Oncogenesis Caused by Loss of the SNF5 Tumor Suppressor Is Dependent on Activity of BRG1, the ATPase of the SWI/SNF Chromatin Remodeling Complex

Xi Wang,[1] Courtney G. Sansam,[1] Christopher S. Thom,[1] Daniel Metzger,[2] Julia A. Evans,[1] Phuong T.L. Nguyen,[1] and Charles W.M. Roberts[1]

[1]Department of Pediatric Oncology, Dana-Farber Cancer Institute; Division of Hematology/Oncology, Children's Hospital Boston, Department of Pediatrics, Harvard Medical School, Boston, Massachusetts and [2]Institut de Génétique et de Biologie Moléculaire et Cellulaire, Centre National de la Recherche Scientifique, Institut National de la Santé et de la Recherche Médicale, Université Louis Pasteur, Illkirch, France

Abstract

Alterations in chromatin play an important role in oncogenic transformation, although the underlying mechanisms are often poorly understood. The SWI/SNF complex contributes to epigenetic regulation by using the energy of ATP hydrolysis to remodel chromatin and thus regulate transcription of target genes. SNF5, a core subunit of the SWI/SNF complex, is a potent tumor suppressor that is specifically inactivated in several types of human cancer. However, the mechanism by which *SNF5* mutation leads to cancer and the role of SNF5 within the SWI/SNF complex remain largely unknown. It has been hypothesized that oncogenesis in the absence of SNF5 occurs due to a loss of function of the SWI/SNF complex. Here, we show, however, distinct effects for inactivation of Snf5 and the ATPase subunit Brg1 in primary cells. Further, using both human cell lines and mouse models, we show that cancer formation in the absence of SNF5 does not result from SWI/SNF inactivation but rather that oncogenesis is dependent on continued presence of BRG1. Collectively, our results show that cancer formation in the absence of SNF5 is dependent on the activity of the residual BRG1-containing SWI/SNF complex. These findings suggest that, much like the concept of oncogene addiction, targeted inhibition of SWI/SNF ATPase activity may be an effective therapeutic approach for aggressive SNF5-deficient human tumors. [Cancer Res 2009; 69(20):8094–101]

Introduction

SNF5 (SMARCB1/INI1/BAF47) has potent tumor suppressor activity. Specific inactivating mutations in *SNF5* are present in the large majority of malignant rhabdoid tumors (MRT), highly aggressive cancers that strike young children (1, 2). These tumors have a median age of onset of 11 months and most of children die from the disease within 1 year of diagnosis despite the use of intensive therapies. Familial cases occur and arise due to inheritance of a mutant *SNF5* allele, a condition termed the "rhabdoid predisposition syndrome" (3). Biallelic inactivating mutations in *SNF5* with resultant loss of protein expression also occur in epithelioid sarcomas, small cell hepatoblastomas, extraskeletal myxoid chondrosarcomas, and undifferentiated sarcomas (4–7). Loss of SNF5 protein was also recently found to occur in all tested cases of renal medullary carcinoma possibly due to epigenetic silencing (8). Inheritance of mutations predicted to have a milder effect on SNF5 function has recently been identified as the basis of familial schwannomatosis (9). Additionally, moderately reduced expression of SNF5, without mutation, is associated with steroid-resistant refractory acute lymphoblastic leukemia (10).

In mice, homozygous deletion of *Snf5* results in early embryonic lethality, whereas heterozygotes, similar to humans, are predisposed to develop aggressive sarcomas (11–13). Conditional inactivation of Snf5 results in profound cancer susceptibility, with all mice developing cancer at a median of 11 weeks (14). The rapidity with which cancer develops is remarkable for inactivation of a single gene. In comparison, p53 loss leads to cancer at 20 weeks, p19Arf deficiency at 38 weeks, and p16Ink4a deficiency at 60 weeks. Thus, the 11-week median cancer onset shows a potent role for Snf5 in tumor suppression.

SNF5 is a core member of the SWI/SNF chromatin remodeling complex, which is a conserved modulator of chromatin structure. *In vitro*, Swi/Snf complexes are capable of disrupting DNA-histone contacts, but the complexes have also been implicated in larger-scale regulation of chromatin structure *in vivo* (15). Mammalian SWI/SNF complexes consist of at least nine subunits, including either of two mutually exclusive core ATPases, BRG1 or BRM, invariant core subunits including SNF5, BAF155, and BAF170, as well as a variety of lineage-restricted subunits (16, 17). Although the BRG1 or BRM ATPase subunit of the SWI/SNF complex is itself capable of remodeling nucleosomes *in vitro* (18), the function of SNF5 within the complex and the mechanism by which SNF5 loss contributes to oncogenic transformation remains unclear. In yeast, *Snf5* mutants behave similarly to other Swi/Snf mutants and strains carrying mutations in multiple Swi/Snf genes also have similar phenotypes (19), suggesting that inactivation of Snf5 is akin to inactivating the entire complex. In mammals, SNF5 is present in stoichiometric amounts and essentially all SNF5 copurifies with the SWI/SNF complex (20). Further, both Brg1 and Snf5 inactivation in mice lead to early embryonic lethality (11–13, 21). Therefore, it has been hypothesized that oncogenesis in the absence of SNF5 occurs due to loss of function of the SWI/SNF complex. However, this view has been challenged by several findings. First, Snf5 loss leads to effects more frequently associated with oncogene activation than tumor suppressor loss. Loss of function of most tumor suppressors confers either an immediate proliferative advantage or a later selective advantage in primary cells, whereas Snf5 loss leads to cell

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

Requests for reprints: Charles W.M. Roberts, Department of Pediatric Oncology, Dana-Farber Cancer Institute, Mayer 657, 44 Binney Street, Boston, MA 02115. Phone: 617-632-2072; Fax: 617-582-8096; E-mail: charles_roberts@dfci.harvard.edu.

