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## Oncogenesis caused by loss of the SNF5 tumor suppressor is dependent upon activity of BRG1, the ATPase of the SWI/SNF chromatin remodeling complex

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### 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 utilizing 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 remains 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 upon continued presence of BRG1. Collectively, our results demonstrate that cancer formation in the absence of SNF5 is dependent upon 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.

### Keywords

SNF5; BRG1; SWI/SNF; tumorigenesis; epigenetics

### 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 (MRTs), 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 one year of diagnosis, despite the use of intensive therapies. Familial cases occur and arise due to inheritance of a

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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, extraskelatal 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 upon 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 while 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 demonstrates 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). While 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 co-purifies 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, while *Snf5* loss leads to cell 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, while a comprehensive analysis of human tumors looking for BRG1 and BRM mutations has yet to be published, so far mutations of BRG1/BRM or SNF5 are correlated with different tumor spectrums and BRG1/BRM mutations have not been detected in MRTs (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 upon 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 (MEFs) 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 demonstrate 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 previously described (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 (Figure 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, anti-Brm and anti-Actin antibodies were purchased from Abcam.

### FACS analysis of T cells

Fluorochrome-conjugated mAbs against B220 (2D1), CD3 $\epsilon$  (145-2C11), CD4 (RM4-5), and CD8 $\alpha$  (53-6.7) were purchased from BD Pharmingen. Data was collected using a FacsCalibur cytometer (Becton-Dickinson) and analyzed with FlowJo software (Tree Star, Ashland, OR).

### RNAi and siRNA

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

### RNA purification and RT-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 standard deviations 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 while Brm tends to be expressed in differentiated cells and is dispensable 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 (Figure 1A). Loss of either Snf5 or Brg1 was detrimental to proliferation of MEFs (Figure 1B). Both Snf5 deficient and Brg1 deficient cells stopped proliferating beginning five days after deletion. Importantly, following several days of arrest, Brg1 deficient cells ultimately re-entered the cell cycle and proliferated well (Figure 1C and Supplemental Figure 1). In contrast, cells deficient in Snf5 never re-entered the cell cycle. Although we observed occasional colonies growing on the Snf5<sup>fl/fl</sup> + 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 6 randomly selected genes that had been previously used as a validation cohort for genes up-regulated following Snf5 inactivation in MEFs (22). While 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 (Figure 1D).

To investigate the mechanism underlying the differential effect of Snf5 and Brg1 inactivation upon 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 (Figure 2A). Consistent with this observation, MEFs in which Brg1 and Brm were simultaneously inhibited by RNAi were non-viable (Figure 2B 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 upon Snf5-deficient MEFs. Co-inactivation of Brg1 had an immediate negative effect upon Snf5-deficient MEFs and lead to rapid cell death (Figure 1B and 3A). This synergy also occurred in T cells where deletion of Brg1 exacerbated the negative effects of Snf5 loss (Figure 3B).

### SNF5 deficient tumor cells are BRG1 dependent

We next evaluated whether BRG1 had a role in tumorigenesis. While 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 (Figure 4). We inactivated BRG1 in human SNF5-deficient MRT cell lines using a retroviral-mediated RNA interference (RNAi) strategy. This approach results in the stable integration and expression of short RNAi hairpins accompanied by expression of a GFP reporter and puromycin resistance. To exclude the possibility that up-regulation of BRM might compensate for the loss of BRG1, we used an RNAi designed to target both BRM and BRG1 simultaneously (28).

The RNAi construct efficiently blocked expression of both BRG1 and BRM proteins (Figure 5A). To evaluate function in the absence of SNF5, we quantified the mRNA levels of two genes known to be dependent upon 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 demonstrating that BRG1 retained activity in the absence of SNF5 (Figure 5B). We next evaluated the effects of BRG1 loss upon the proliferation of SNF5 deficient MRT cells and other cell lines. While knockdown of BRG1 had no effect upon 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 (Figure 5C) accompanied by decreased BrdU incorporation, G1 arrest and cell death (Figure 5D).

