# Targeted Knockout of *BRG1* Potentiates Lung Cancer Development

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## Abstract

**Brahma-related gene 1 (BRG1) is a catalytic subunit of the switch in mating type/sucrose nonfermentation complex and plays an important role in cancer development. Mouse homozygous knockout experiments testing the role of BRG1 in tumorigenesis have been hampered because BRG1 inactivation is embryonic lethal. To bypass this constraint, we developed a lung-specific conditional knockout of *BRG1* and examined the effect of *BRG1* inactivation in an ethyl carbamate lung carcinogenesis mouse model. We found that the heterozygous loss of BRG1 resulted in increases in both the number and size of tumors when compared with controls. In contrast, when both *BRG1* alleles were inactivated, neither the number nor the size of tumors increased compared with controls. In mouse lung tissue where *BRG1* was homozygously inactivated, immunostaining for apoptotic markers showed significant increase in Apo-BrdUrd and cleaved caspase-3. These data indicate that a loss of cell viability underlies why biallelic inactivation of *BRG1* does not increase tumorigenesis. We also examined mice when exposed to the carcinogen ethyl carbamate and then subjected to BRG1 inactivation. In these cells, loss of BRG1 after carcinogen exposure potentiated tumor development. A subset of tumors retained BRG1 expression, whereas others showed either partial or complete loss of BRG1 expression. Tumors completely devoid of BRG1 expression were significantly larger and expressed higher levels of two markers of proliferation, proliferating cell nuclear antigen and Ki67. Although biallelic inactivation of *BRG1* could not initiate tumor development in untransformed cells, our results indicate that transformation and tumor progression are greatly affected by loss of BRG1.** [Cancer Res 2008;68(10):3689–96]

## Introduction

The yeast switch in mating type (SWI)/sucrose nonfermentation (SNF) complex is one of several chromatin remodeling complexes that have been implicated in the development of human cancer. Yet, its role in tumorigenesis is still largely undefined. The SWI/SNF complex was first discovered through yeast genetic screens that uncovered a set of genes necessary for the two phenotypes: SWI and SNF (1–4). Subsequent studies in both drosophila and mammalian cells have shown that this complex is essential for both cellular differentiation and development. Because SWI/SNF is linked to many important cellular processes, it is not surprising that it is targeted during cancer development. Specifically, it has been shown that the SWI/SNF subunit, *BAF47* (Ini1/SNF5), is a *bona fide* tumor suppressor gene. The loss of this gene is one of the main underlying defects in the development of several different types of sarcomas, in particular, pediatric rhabdoid sarcoma. In these tumors, *BAF47* can be mutated or deleted, it can undergo splicing alteration, and it can be epigenetically silenced (5–8), and mouse knockout experiments show that inactivation of this SWI/SNF subunit is highly tumorigenic (9).

In addition to BAF47, the SWI/SNF complex contains 9 to 12 different subunits that assemble into at least three separate complexes containing one ATPase subunit, either Brahma (BRM) or Brahma-related gene 1 (BRG1; refs. 10–12). These subunits have been linked to cellular processes that oppose cancer development, such as differentiation and growth control (reviewed in refs. 13, 14). As such, their loss will inactivate SWI/SNF complex activity and promote cancer development by abrogating anticancer proteins and pathways that are functionally dependent on SWI/SNF. We have found that these subunits are concomitantly silenced in 30% to 40% of lung cancer cell lines and 10% to 20% of primary lung tumors, as well as in other cancer types (15–17). The SWI/SNF complex is further required for retinoblastoma-mediated growth arrest, and cell lines devoid of both BRG1 and BRM expression show a high degree of resistance to the growth inhibitory effects of exogenous retinoblastoma expression, as well as p16, an upstream activator of retinoblastoma (17–20). Conversely, re-expression of BRG1 is sufficient to restore retinoblastoma-mediated growth inhibition. SWI/SNF also interacts with the retinoblastoma family members p130 and p107. In addition, a number of anticancer proteins, such as BRCA1, p53, and Fanconi anemia protein, have been functionally linked to BRG1 (17, 18, 20, 21), further suggesting BRG1 loss can hasten cancer development.