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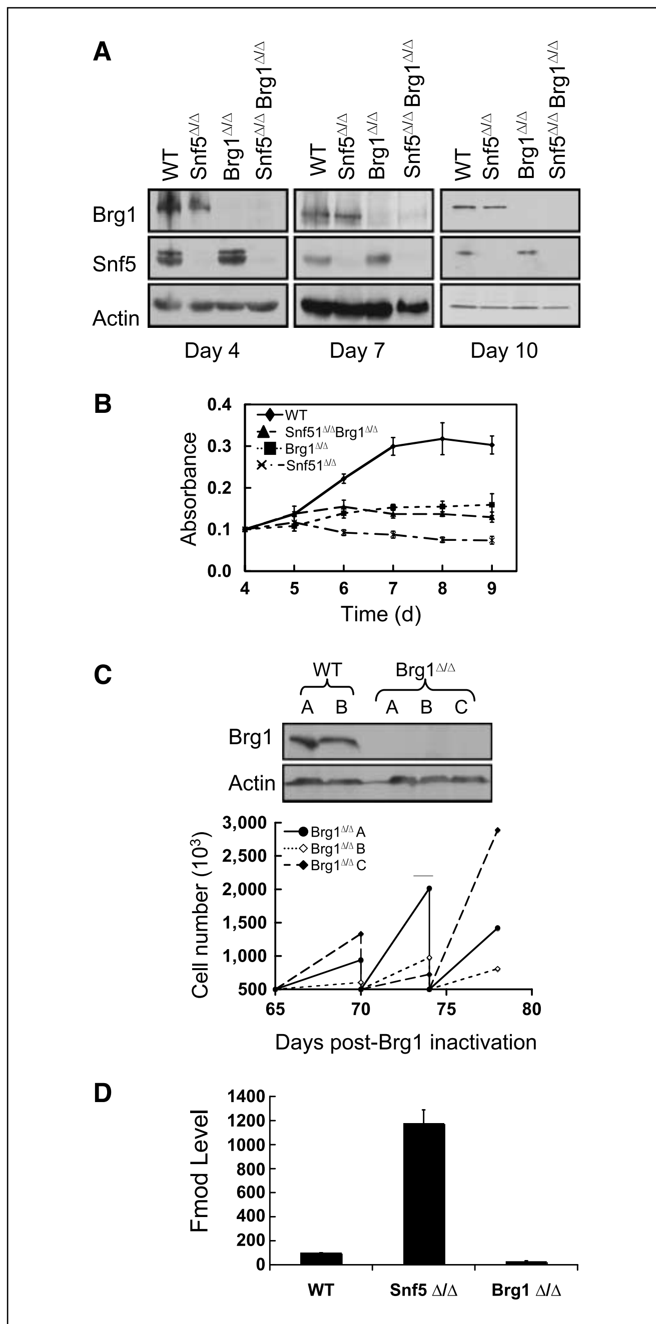

Figure 1. Inactivation of BRG1 and SNF5 have distinct effects on cell survival and gene expression. *A,* immunoblots showing that Snf5 and Brg1 protein are efficiently ablated in *Snf5*$^{fl/fl}$ or *Brg1*$^{fl/fl}$ cells exposed to Cre-recombinase. *B,* following treatment with Cre, proliferation of wild-type (*WT*), Snf5-deficient (Snf5$^{\Delta/\Delta}$), Brg1-deficient (Brg1$^{\Delta/\Delta}$), and Brg/Snf5 doubly deficient cells (Snf5$^{\Delta/\Delta}$ Brg1$^{\Delta/\Delta}$) was monitored. Whereas cells lacking either Brg1 or Snf5 alone underwent growth arrest, combined loss of both Brg1 and Snf5 led to rapid cell death. *C,* primary MEFs can survive and proliferate in the absence of Brg1. MEFs isolated from three *Brg1*$^{fl/fl}$ embryos (*A-C*) were exposed to a retrovirus expressing Cre-recombinase as in *A* and then maintained in selection. MEFs continue to stably proliferate in the absence of Brg1. To establish that MEFs continue to stably proliferate long-term in the absence of Brg1, cells were monitored for proliferation. Representative proliferation data, from the days 65 to 78 time frame, are shown including serial replating on days 70 and 74. Immunoblots of Brg1 and actin at day 80 post-infection is shown to confirm Brg1 absence. *D,* expression of *Fmod* reveals differences in Snf5 loss compared with Brg1 loss. Following adeno-Cre treatment of conditional MEFs, real-time PCR reveals that Fmod expression is increased in Snf5-deficient MEFs but decreased in Brg1-deficient MEFs. Level of expression of Fmod normalized to actin message in three independent experiments ± SE.

cycle arrest and apoptosis (22, 23). These inhibitory effects are more commonly associated with gain-of-function activation of oncogenes. For example, overexpression of *MYC* or mutant *RAS* triggers cell cycle checkpoints and leads to senescence or apoptosis in primary cells (24, 25). Second, although a comprehensive analysis of human tumors looking for *BRG1* and *BRM* mutations has yet to be published, thus far, mutations of *BRG1/BRM* or *SNF5* are correlated with different tumor spectrums and BRG1/BRM mutations have not been detected in MRT (14, 21). Third, BRG1 can associate with at least some other SWI/SNF subunits in the absence of SNF5 and some genes thought to be dependent on BRG1 for expression are expressed in MRT cell lines (26). We therefore hypothesized that SNF5 loss does not equate to inactivation of the SWI/SNF complex but rather results in residual activity of the complex that promotes oncogenic transformation.

Here, we have tested the activities of SNF5 within the SWI/SNF complex. We show, in primary mouse embryonic fibroblasts (MEF) and T cells, that deletion of Brg1 is not redundant to Snf5 loss but instead exacerbates the effects of Snf5 loss. Such synergy between Brg1 loss and Snf5 loss suggests functional activity of the residual Swi/Snf complex in the absence of Snf5. With respect to oncogenesis, if SNF5 loss impaired the function of the complex, it would be predicted that inactivation of BRG1 should either be redundant or even lead to synergistic tumor formation by removing any residual function. In contrast, we find that BRG1 loss is antagonistic to the oncogenesis caused by SNF5 loss. Lastly, we show that Brg1 is essential for tumor formation caused by Snf5 loss *in vivo*.