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 short interfering RNAs (siRNA) against BRG1. Addition of either siRNA led to growth arrest of G401 and A204, but not SW13 cells (Supplemental Figure 2). Thus, inactivation of BRG1 was not redundant with SNF5 loss but instead had specific synergistic effects upon 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 previously shown 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<sup>4</sup>. To investigate whether Brg1 played a role in tumorigenesis caused by Snf5 loss, we bred Brg1<sup>fl/fl</sup> to Lck-Cre Snf5<sup>fl/fl</sup> to create three strains: Lck-Cre Snf5<sup>fl/fl</sup>, Lck-Cre Brg1<sup>fl/fl</sup>, and Lck-Cre Brg1<sup>fl/fl</sup> Snf5<sup>fl/fl</sup>. We aged cohorts of mice to monitor tumor formation. As with our previous report, Lck-Cre Snf5<sup>fl/fl</sup> mice were cancer prone and 100% developed aggressive lymphoma with an early median onset of only 14 weeks (4 and Figure 6A). In contrast, none of the Lck-Cre Brg1<sup>fl/fl</sup> mice developed tumors in their two-year lifespan (Figure 6A). 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 (Supplemental Figure 3).

To evaluate whether Brg1 loss would synergize, antagonize or have no effect upon oncogenesis in the absence of Snf5, we evaluated tumor formation in the Lck-Cre Brg1<sup>fl/fl</sup> Snf5<sup>fl/fl</sup> 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 to the Snf5<sup>fl/fl</sup> mice (Figure 6A). Strikingly, in the tumors that did develop, Brg1 expression was retained at both the RNA and protein levels (Figure 6B, and left panel of 6C). Thus, these tumors are derived from rare cells that underwent Cre-mediated recombination at the Snf5 locus but not at the Brg1 locus (Figure 6D). This model is further supported by the finding that in addition to retention of Brg1 in the Lck-Cre model, the cancers from Snf5<sup>fl/fl</sup> Brg1<sup>fl/fl</sup> mice in both the CD4-Cre and Mx-Cre models have lost Snf5 but retained Brg1. (Figure 6C, right panel and data not shown). Thus, oncogenesis in the absence of SNF5 is dependent upon 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 utilizing 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 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 to related genes. Based upon data from yeast in which inactivation of Snf5 results in phenotypes similar to inactivation to the SWI2 ATPase subunit (19) and upon data

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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, knockdown of BRG1 affects the expression of BRG1-dependent genes even in the absence of SNF5 (Figure 5B). Consequently, our results demonstrate 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 knock-down of BRG1 in SNF5 deficient MRT cells leads to cell cycle arrest at G0/G1 followed by cell death. Notably, re-introduction of SNF5 into MRT cells similarly leads to G0/G1 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 upon BRG1. As BRG1 consistently co-purifies 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. While data for SNF5 is most definitive, mutations of BAF180 and BAF250 have been identified in subsets of breast cancers and BRM mutations have been found in non-melanomatous 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 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 upon 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 the fact that mutation of Snf5 leads to extremely rapid cancer formation *in vivo*, the negative effects of Snf5 loss upon MEF growth are profound.