Re-expression of BRG1 in cell lines lacking its expression results in a slow, progressive growth arrest and a flattened, differentiated phenotype (22). Mice studies also support a role for BRG1 loss in cancer development (23, 24). Heterozygous *BRG1* knockouts have shown that 10% of mice develop tumors within a year. However, experiments directly testing the effect of biallelic *BRG1* loss on cancer development have been hampered because the complete loss of BRG1 expression is embryonic lethal. To circumvent this difficulty, we have developed a lung-specific, conditional BRG1 knockout model system. Our data indicate that heterozygous loss of *BRG1* can serve as an initiating event in cancer development, whereas complete loss of BRG1 can promote tumor progression rather than tumor initiation. This model system provides insight into how loss of BRG1 and inactivation of the SWI/SNF complex can serve to further tumor development and progression.

## Materials and Methods

**Mice.** Green fluorescent protein (GFP) mice (129SF2/J) were purchased from Jackson Labs, and $BRG1^{F+}$ mice were a gift from Pierre Chambon

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

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doi:10.1158/0008-5472.CAN-07-6652

(Institut de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch, France; ref. 25). The CCSP/rtTA/Cre mice were initially a FVB/N background and crossed at least 10 times with 129/Sv background strain. The $BRG1^{F+}$ are of a 129/Sv background as described (1). CCSP/rtTA/Cre was obtained from Jeff Whitsett (Division of Pulmonary Biology, Children's Hospital Medical Center, Cincinnati, OH; ref. 26). These mice were maintained at the University of Michigan Unit of Laboratory Animal Medicine facility, and all experimental procedures were reviewed and approved by the University Committee on Use and Care of Animals at the University of Michigan. Mice were injected once with ethyl carbamate (1 mg/kg i.p.; Sigma). Genotyping for $BRG1^{F+}$ and GFP has been previously described (25). Ten mice were used for both test and control conditions. The tumor size determined by measuring the greatest diameter of each tumor in millimeter.

**Immunohistochemical analysis.** Tissue sections were processed by standard deparaffinization with xylene and hydrated in a descending ethanol series to double-distilled water. Antigen retrieval on tissue section was done using either 10 mmol/L Tris-buffer (pH 10.0) or Sodium-Citrate buffer (pH 6.0). Slides were then incubated with either anti-mouse BRG1 (1:1,000; gift from Pierre Chambon), anti-rabbit GFP (1:1,000; Molecular Probes), anti-rabbit PCNA (proliferating Cell Nuclear Antigen; 1:500; Labvision), anti-rabbit Ki67 (1:300; Abcam), or anti-rabbit cleaved caspase-3 (1:100; Cell Signaling) and stained using the M.O.M (Vector Laboratories) staining kit according to the manufacturer's instructions. Alternatively, deparaffinized lung tissue was labeled with BrdUrd and subsequently probed with anti-mouse BrdUrd antibody using the APO-BrdUrd [immunohistochemical staining (IHC)] kit (Millipore). Finally, sections were counterstained with Harris Hematoxlyin (Fisher), dehydrated, and mounted with Permount (Fisher).

**RNA preparation, reverse transcription-PCR, and quantitative PCR.** DNA and mRNA were respectively extracted using the DNAeasy kit or RNAeasy kits (Quiagen). Reverse transcription-PCR (RT-PCR) was performed using the Superscript III first strand synthesis kit (Invitrogen). DNA was examined for BRG1 rearrangement by semi-quantitative PCR or quantitative PCR using SYBR-green microprobes (Applied Biosystems Foster City) and primers, which flank the loxP BRG1 exons as previously described (15). Primers used for genotyping are as follows: 5′-(LoxP 2)-GCC TTG TCT CAA ACT GAT AAG' and 3′-(LoxP3)'-CGGTGGTCTCAA ACTGAT AGG. Primers used for QPCR are as follows: 5′-(LoxP1)-GATCAGCTC ATG CCCTAA-5′GAPDH-ACAACTTTGGCATTGTGGAA and 3′-GAPDH-AGGG-CTCATGGTATGTAGGC.