Materials and Methods

Cell culture. Primary MEFs were harvested and cultured as described previously (22). All cell lines have been in the possession of the Roberts laboratory for several years. All MRT cell lines have been validated as SNF5-deficient and the SW13 lines as BRG1/BRM-deficient by use of immunoblots (Fig. 4).

Antibodies used for Western blot. Anti-Snf5 antibody was purchased from BD Biosciences. Anti-Brg1, BAF155, and BAF170 antibodies were purchased from Santa Cruz Biotechnology. Anti-Brm and anti-actin antibodies were purchased from Abcam.

Fluorescence-activated cell sorting analysis of T cells. Fluorochrome-conjugated monoclonal antibodies against B220 (2D1), CD3ε (145-2C11), CD4 (RM4-5), and CD8α (53-6.7) were purchased from BD Pharmingen. Data were collected using a FACSCalibur cytometer (Becton Dickinson) and analyzed with FlowJo software (Tree Star).

RNA interference and small interfering RNA. The RNA interference (RNAi) construct against BRG1/BRM and the control virus were obtained from Stephen Smale (UCLA). The small interfering RNA reagents were ordered from Dharmacon and the sequences are available upon request.

RNA purification and reverse transcription-PCR. Total RNA was extracted using Trizol reagent (Invitrogen) and reverse-transcribed by the Reverse Transcription System (Promega). Gene expression was normalized to RPS8. Error bars in RNA analysis represent SD of mean expression or fold changes based on at least three independent RNA isolations or as indicated in figure legends. Primer sequences are available upon request.

Results

Residual Swi/Snf activity in the absence of Snf5. We first asked whether loss of Snf5 equates to inactivation of the Swi/Snf complex in primary cells. All Swi/Snf complexes contain one of two mutually exclusive ATPases: Brg1 or Brm. Brg1 is expressed in proliferating cells and is essential for mouse development, whereas Brm tends to be expressed in differentiated cells and is dispensable

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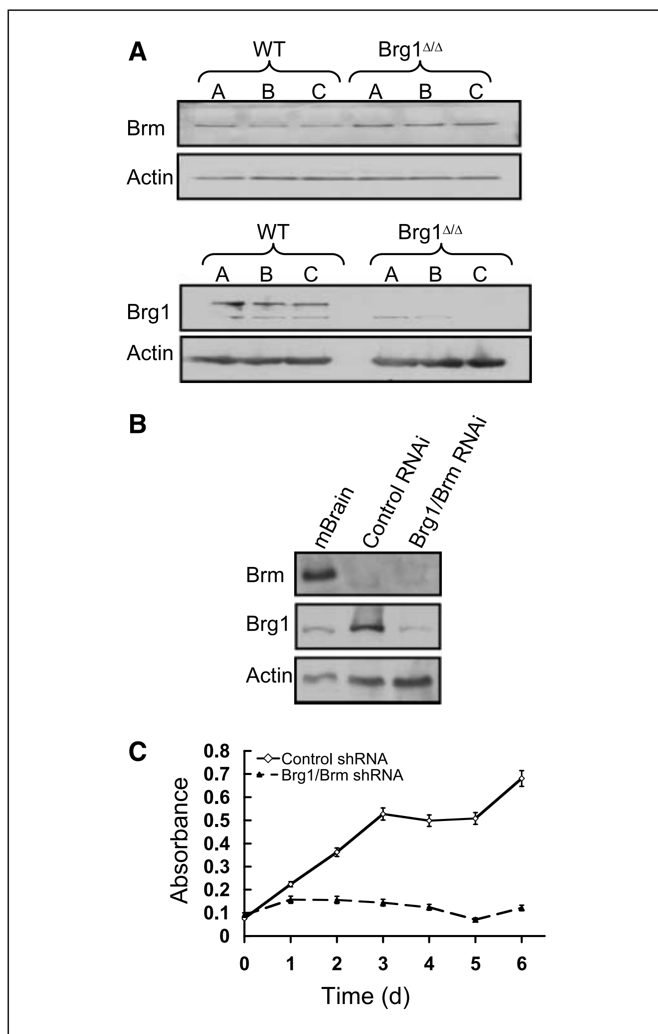

Figure 2. Brm can partially compensate for Brg1 loss in MEFs. *A,* Brm protein levels modestly increase following the knockdown of Brg1 in MEFs. MEFs isolated from three wild-type and three $Brg1^{fl/fl}$ embryos were exposed to a retrovirus expressing Cre-recombinase and under selection for 40 d. Immunoblots of Brm and Brg1 are shown. *B,* MEFs isolated from three wild-type embryos were infected with either a RNAi-green fluorescent protein-puro^r virus against both Brg1/Brm or a control green fluorescent protein-puro^r virus. The following day, green fluorescent protein–positive cells were sorted by flow cytometry and selected in puromycin for 2 d. Immunoblots show that the Brg1/BRM RNAi effectively suppresses Brg1 and Brm expression. Mouse brain was used as a positive staining control. *C,* MEFs deficient for both Brg1 and Brm failed to proliferate and died. Average ± SE of three independent experiments.

for mouse development and survival (21, 27). We evaluated the effects of *Snf5* deletion, *Brg1* deletion, or deletion of both in conditionally targeted primary MEFs (Fig. 1*A*). Loss of either Snf5 or Brg1 was detrimental to proliferation of MEFs (Fig. 1*B*). Both Snf5-deficient and Brg1-deficient cells stopped proliferating beginning 5 days after deletion. Importantly, following several days of arrest, Brg1-deficient cells ultimately reentered the cell cycle and proliferated well (Fig. 1*C*; Supplementary Fig. S1). In contrast, cells deficient in Snf5 never reentered the cell cycle. Although we observed occasional colonies growing on the $Snf5^{fl/fl}$ + Cre plates after several weeks, in all cases, these colonies were derived from cells that had escaped deletion and still expressed Snf5 (data not shown). Differences between Snf5 loss and Brg1 loss were also detectable at the gene expression level. We examined expression of a panel of six randomly selected genes that had been used previously as a validation cohort for genes up-regulated following Snf5 inactivation in MEFs (22). Whereas four of the six genes displayed similar changes following loss of either Snf5 or Brg1, the other two did not. In particular, Fmod was up-regulated 12-fold following Snf5 loss but down-regulated 5-fold following Brg1 loss (Fig. 1*D*).

