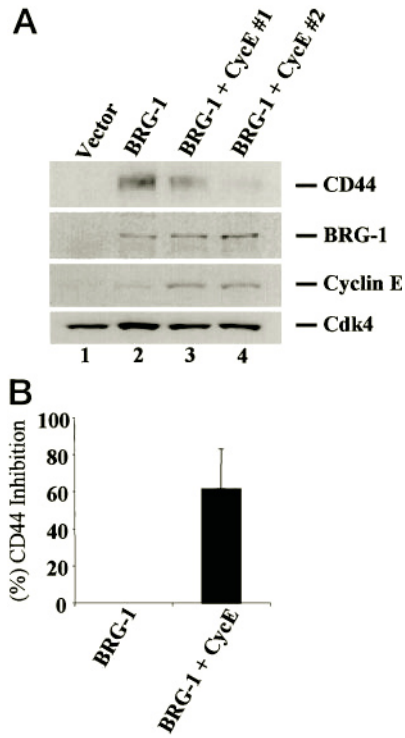

FIG. 6. **Cyclin E antagonizes BRG-1 transcriptional regulation of CD44.** C33A cells were transfected with (1 μg) pBabe-Puro and (15 μg) of vector (*lane 1*), BRG-1 (*lane 2*), or BRG-1 + Cyclin E (*lanes 3* and *4*). Following puromycin selection, equal total protein was resolved by SDS-PAGE and then immunoblotted for CD44, BRG-1, Cyclin E, and Cdk4. The level of CD44 protein was normalized to that of Cdk4. The results are representative of two independent experiments.

transcription of specific genes (22, 26–30, 32). BRG-1 is an ATP-dependent chromatin remodeler that regulates transcription through its ability to modify nucleosome structure (37). Activation of CD44 expression is apparently dependent on chromatin remodeling, because the dominant-negative BRG-1 used in this study specifically disrupts the ATPase activity of BRG-1. In the case of CD44, BRG-1 likely converts the CD44 promoter from a transcriptionally inactive state to an active one. The finding that transiently transfected CD44 reporter constructs maintained high basal activity in the absence of BRG-1 suggests that the plasmid-borne CD44 promoter construct already exists in a transcriptionally active state, thus masking any BRG-1 stimulatory effect at the level of chromatin (data not shown).

The loss of CD44 expression by some tumors correlates with increased metastatic potential (1). The mechanism of CD44 loss in these tumors is largely unknown; however, in prostate carcinomas, CD44 expression is repressed through the methylation of the 5′-CpG islands in the CD44 promoter (40). Interestingly, loss of heterozygosity mapped to the 19p locus (which overlaps with BRG-1) also occurs as a late-stage event in prostate cancer (41). There are numerous ties between transcriptional regulation, chromatin structure, and methylation. Three SWI2/SNF2-like proteins, Mi-2, ATRX, and DDM1, have been implicated in DNA methylation (42, 43). Specifically, DDM1 maintains genomic methylation through its ability to either modify histones or increase the accessibility of DNA methyltransferase. The chromatin-remodeling activity of Mi-2 and ATRX is thought to be targeted specifically to DNA to initiate or maintain methylation-mediated transcriptional control. However, it remains to be determined whether the recruitment of SWI/SNF components to chromatin stimulates methylation or if methylation is causative for the recruitment of specific chromatin-remodeling activities. Therefore, determining whether BRG-1 influences the methylation of the CD44 promoter would be important in defining its mechanism of gene regulation.

The importance of SWI/SNF in cancer is becoming increasingly apparent. Recently, BRG-1 has been linked to breast cancer by the finding that the coactivator function of BRCA1 on p53 transcription is mediated through BRG-1 (30). In addition, it has been shown that BRG-1 is required for RB signaling to specific cell cycle targets (22, 32). Alteration of another SWI/SNF subunit was identified after a genetic screen in malignant rhabdoid tumors, where the *hSNF5/INI1* gene located on the long arm of chromosome 22, is either mutated or deleted in rhabdoid tumors (44, 45). In addition, there is evidence that loss of other SWI/SNF subunits like Baf 47, Baf 57, and Baf 155 occur in a variety of tumors; however, it remains to be determined whether loss of these other subunits affect tumorigenesis or CD44 expression (46).[2] This study shows that BRG-1 is required for enabling CD44 expression and provides a mechanism through which CD44 is lost in cancer. These findings suggest that BRG-1 loss may contribute to the formation of the metastatic phenotype.