## Results

**Conditional inactivation of *BRG1* in the murine lung.** As homozygous inactivation of *BRG1* is lethal to embryos, it has not

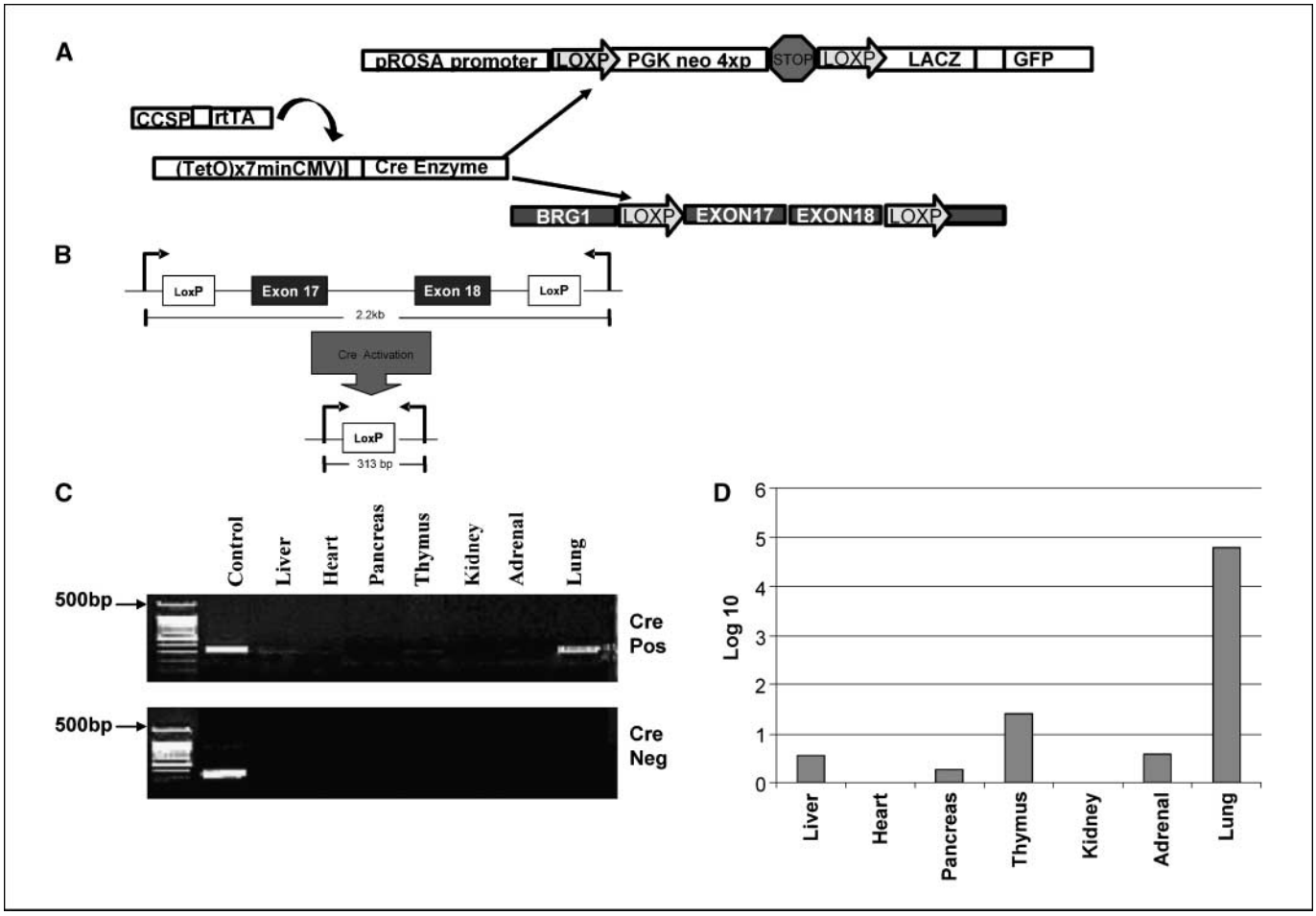



**Figure 1.** *A,* diagram describing the conditional inactivation of BRG1 in the mouse lung. In the presence of tetracycline, the CCSP-rtTA transgene binds tet and then activates the (Tet-O)7-CMV promoter–regulated Cre transgene. The activated Cre then excises the floxed exons 23 and 24 of BRG1 gene. Also, activation of Cre can be detected by a Cre-dependent "knock in" GFP reporter. *B,* in the presence of Cre, two BRG1 exons are excised inactivating BRG1. These changes can be detected by PCR as shown by the location of flanking PCR primers that are normally 2.2 kb apart but become separated by only 313 bp after Cre-mediated rearrangement of BRG1. *C* and *D,* BRG1 rearrangement in various tissues of Cre-positive (*Cre pos*) and Cre-negative (*Cre neg*) mice. $CCSP/rtTA^{+/-}Cre^{+/-}$ $BRG1^{F+/wt}$ mice were treated with Tet for 48 h and then DNA was extracted from various tissues. BRG1 rearrangement was examined by semi-quantitative PCR and by quantitative PCR. *C,* PCR detection of BRG1 rearrangement in various tissues. The presence of a band(s) in lung tissue is confirmatory that BRG1 is being rearranged. *D,* quantitative PCR results for BRG1 rearrangement from each tissue. BRG1 rearrangement is standardized to the GADPH.