To investigate the mechanism underlying the differential effect of Snf5 and Brg1 inactivation on proliferation, we examined Brm, the other Swi/Snf ATPase. The proliferation of Brg1-deficient MEFs was accompanied by up-regulation of Brm, suggesting that Brm can partially compensate for Brg1 loss (Fig. 2*A*). Consistent with this observation, MEFs in which Brg1 and Brm were simultaneously inhibited by RNAi were nonviable (Fig. 2*B* and *C*).

Next, we further examined whether Brg1 had functional activity in the absence of Snf5; we deleted Brg1 to determine whether this would have any effect on Snf5-deficient MEFs. Coinactivation of Brg1 had an immediate negative effect on Snf5-deficient MEFs and lead to rapid cell death (Figs. 1*B* and 3*A*). This synergy also occurred in T cells where deletion of Brg1 exacerbated the negative effects of Snf5 loss (Fig. 3*B*).

SNF5-deficient tumor cells are BRG1-dependent. We next evaluated whether BRG1 had a role in tumorigenesis. Whereas BRM is absent in both human cancer cell lines and primary murine tumors, BRG1 protein is readily detectable and binds to other SWI/SNF components in the absence of SNF5 (Fig. 4). We inactivated BRG1 in human SNF5-deficient MRT cell lines using a retroviral-mediated RNAi strategy. This approach results in the stable integration and expression of short RNAi hairpins accompanied by

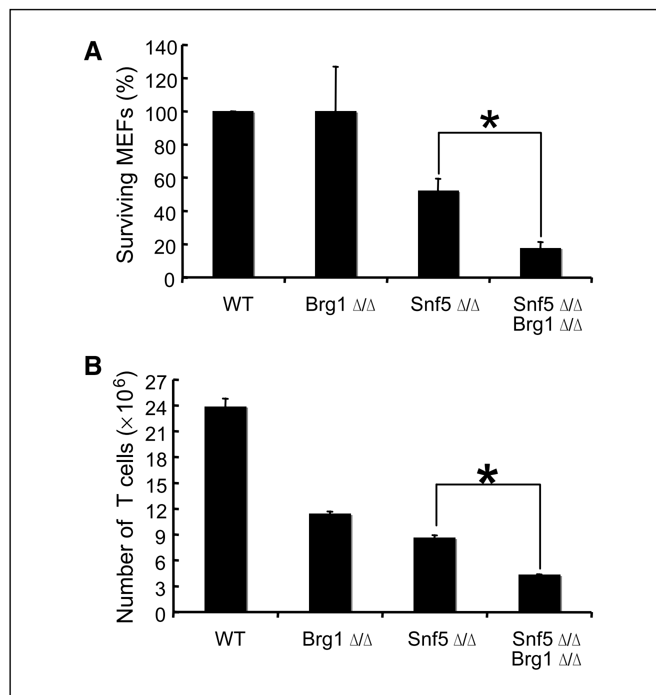

Figure 3. Coinactivation of BRG1 and SNF5 affects cell survival and proliferation more severely than loss of either one alone. *A,* loss of Brg1 exacerbates the negative effects of Snf5 loss on MEF survival. Average ± SE survival from six independent experiments. *, $P < 0.002$. *B,* inactivation of Brg1 in T cells *in vivo* similarly exacerbates the effects of Snf5 loss. Splenocytes were isolated from CD4-Cre $Brg1^{fl/fl}$, CD4-Cre $Snf5^{fl/fl}$, and CD4-Cre $Snf5^{fl/fl}$, $Brg1^{fl/fl}$ mice and $CD3^+$ T cells were counted by flow cytometry. Average ± SE number of cells from four mice of each genotype. *, $P < 0.002$.

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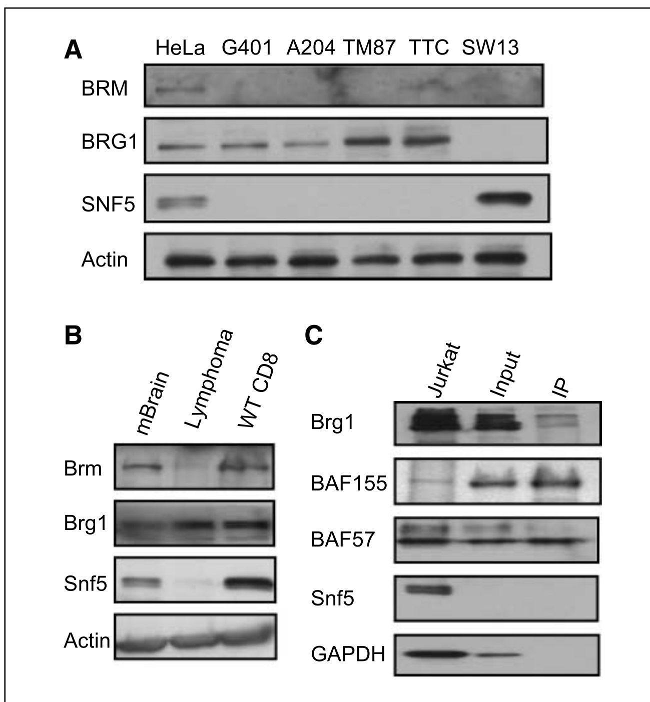