Consequently, the marked negative effects of Snf5 loss upon cell proliferation and viability, even within the T cell lineage where Snf5 has potent anti-cancer activity<sup>4</sup>, 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 (Figure 3B and (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 upon 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.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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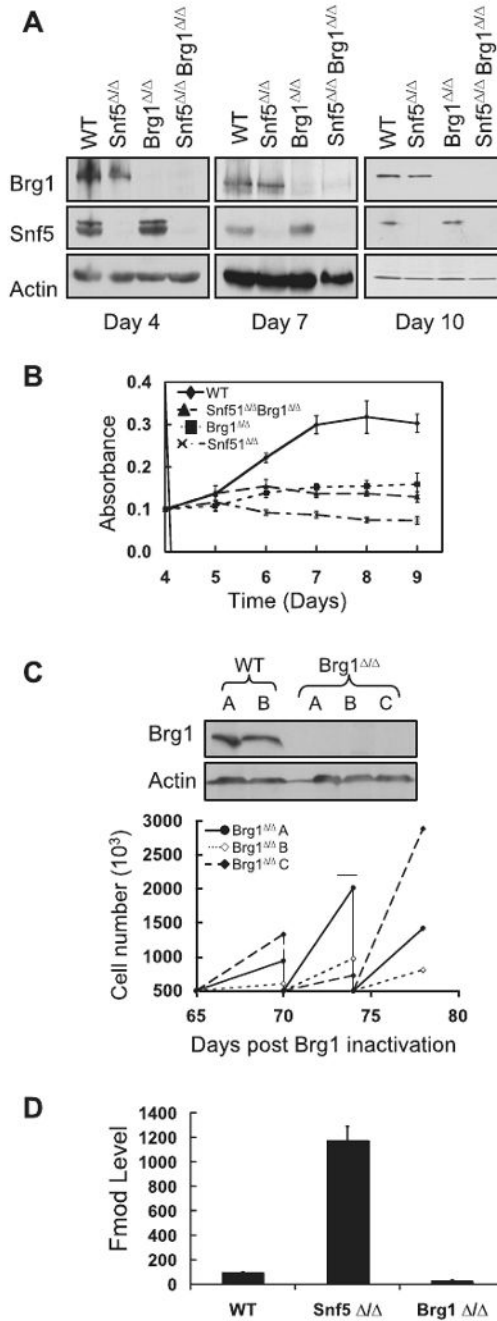
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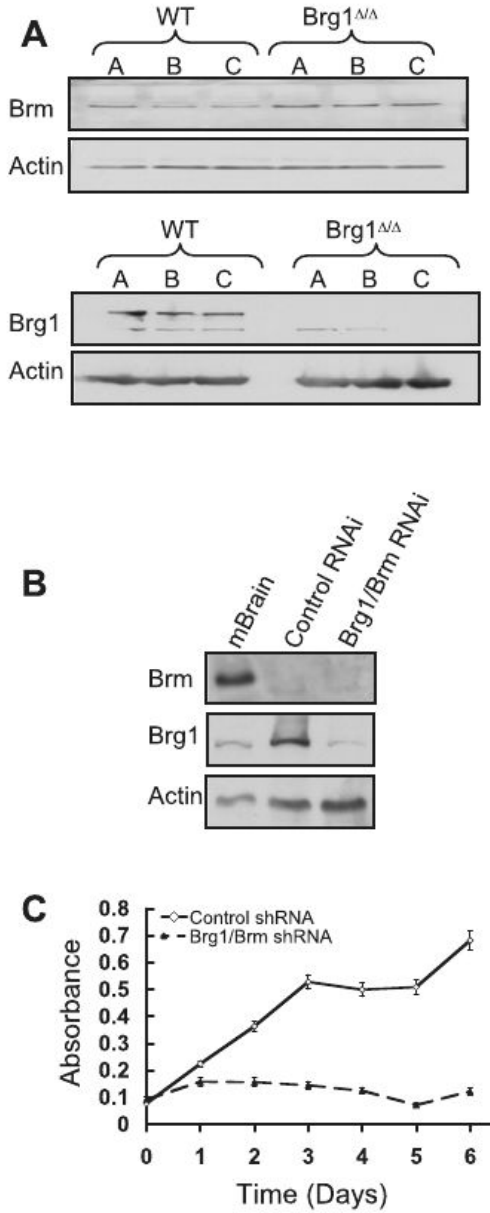
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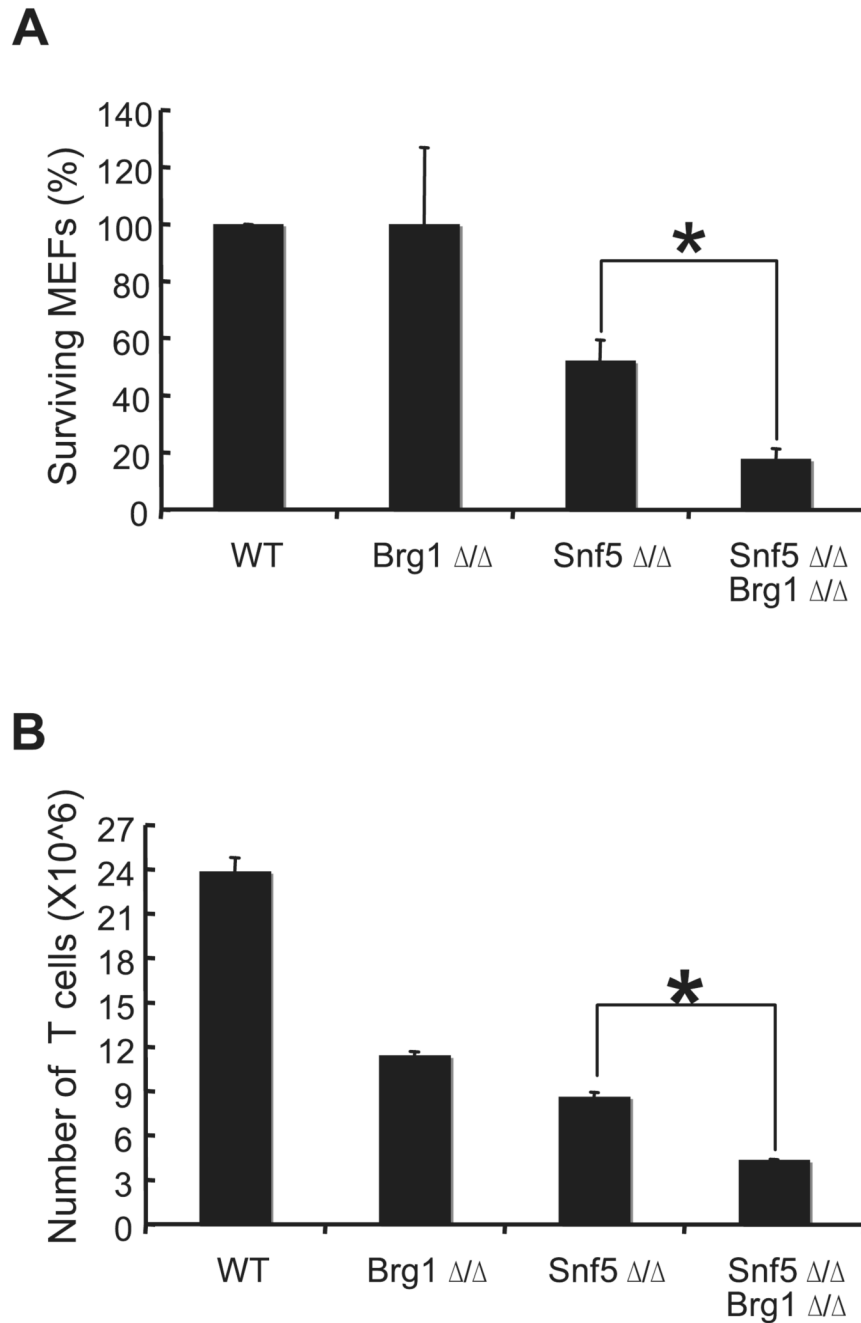
**Figure 1. Inactivation of BRG1 and SNF5 have distinct effects upon cell survival and gene expression**