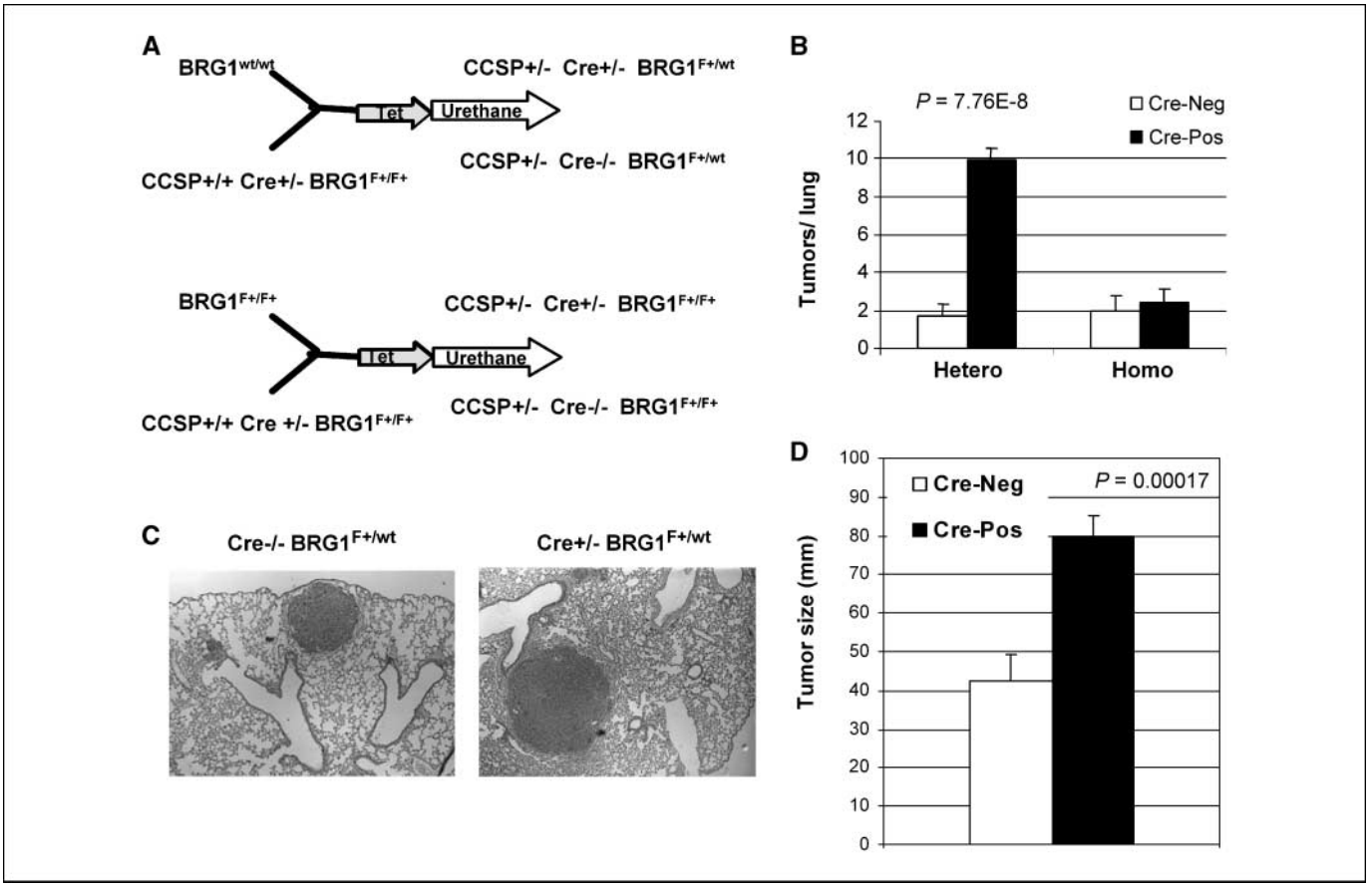



**Figure 2.** *A, top,* the breeding scheme to determine if a single allelic knockout of $BRG1^{F+}$ can potentiate adenoma formation. To determine the effect of heterozygous BRG1 loss on carcinogen-induced lung tumors, pups were treated with Tet for 48 h after birth and then treated with ethyl carbamate at 8 wk. After 12 wk, the number of adenomas in the test ($n$ = 10) versus the control group ($n$ = 10) was examined. *Bottom,* the breeding scheme to determine if biallelic inactivation of BRG1 potentiates tumor development. Pups were treated with Tet for 48 h after birth and then treated with ethyl carbamate at 8 wk. After 12 wk, the number of adenomas was examined in the test ($n$ = 10) and control ($n$ = 10) group. *B,* the relative of surface adenomas observed in test Cre-positive (BRG1 inactivation) and control Cre-negative pups; $n$ = 10; $P$ = 7.67E-8. *Columns,* mean; *bars,* SE. *C,* pictures of the adenomas that arise in the lungs. *D,* with heterozygous inactivation of BRG1, 2-fold larger adenomas were present in Cre-positive than in Cre-negative ($P$ = 0.0017; $n$ = 13); *Columns,* mean; *bars,* SE.

been possible to examine how the loss of this gene might promote cancer development. We therefore developed a lung-specific, conditional murine knockout model. In this system, the transgenic lung-specific promoter Clara cell–secreted protein (CCSP) is linked to the reverse tetracycline transactivator (rtTA) gene (Fig. 1*A*), and the multiple rtTA binding sites adjoining a minimal cytomegalovirus (CMV) promoter $(TetO)_7$-CMV. This promoter is linked to the *Cre* transgene, allowing temporal and lung-specific activation of Cre recombinase (Fig. 1*A*; refs. 26–28). To specifically target *BRG1* inactivation, we used transgenic mice carrying the Cre recognition site, LoxP, flanking the murine BRG1 exons 23 and 24 (Fig. 1*B*; ref. 25). As these exons are essential for the ATPase catalytic activity of BRG1, their Cre-mediated excision results in loss of BRG1 function (29, 30). For all experiments, we used mice that were homozygous for CCSP/rtTA and $BRG1^{F+}$, but heterozygous for Cre, which we designated as "CCB" mice.