Figure 4. Brg1, not Brm, is present and binds to other Swi/Snf components in Snf5-deficient tumors. *A,* Western analysis of BRG1, BRM, and SNF5 in human MRT cell lines. HeLa cells were used as a positive control for expression. *B,* Western analysis of Brg1, Brm, and a Snf5 in Snf5-deficient murine lymphoma. $CD8^+$ T cells and murine brain (*mBrain*) extracts were used as positive controls. Brg1 is present, whereas Brm is not. *C,* Brg1 binds to other Swi/Snf components in Snf5-deficient lymphomas. Whole-cell extracts from murine Snf5-deficient lymphomas were subjected to Brg1 immunoprecipitation (*IP*) followed by immunoblotting. Jurkat cells were used as positive controls.

expression of a green fluorescent protein reporter and puromycin resistance. To exclude the possibility that up-regulation of BRM might compensate for the loss of BRG1, we used a RNAi designed to target both BRM and BRG1 simultaneously (28).

The RNAi construct efficiently blocked expression of both BRG1 and BRM proteins (Fig. 5*A*). To evaluate function in the absence of SNF5, we quantified the mRNA levels of two genes known to be dependent on BRG1 for expression, *CSF1* and *SPARC* (26, 29). Both genes were expressed in the absence of SNF5 and were down-regulated following the inactivation of BRG1, showing that BRG1 retained activity in the absence of SNF5 (Fig. 5*B*). We next evaluated the effects of BRG1 loss on the proliferation of SNF5-deficient MRT cells and other cell lines. Whereas knockdown of BRG1 had no effect on cell lines SW13 (BRG1/BRM-deficient, SNF5-positive adrenal carcinoma control to rule out off-target effects), HeLa (BRG1/BRM- and SNF5-positive cervical cancer), or MDA231 (BRG1/BRM- and SNF5-positive breast cancer), knockdown in SNF5-deficient MRT cell lines resulted in reduced proliferation (Fig. 5*C*) accompanied by decreased bromodeoxyuridine incorporation, G_1 arrest, and cell death (Fig. 5*D*).

To confirm that the growth arrest effects were not due to off-target toxicity, we transduced G401, A204 (another SNF5-deficient MRT cell line), and SW13 with two different synthetic small interfering RNA against BRG1. Addition of either small interfering RNA led to growth arrest of G401 and A204 but not SW13 cells (Supplementary Fig. S2). Thus, inactivation of BRG1 was not redundant with SNF5 loss but instead had specific synergistic effects on SNF5-deficient cancer cell lines.

Brg1 inactivation prevents the tumor formation following Snf5 loss in mice. To determine whether expression of Brg1 was essential for tumor formation *in vivo*, we turned to our mouse models. We have shown previously that inactivation of Snf5 in mice using the inducible Mx-Cre transgene leads to the rapid onset of lymphomas or rhabdoid tumors (14). Restricting inactivation of Snf5 to the T-cell lineage using the Lck-Cre transgene similarly results in rapid lymphoma onset with complete penetrance.[3] To investigate whether Brg1 played a role in tumorigenesis caused by Snf5 loss, we bred *Brg1*$^{fl/fl}$ to Lck-Cre *Snf5*$^{fl/fl}$ to create three strains: Lck-Cre *Snf5*$^{fl/fl}$, Lck-Cre *Brg1*$^{fl/fl}$, and Lck-Cre *Brg1*$^{fl/fl}$ *Snf5*$^{fl/fl}$. We aged cohorts of mice to monitor tumor formation. As with our previous report, Lck-Cre *Snf5*$^{fl/fl}$ mice were cancer-prone and 100% developed aggressive lymphoma with an early median onset of only 14 weeks (Fig. 6*A*).[3] In contrast, none of the Lck-Cre *Brg1*$^{fl/fl}$ mice developed tumors in their 2-year lifespan (Fig. 6*A*). This all (Snf5)-or-none (Brg1) cancer effect was also observed when we used CD4-Cre transgene to induce loss of Snf5 or Brg1 in T cells (Supplementary Fig. S3).

To evaluate whether Brg1 loss would synergize, antagonize, or have no effect on oncogenesis in the absence of Snf5, we evaluated tumor formation in the Lck-Cre *Brg1*$^{fl/fl}$ *Snf5*$^{fl/fl}$ conditional mice. The presence of the Brg1 conditional alleles markedly impaired tumor formation caused by Snf5 loss. These mice displayed a reduced penetrance (78%) and prolonged onset (27 weeks) compared with the *Snf5*$^{fl/fl}$ mice (Fig. 6*A*). Strikingly, in the tumors that did develop, Brg1 expression was retained at both RNA and protein levels (Fig. 6*B* and *C, left*). Thus, these tumors are derived from rare cells that underwent Cre-mediated recombination at the *Snf5* locus but not at the *Brg1* locus (Fig. 6*D*). This model is further supported by the finding that, in addition to retention of Brg1 in the Lck-Cre model, the cancers from *Snf5*$^{fl/fl}$ *Brg1*$^{fl/fl}$ mice in both CD4-Cre and Mx-Cre models have lost Snf5 but retained Brg1 (Fig. 6*C, right*; data not shown). Thus, oncogenesis in the absence of SNF5 is dependent on BRG1 expression.

Discussion

It has become increasingly clear that epigenetic misregulation plays a critical role in oncogenesis. For example, proteins functioning in DNA methylation, histone modifications, and chromatin remodeling have been extensively implicated in tumorigenesis. By using the energy of ATP hydrolysis to remodel chromatin and modulate transcription, the SWI/SNF complex is a key player in the process of epigenetic regulation. SNF5 and the SWI/SNF complex serve particularly potent roles as tumor suppressors as evidenced by the variety of cancers involved, by the extremely rapid cancer onset that occurs following Snf5 inactivation, and by the fact that both human and murine Snf5-deficient cancers, despite being highly aggressive, frequently metastatic, and rapidly fatal, lack genome instability (30). This latter fact suggests that the epigenetic changes induced by Snf5 loss are extremely potent transforming events and do not require genome instability.