Acknowledgments—We thank Dr. Karen Knudsen, Dr. Kenji Fukasawa, Steve Angus, and Yelena Whetherhill for critical reading of the manuscript. We are also grateful to Dr. S. Goff for supplying us with the BRG-1 plasmid and Dr. A. Imbalzano for providing the BO5-1 cells.

REFERENCES

1. Naot, D., Sionov, R. V., and Ish-Shalom, D. (1997) *Adv. Cancer Res.* **71,** 241–319
2. Goodison, S., Urquidi, V., and Tarin, D. (1999) *Mol. Pathol.* **52,** 189–196
3. Sherman, L., Sleeman, J., Dall, P., Hekele, A., Moll, J., Ponta, H., and Herrlich, P. (1996) *Curr. Top. Microbiol. Immunol.* **213,** 249–269
4. Herrlich, P., Morrison, H., Sleeman, J., Orian-Rousseau, V., Konig, H., Weg-Remers, S., and Ponta, H. (2000) *Ann. N. Y. Acad. Sci.* **910,** 106–118; discussion, 118–120
5. Gunthert, U., Hofmann, M., Rudy, W., Reber, S., Zoller, M., Haussmann, I., Matzku, S., Wenzel, A., Ponta, H., and Herrlich, P. (1991) *Cell* **65,** 13–24
6. Rudy, W., Hofmann, M., Schwartz-Albiez, R., Zoller, M., Heider, K. H., Ponta, H., and Herrlich, P. (1993) *Cancer Res.* **53,** 1262–1268
7. Sleeman, J. P., Arming, S., Moll, J. F., Hekele, A., Rudy, W., Sherman, L. S., Kreil, G., Ponta, H., and Herrlich, P. (1996) *Cancer Res.* **56,** 3134–3141
8. Hofmann, M., Rudy, W., Gunthert, U., Zimmer, S. G., Zawadzki, V., Zoller, M., Lichtner, R. B., Herrlich, P., and Ponta, H. (1993) *Cancer Res.* **53,** 1516–1521
9. Jamal, H. H., Cano-Gauci, D. F., Buick, R. N., and Filmus, J. (1994) *Oncogene* **9,** 417–423
10. Lamb, R. F., Hennigan, R. F., Turnbull, K., Katsanakis, K. D., MacKenzie, E. D., Birnie, G. D., and Ozanne, B. W. (1997) *Mol. Cell. Biol.* **17,** 963–976
11. Ladeda, V., Aguirre Ghiso, J. A., and Bal de Kier Joffe, E. (1998) *Exp. Cell Res.* **242,** 515–527
12. Goodison, S., and Tarin, D. (1998) *Front. Biosci.* (*Online*) **3,** E89–E109
13. Kohlberger, P. D., Kieback, D. G., Bancher, D., Stickeler, E., Heinzl, H., Gitsch, G., Breitenecker, G., and Kainz, C. (1997) *Gynecol. Oncol.* **66,** 227–232
14. Saegusa, M., Hashimura, M., Machida, D., and Okayasu, I. (1999) *J. Pathol.* **187,** 173–183
15. Kallakury, B. V., Yang, F., Figge, J., Smith, K. E., Kausik, S. J., Tacy, N. J., Fisher, H. A., Kaufman, R., Figge, H., and Ross, J. S. (1996) *Cancer* **78,** 1461–1469
16. Li, H., Liu, J., Hofmann, M., Hamou, M. F., and de Tribolet, N. (1995) *Br. J. Cancer* **72,** 160–163
17. Karjalainen, J. M., Tammi, R. H., Tammi, M. I., Eskelinen, M. J., Agren, U. M., Parkkinen, J. J., Alhava, E. M., and Kosma, V. M. (2000) *Am. J. Pathol.* **157,** 957–965
18. Meraldi, P., Lukas, J., Fry, A. M., Bartek, J., and Nigg, E. A. (1999) *Nat. Cell Biol.* **1,** 88–93
19. Khavari, P. A., Peterson, C. L., Tamkun, J. W., Mendel, D. B., and Crabtree, G. R. (1993) *Nature* **366,** 170–174
20. Kwon, H., Imbalzano, A. N., Khavari, P. A., Kingston, R. E., and Green, M. R. (1994) *Nature* **370,** 477–481
21. Imbalzano, A. N., Kwon, H., Green, M. R., and Kingston, R. E. (1994) *Nature* **370,** 481–485
22. 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
23. 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
24. Trouche, D., Le Chalony, C., Muchardt, C., Yaniv, M., and Kouzarides, T.