To determine whether *BRG1* can specifically be inactivated in the lung, CCB mice were crossed with mice that carry a Cre-activated GFP reporter gene. All resultant pups from this breeding were positive for CCSP/rtTA, $BRG1^{F+}$, and $GFP^{F+}$ but half lacked the *Cre* transgene. In this way, we specifically bred a control group (Cre-negative) and a test group (Cre-positive). The pups were treated with tetracycline through the mothers' drinking water for 48 h, and the lungs were removed 24 h later. Lung-specific activation of *Cre* was confirmed by indirect IHC for GFP in formalin-fixed, paraffin-embedded tissue (Supplementary Fig. S1). To further determine the specificity of this system, we also examined Cre-mediated $BRG1^{F+}$ allelic rearrangement in various tissues. Tet-induced BRG1 rearrangement was detectable by RT-PCR in the lungs of Cre-positive mice, with lower levels observed in the pancreas and thymus. In contrast, *BRG1* rearrangement was absent in all tissues from Cre-negative mice (Fig. 1*C*). By quantitative PCR, using the same primers, *BRG1* rearrangement in Cre-positive mice was between $1 \times 10^3$ and $1 \times 10^5$ more abundant in the lung when compared with other tissues (Fig. 1*D*). Consistent with these PCR results, Cre-induced GFP expression was detected by immunostaining in the lungs, although not in the heart, kidney, brain, liver, adrenal, intestine, or spleen (data not shown).

**Heterozygous *BRG1* inactivation promoted tumor development.** To investigate the effect of *BRG1* inactivation on cancer development, we used an ethyl carbamate carcinogen mouse model. To examine the effect of heterozygous *BRG1* loss in lung tumor development, CCB mice were crossed with outbred $BRG1^{wt/wt}$ mice (129s/v background), as shown in Fig. 2*A*. The resultant pups were heterozygous for CCSP/rtTA and floxed BRG1, and either Cre

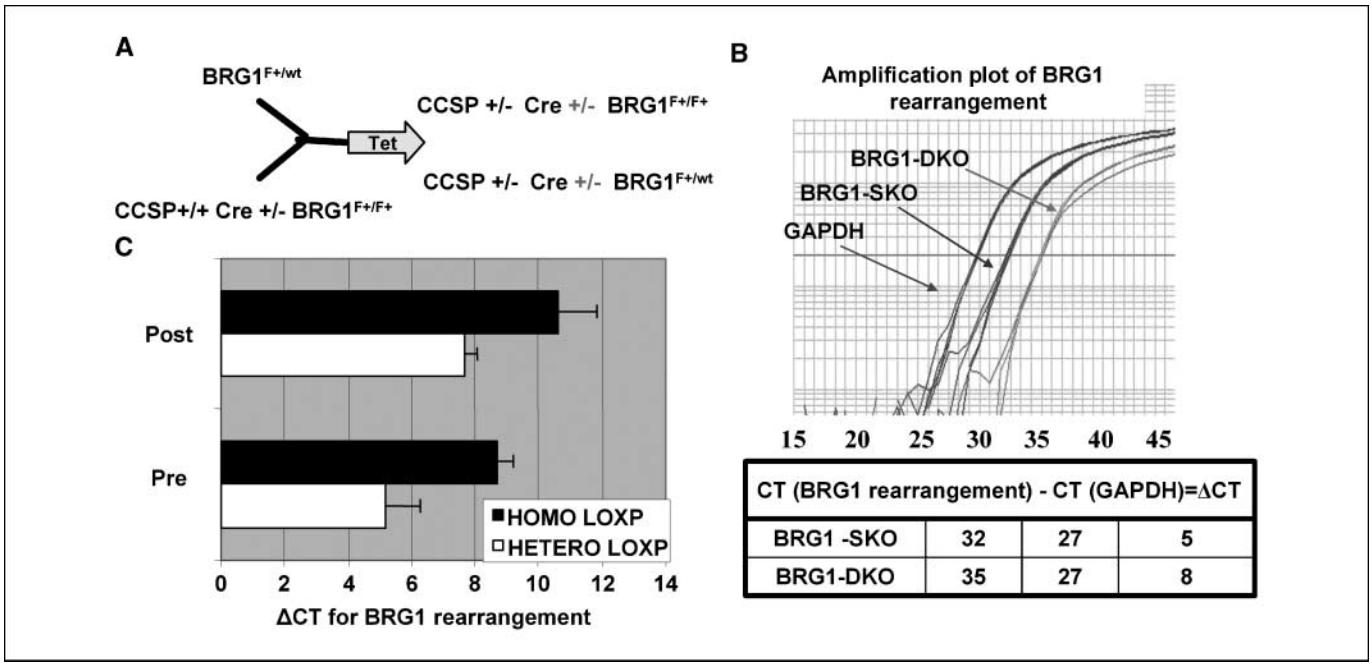