(A) Immunoblots showing that Snf5 and Brg1 protein are efficiently ablated in Snf5<sup>fl/fl</sup> or Brg1<sup>fl/fl</sup> cells exposed to Cre recombinase. (B) Following treatment with Cre, proliferation of wild-type (WT), Snf5-deficient (Snf5<sup>Δ/Δ</sup>), Brg1-deficient (Brg1<sup>Δ/Δ</sup>), and Brg1/Snf5 doubly-deficient cells (Snf5<sup>Δ/Δ</sup> Brg1<sup>Δ/Δ</sup>) were monitored. While 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<sup>fl/fl</sup> embryos (A, B, C) were exposed to a retrovirus expressing Cre-recombinase as in panel 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, MTT assays were performed on Brg1-deficient and control MEFs. Representative proliferation data, from the day 65 to 78 time frame, is shown including serial re-plating 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 to 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. Data represents level of expression of Fmod normalized to Actin message in three independent experiments  $\pm$  S.E.

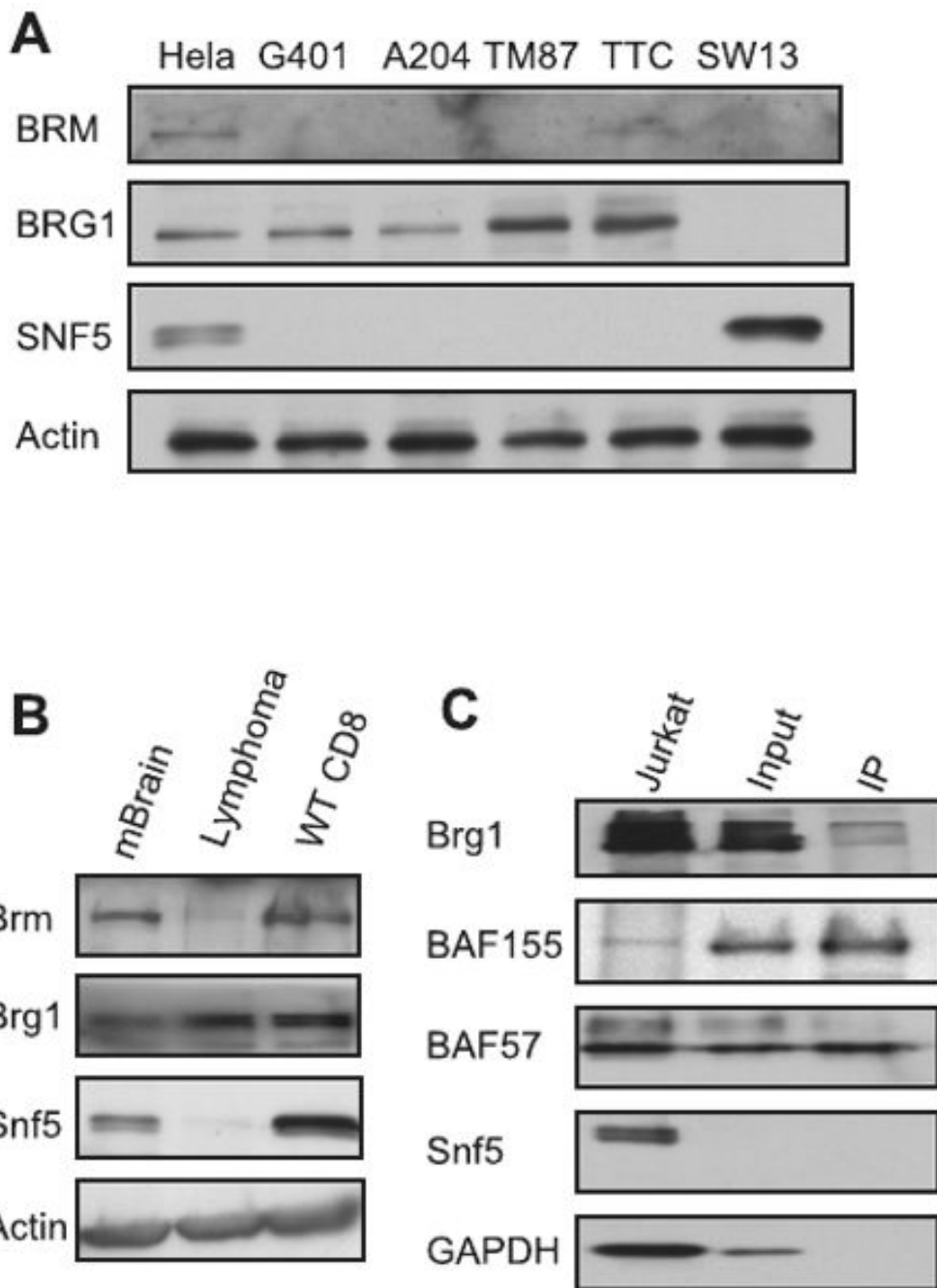


**Figure 2. Brm can partially compensate for Brg1 loss in MEFs**  
 (A) Brm protein levels modestly increase following the knockdown of Brg1 in MEFs. MEFs isolated from three wild-type (WT) and three Brg1<sup>fl/fl</sup> embryos were exposed to a retro-virus expressing Cre-recombinase and under selection for 40 days. Immunoblots of Brm and Brg1 are shown. (B) MEFs isolated from three WT embryos were infected with either an RNAi-GFP-puro<sup>r</sup> virus against both Brg1/Brm or a control GFP-puro<sup>r</sup> virus. The following day, GFP<sup>+</sup> cells were sorted by flow cytometry, and selected in puromycin for 2 days. 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. Graph represents the average of three independent experiments ± S.E.