[2] B. Weissman, personal communication.

(1997) *Proc. Natl. Acad. Sci. U. S. A.* **94,** 11268–11273
25. Murphy, D. J., Hardy, S., and Engel, D. A. (1999) *Mol. Cell. Biol.* **19,** 2724–2733
26. DiRenzo, J., Shang, Y., Phelan, M., Sif, S., Myers, M., Kingston, R., and Brown, M. (2000) *Mol. Cell. Biol.* **20,** 7541–7549
27. Fryer, C. J., and Archer, T. K. (1998) *Nature* **393,** 88–91
28. de La Serna, I. L., Carlson, K. A., Hill, D. A., Guidi, C. J., Stephenson, R. O., Sif, S., Kingston, R. E., and Imbalzano, A. N. (2000) *Mol. Cell. Biol.* **20,** 2839–2851
29. Cheng, S. W., Davies, K. P., Yung, E., Beltran, R. J., Yu, J., and Kalpana, G. V. (1999) *Nat. Genet.* **22,** 102–105
30. 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
31. Chen, C., and Okayama, H. (1987) *Mol. Cell. Biol.* **7,** 2745–2752
32. Strobeck, M. W., Fribourg, A. F., Puga, A., and Knudsen, E. S. (2000) *Oncogene* **19,** 1857–1867
33. Knudsen, E. S., and Wang, J. Y. (1997) *Mol. Cell. Biol.* **17,** 5771–5783
34. Knudsen, E. S., Buckmaster, C., Chen, T. T., Feramisco, J. R., and Wang, J. Y. (1998) *Genes Dev.* **12,** 2278–2292
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. 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
37. Muchardt, C., and Yaniv, M. (1999) *Semin. Cell Dev. Biol.* **10,** 189–195
38. Shanahan, F., Seghezzi, W., Parry, D., Mahony, D., and Lees, E. (1999) *Mol. Cell. Biol.* **19,** 1460–1469
39. Sherr, C. J. (2000) *Cancer Res.* **60,** 3689–3695
40. Lou, W., Krill, D., Dhir, R., Becich, M. J., Dong, J. T., Frierson, H. F., Jr., Isaacs, W. B., Isaacs, J. T., and Gao, A. C. (1999) *Cancer Res.* **59,** 2329–2331
41. Nupponen, N. N., Hyytinen, E. R., Kallioniemi, A. H., and Visakorpi, T. (1998) *Cancer Genet. Cytogenet.* **101,** 53–57
42. Gibbons, R. J., McDowell, T. L., Raman, S., O'Rourke, D. M., Garrick, D., Ayyub, H., and Higgs, D. R. (2000) *Nat. Genet.* **24,** 368–371
43. Jeddeloh, J. A., Stokes, T. L., and Richards, E. J. (1999) *Nat. Genet.* **22,** 94–97
44. Versteege, I., Sevenet, N., Lange, J., Rousseau-Merck, M. F., Ambros, P., Handgretinger, R., Aurias, A., and Delattre, O. (1998) *Nature* **394,** 203–206
45. DeCristofaro, M. F., Betz, B. L., Wang, W., and Weissman, B. E. (1999) *Oncogene* **18,** 7559–7565
46. DeCristofaro, M. F., Rorie, C. J., Reisman, D. N., Wang, W., and Weissman, B. E. (2001) *J. Cell. Physiol.* **186,** 136–145