positive or Cre negative (Fig. 2*A, 1*). These pups were treated at birth with Tet for 48 hours to heterozygously inactivate BRG1 and then, at ages 8 weeks, were treated once with the lung-specific carcinogen ethyl carbamate. Pairs of test and control mice were sacrificed every 4 weeks until we began to detect adenomas in the control mice. At this time, we examined the number of adenomas visible on the surface of the lungs in both test and control mice. Control mice (Cre negative) had low numbers of adenomas (~2 per mouse), whereas the Cre-positive BRG1 heterozygous mice had 5-fold more adenomas (~10 per mouse; Fig. 2*B*). Further examination revealed that adenomas from the Cre-positive mice were also ~2-fold larger ($P$ = 0.00017) than the adenomas arising in the control (Cre negative) mice (Fig. 2*A, 1* and *D*). However, comparing Cre-positive and Cre-negative mice revealed no difference in histologic appearance or differentiation status. To confirm that these changes were due to *BRG1* inactivation, we isolated DNA from adenomas that arose in both the Cre-positive and Cre-negative mice. PCR analysis revealed that *BRG1* rearrangement was detected in the majority of tumors (70%) from Cre-positive mice, whereas the tumors of Cre-negative mice were devoid of *BRG1* rearrangement. Ten mice were in each group, which had a $P$ value of 7.67E-8. These data indicate that single allelic loss of *BRG1* can promote tumor development (data not shown).