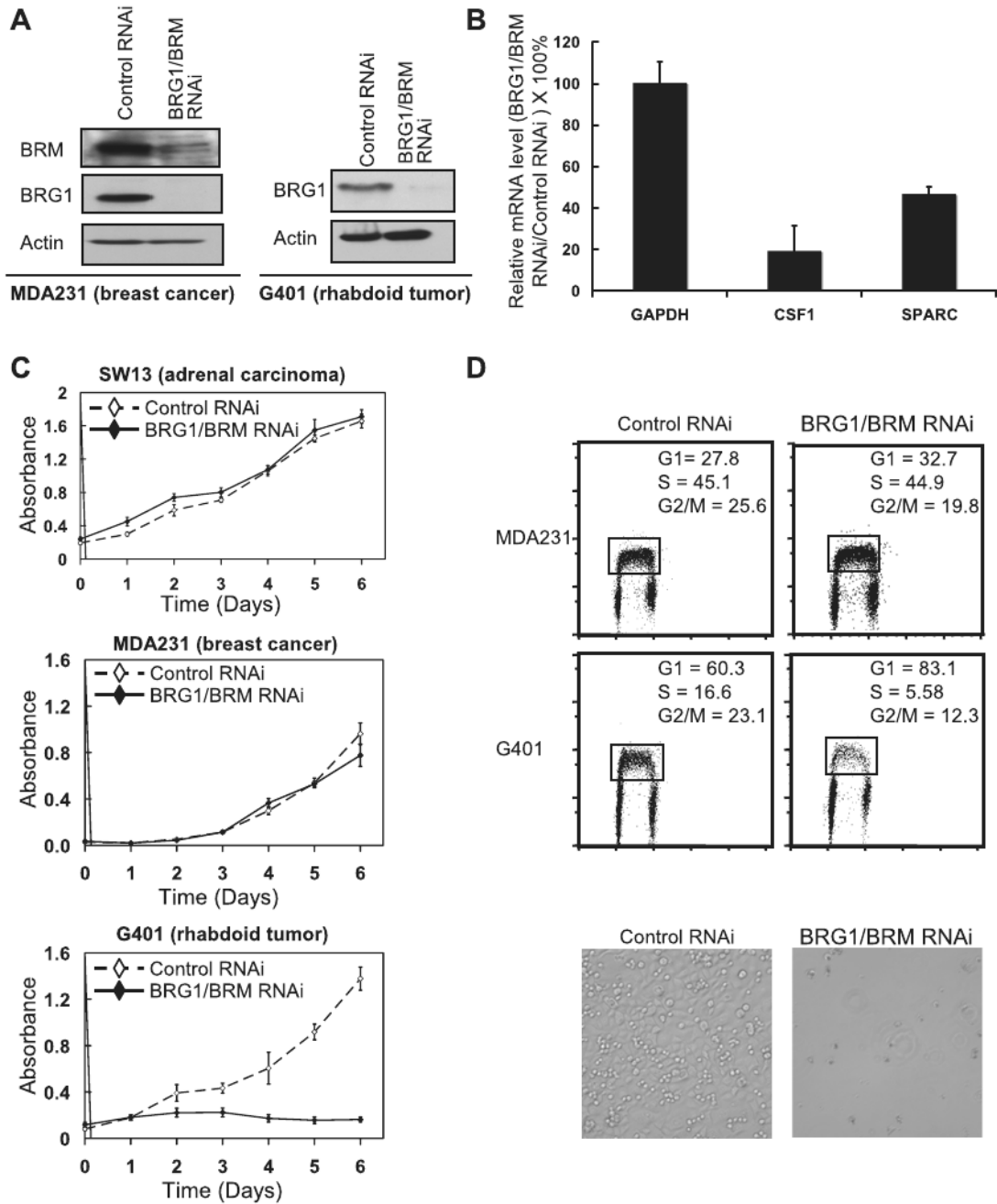


**Figure 3. Co-inactivation of BRG1 and SNF5 affects cell survival and proliferation more severely than loss of either one alone**

(A) Loss of Brg1 exacerbates the negative effects of Snf5 loss upon MEF survival. Graph represents the average survival  $\pm$  S.E. from six independent experiments. The asterisk indicates statistical significance,  $p < 0.002$ . (B) Inactivation of Brg1 in T cells in vivo similarly exacerbates the effects of Snf5 loss. Splenocytes were isolated from CD4-Cre Brg1<sup>fl/fl</sup>, CD4-Cre Snf5<sup>fl/fl</sup>, and CD4-Cre Snf5<sup>fl/fl</sup>, Brg1<sup>fl/fl</sup> mice and CD3<sup>+</sup> T cells were counted by flow cytometry. The graph represents the average number of cells  $\pm$  S.E. from four mice of each genotype. The asterisk indicates statistical significance,  $p < 0.002$ .



**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 extracts were used as positive controls. Brg1 is present while 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 IP followed by immunoblotting. Jurkat cells were used as positive controls.