In spite of its potent tumor suppressor activity, the role of SNF5 within the SWI/SNF complex and the mechanism by which SNF5 mutation leads to cancer remain largely unknown. The structure of SNF5 lends little insight into its function. Its most evolutionary

[3] X. Wang, M. Werneck, et al., in preparation.

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conserved feature consists of two imperfect repeat domains, to which protein-protein interactions map, but otherwise lack known function (31). The remainder of the protein also lacks obvious functional domains. Additionally, there are no paralogs of SNF5 in metazoans, thus precluding insight from comparison with related genes. Based on data from yeast in which inactivation of Snf5 results in phenotypes similar to inactivation to the SWI2 ATPase subunit (19) and on data from mice in which both Brg1 and Snf5 inactivation lead to early embryonic lethality (11–13, 21), it has been hypothesized that the function of SNF5 is integral to SWI/SNF function. However, our findings show that both Snf5-deficient MEFs and T cells are further impaired by Brg1 deletion. In addition,

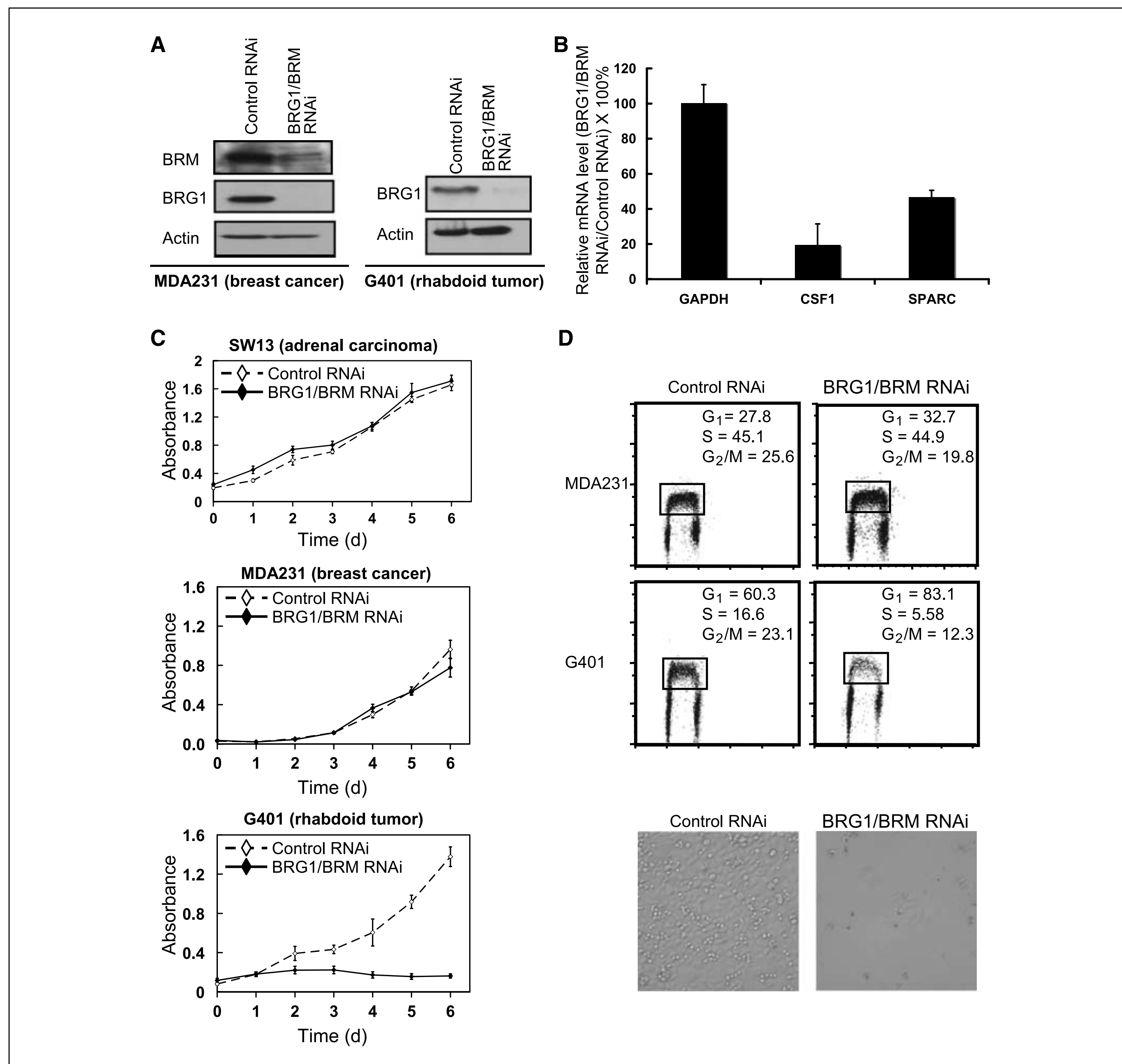

Figure 5. Knockdown of BRG1 is lethal to SNF5-deficient human tumor cell lines. *A,* RNAi against BRG1 and BRM leads to stable knockdown of protein expression. Control SNF5-positive MDA321 breast adenocarcinoma cell lines or SNF5-deficient G401 MRT cell lines were infected with a retrovirus containing a RNAi against BRG1/BRM or with a control retrovirus. Knockdown of BRG1 and BRM was confirmed by Western following infection. BRM expression is not detectable in G401 cells (Fig. 4). *B, CSF1* and *SPARC* remain dependent on BRG1 for expression even in the absence of SNF5. Real-time PCR was used to detect *CSF1* and *SPARC* gene expression following transduction of SNF5-deficient G401 cells with either BRG1/BRM or control RNAi. Level of expression in the presence of BRG1/BRM RNAi compared with control RNAi normalized to *glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*) message in three independent experiments ± SE. *C,* SNF5-deficient MRT cells are dependent on the presence of BRG1 for cell growth. G401 MRT cells or SNF5-positive SW13 and MDA231 cells were infected with either BRG1/BRM or control RNAi and monitored for proliferation. Mean ± SE of four independent experiments. *D, top,* bromodeoxyuridine cell cycle analysis reveals that loss of Brg1 in SNF5-deficient G401 MRT cells, but not control MDA231 breast cancer cells, leads to decreased S ($P = 2 \times 10^{-7}$) and G_2-M ($P = 0.001$) phases accompanied by an increased G_1 phase ($P = 2 \times 10^{-6}$), indicating a G_1 cell cycle arrest. *Bottom,* MRT cells die on BRG1 knockdown. Photomicrographs following infection with either BRG1/BRM RNAi or control vector and 7 d of selection.

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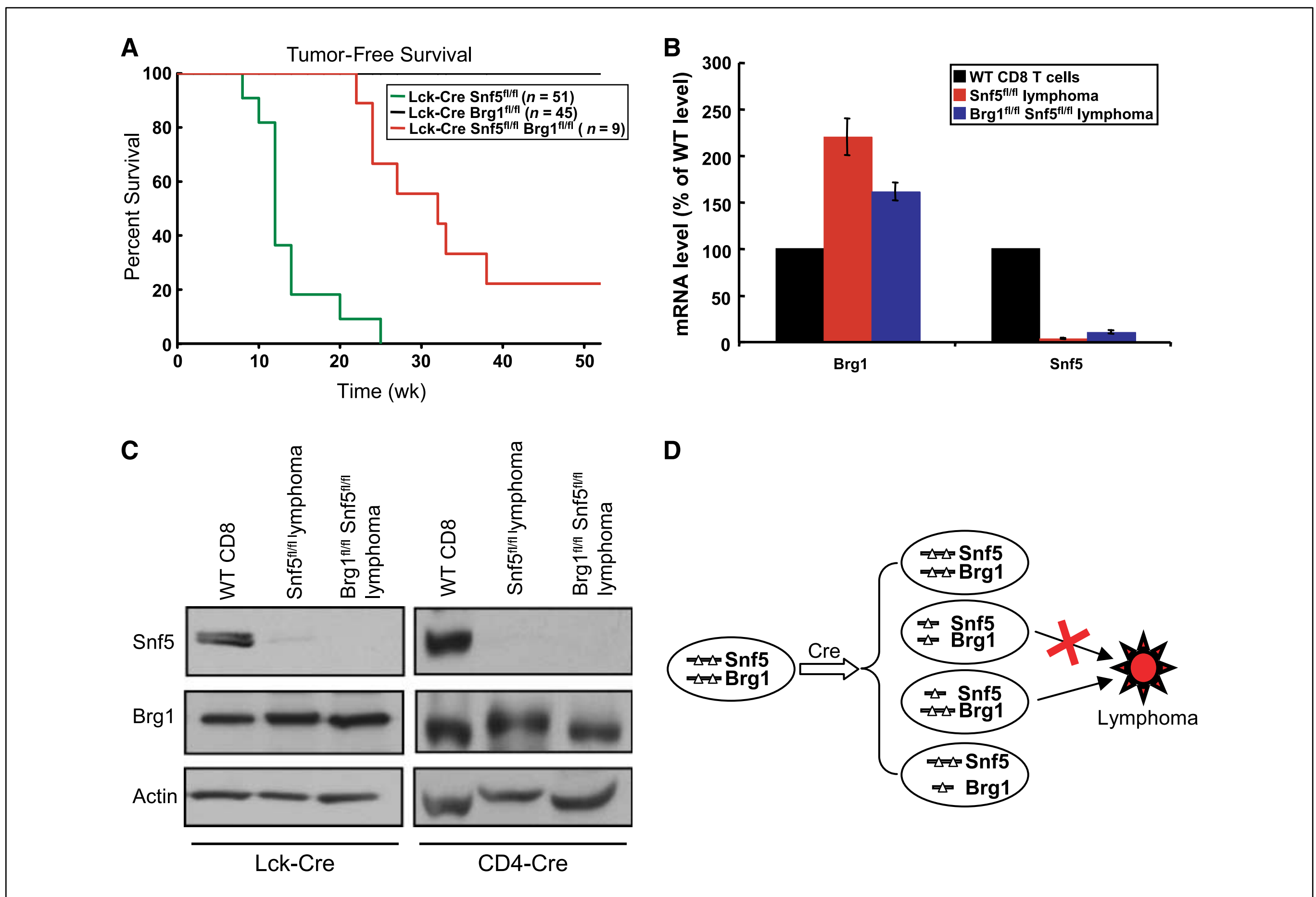

Figure 6. Inactivation of Brg1 blocks tumor formation in Snf5 conditional mice. *A,* tumor-free survival curves of Lck-Cre *Snf5*$^{fl/fl}$ (n = 51), Lck-Cre *Brg1*$^{fl/fl}$ (n = 45), and Lck-Cre *Snf5*$^{fl/fl}$ *Brg1*$^{fl/fl}$ (n = 9) mice. *B,* relative mRNA expression of Brg1 and Snf5 in CD8$^+$ lymphomas obtained from *Snf5*$^{fl/fl}$ or *Snf5*$^{fl/fl}$ *Brg1*$^{fl/fl}$ mice compared with expression in wild-type CD8$^+$ T cells. Whereas Snf5 expression is gone in the lymphomas, Brg1 is maintained. qPCR data are shown as mean ± SE of two independent experiments normalized to RPS8 control message. *C,* immunoblot showing that the lymphomas that ultimately arise in *Snf5*$^{fl/fl}$ *Brg1*$^{fl/fl}$ conditional mice are derived from cells in which Snf5 has been deleted but Brg1 retained. *D,* a model showing that Cre induction in Lck-Cre or CD4-Cre *Snf5*$^{fl/fl}$ *Brg1*$^{fl/fl}$ mice can lead to four possible outcomes: (*a*) neither *Snf5* nor *Brg1* get excised; (*b*) both *Snf5* and *Brg1* get excised; (*c*) only *Snf5*, but not *Brg1*, gets excised; or (*d*) only *Brg1*, but not *Snf5*, gets excised following Cre induction. Our data reveal that lymphoma can only develop from the rare population of cells in which *Snf5*, but not *Brg1*, is excised and inactivated.

knockdown of BRG1 affects the expression of BRG1-depenent genes even in the absence of SNF5 (Fig. 5*B*). Consequently, our results show that Brg1 serves important functional roles in the absence of Snf5.