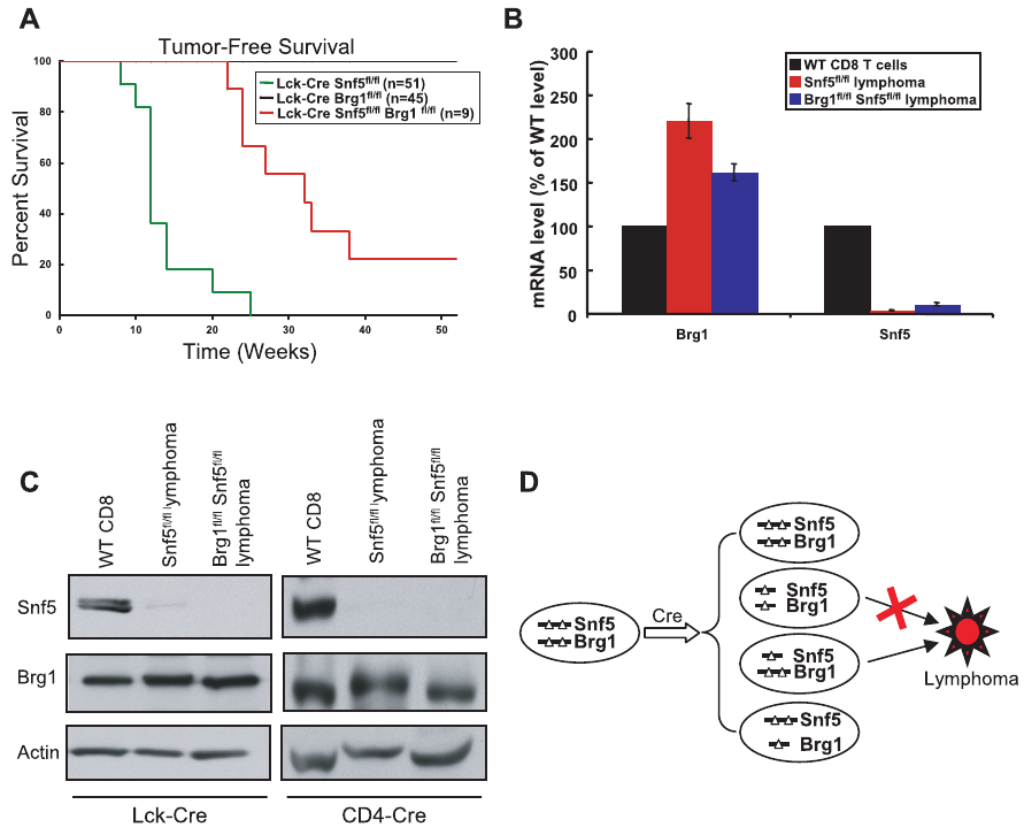


**Figure 5. Knockdown of BRG1 is lethal to SNF5-deficient human tumor cell lines**

(A) RNAi against BRG1 and BRM leads to stable knockdown of protein expression. Control SNF5 positive MDA231 breast adenocarcinoma cell lines or SNF5-deficient G401 MRT tumor cells lines were infected with a retrovirus containing an RNAi against BRG1/BRM or with a control retrovirus. Knock-down of BRG1 and BRM were confirmed by western following infection. BRM expression is not detectable in these cells (Figure 4). (B) CSF1 and SPARC remain dependent upon 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. Data represents level of expression in presence of BRG1/BRM RNAi compared to control RNAi normalized to



GADPH message in three independent experiments  $\pm$  S.E. (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. Data represent the means of four independent experiments  $\pm$  SE. (D) Top panel: BrdU 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 phase ( $p = 2 \times 10^{-7}$ ) and G2/M ( $p = 0.001$ ) phases accompanied by a increased G1 phase ( $p = 2 \times 10^{-6}$ ), indicating a G1 cell cycle arrest. Bottom panel: MRT cells die upon BRG1 knockdown. Photomicrographs following infection with either BRG1/BRM RNAi or control vector and seven days of selection.



**Figure 6. Inactivation of Brg1 blocks tumor formation in Snf5-conditional mice**  
 (A) Tumor-free survival curves of Lck-Cre Snf5<sup>fl/fl</sup> (n=51), Lck-Cre Brg1<sup>fl/fl</sup> (n=45), and Lck-Cre Snf5<sup>fl/fl</sup> Brg1<sup>fl/fl</sup> (n=9) mice. (B) Relative mRNA expression of Brg1 and Snf5 in CD8+ lymphomas obtained from Snf5<sup>fl/fl</sup> or Snf5<sup>fl/fl</sup> Brg1<sup>fl/fl</sup> mice compared to expression in WT CD8+ T cells. While Snf5 expression is gone in the lymphomas, Brg1 is maintained. qPCR data is shown as the mean of two independent experiments ± S.E normalized to RPS8 control message. (C) Immunoblot showing that the lymphomas that ultimately arise in Snf5<sup>fl/fl</sup> Brg1<sup>fl/fl</sup> 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<sup>fl/fl</sup> Brg1<sup>fl/fl</sup> mice can lead to four possible outcomes: 1) neither Snf5 nor Brg1 get excised, 2) both Snf5 and Brg1 get excised, 3) only Snf5, but not Brg1, gets excised, or 4) only Brg1, but not Snf5, gets excised following Cre induction. Our data reveals that lymphoma can only develop from the rare population of cells in which Snf5, but not Brg1, is excised and inactivated.