As attempts are made to develop targeted therapies for cancers in which *SNF5* is mutated, it has not been clear whether a therapeutic goal should be to replace lost function of the SWI/SNF complex or whether to inhibit aberrant function of the residual complex. Our findings address this question by defining relative roles for SNF5 and BRG1 in oncogenesis. Here, we show that knockdown of BRG1 in SNF5-deficient MRT cells leads to cell cycle arrest at G_0-G_1 followed by cell death. Notably, reintroduction of SNF5 into MRT cells similarly leads to G_0-G_1 arrest followed by cell death (32–35). Further, our *in vivo* mouse model revealed that inactivation of Brg1 prevents tumor formation that otherwise occurs in the absence of Snf5. Collectively, these data show that SNF5-deficient cancers are dependent on BRG1. As BRG1 consistently copurifies in a large complex with other SWI/SNF subunits and still interacts with most of these subunits in the absence of SNF5 (26), our data indicate that oncogenesis is driven not by loss of SWI/SNF complex function but rather by aberrant residual activity of the BRG1-containing complex.

In considering a model for cancer formation driven by perturbation of the SWI/SNF complex, it is noteworthy that other SWI/SNF subunits have been linked to tumor suppression. Although data for SNF5 are most definitive, mutations of BAF180 and BAF250 have been identified in subsets of breast cancers and BRM mutations have been found in nonmelanomatous skin cancers (36–38). Specific inactivating mutations in BRG1 itself have been identified in some non–small cell lung, breast, and prostate cancer cell lines and from some primary tumors (39–42). Brg1 heterozygous mice are predisposed to a low rate of mammary tumors, entirely distinct from the tumors caused by Snf5 loss, in which the remaining allele of Brg1 is always retained. This latter finding suggests a haploinsufficient mechanism whereby effects on the complex caused by low, but not absent, levels of Brg1 can contribute to tumor formation in a mammary lineage (43). Lastly, altered expression of several SWI/SNF subunits has been shown to convey prognostic significance in a variety of cancers (10, 39, 44, 45). Consequently, disruption of individual subunits may predispose to cancers within specific lineages. A recurrent mechanistic theme involving genes mutated during oncogenic transformation is that many are master regulators of development. It is therefore

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tempting to speculate that lineage specificity derived from mutation of individual SWI/SNF subunits may be related to the role of the complex in control of lineage-specific fate decisions. Via combinatorial incorporation of lineage-restricted subunits, several hundred variants of the Swi/Snf complex may exist in mammals (46). Mechanistically, incorporation of these variant subunits enables the complex to control differentiation and to determine cellular fate in numerous lineages (46–50). Therefore, based on our data and supported by the distinct tumor spectra associated with individual subunits, we propose a model whereby perturbation, rather than loss, of SWI/SNF function is a highly oncogenic event driven by aberrant residual function.

Consistent with this, SNF5 possesses some uncommon features for a tumor suppressor. In general, inactivation of tumor suppressor genes typically leads to either increased proliferation or a latent selective advantage that can be uncovered by subsequent mutations. In contrast, deletion of Snf5 results in cell cycle arrest and apoptosis in most primary cells (22, 23). This effect is similar to gain-of-function activation of oncogenes such as Myc, Ras, and others that, when activated alone in primary cells, typically trigger checkpoints and block proliferation (24, 25). Intriguingly, despite that mutation of Snf5 leads to extremely rapid cancer formation *in vivo*, the negative effects of Snf5 loss on MEF growth are profound. Consequently, the marked negative effects of Snf5 loss on cell proliferation and viability, even within the T-cell lineage where Snf5 has potent anticancer activity,[3] are highly unusual for a tumor suppressor but are consistent with aberrant activity of the residual Swi/Snf complex predisposing to oncogenic transformation and triggering cell cycle arrest.

Our results establish that SNF5 loss has marked effects on the role of the SWI/SNF complex in gene regulation (22). SWI/SNF-dependent genes can be divided into two groups: SNF5-dependent genes such as *p16INK4A* and *cyclin D1* (33) and SNF5-independent genes such as *CD44* and *CSF1* (Fig. 3*B*; ref. 26). Loss of SNF5 therefore causes imbalance between these two groups. BRG1 inactivation in SNF5-deficient cancers additionally disrupts SNF5-independent targets and this may be the mechanism by which it blocks tumor formation.

Accordingly, deregulation of epigenetic transcriptional regulatory activities of the SWI/SNF complex due to SNF5 loss contributes to cancer initiation. This epigenetic effect may be equivalent to multiple genetic mutations and undergo similar selective processes during tumorigenesis. In this model, SNF5 loss may result in defective regulation of nucleosome positioning and thus give rise to "epigenetic instability" in contrast to genetic instability. Oncogenic clonal selection, driven by the epigenetic state rather than genetic state of a cell, may then explain the emergence of malignant cells from the otherwise widespread death caused by SNF5 loss in normal cells. This situation, dependence of SNF5-deficient tumors on the aberrant activity of the residual SWI/SNF complex, an effect not seen in primary MEFs, is in many ways analogous to the phenomenon of oncogene addiction. Our data suggest that inhibition of residual SWI/SNF activity, perhaps via small-molecule inhibition of the BRG1 ATPase, may have therapeutic benefit for SNF5 mutant cancers.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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