**Biallelic loss of *BRG1* in untransformed cells did not potentiate adenoma development.** Next, we investigated whether homozygous, such as heterozygous, loss of BRG1 could also potentiate adenoma development in this murine model system. For these experiments, CCB mice were crossed with mice that were homozygous for the floxed *BRG1* allele. This breeding resulted in pups that were heterozygous for the CCSP/rtTA transgene, homozygous for the floxed *BRG1* allele, and either Cre positive (test group) or Cre negative (control group; Fig. 2*A, 2*). Similar to *BRG1* heterozygous knockout experiments, we administered Tet for 48 hours and then treated the mice once with ethyl carbamate at 8 weeks. To monitor the development of adenomas in these mice, we sacrificed 2 mice from both the test and control groups every 4 weeks. Unlike the previous experiment, where inactivation of a single *BRG1* allele resulted in a ~5-fold increase in the number of adenomas 12 weeks postcarcinogen exposure, we observed no significant increase in the number of adenomas after homozygous inactivation of *BRG1* in this test group (Fig. 3*A*). Instead, ~2 to 3 adenomas per mouse were observed in both the test and control groups 3 months postethyl carbamate exposure. After an additional 3 months, or 6 months post carcinogen exposure, the average number of adenomas in both the control and test groups slightly increased over this 3-month interval (from ~2 per mouse to ~5 per mouse) in both groups. Hence, this increase in the test group was not statistically different from the control group ($n$ = 10 for each group).

**Homozygous inactivation of *BRG1* adversely affected untransformed cells.** That heterozygous but not homozygous inactivation of *BRG1* readily potentiated adenoma development in this model system seemed contradictory. However, previous work has shown that biallelic inactivation of *BRG1* in certain cell types may affect cellular proliferation or viability. Moreover, homozygous inactivation of *BAF47*, another SWI/SNF subunit, has also been reported to induce apoptosis in nontransformed cells (9). Hence, we surmised that biallelic Cre-mediated inactivation of *BRG1* may adversely affect normal untransformed lung cells.

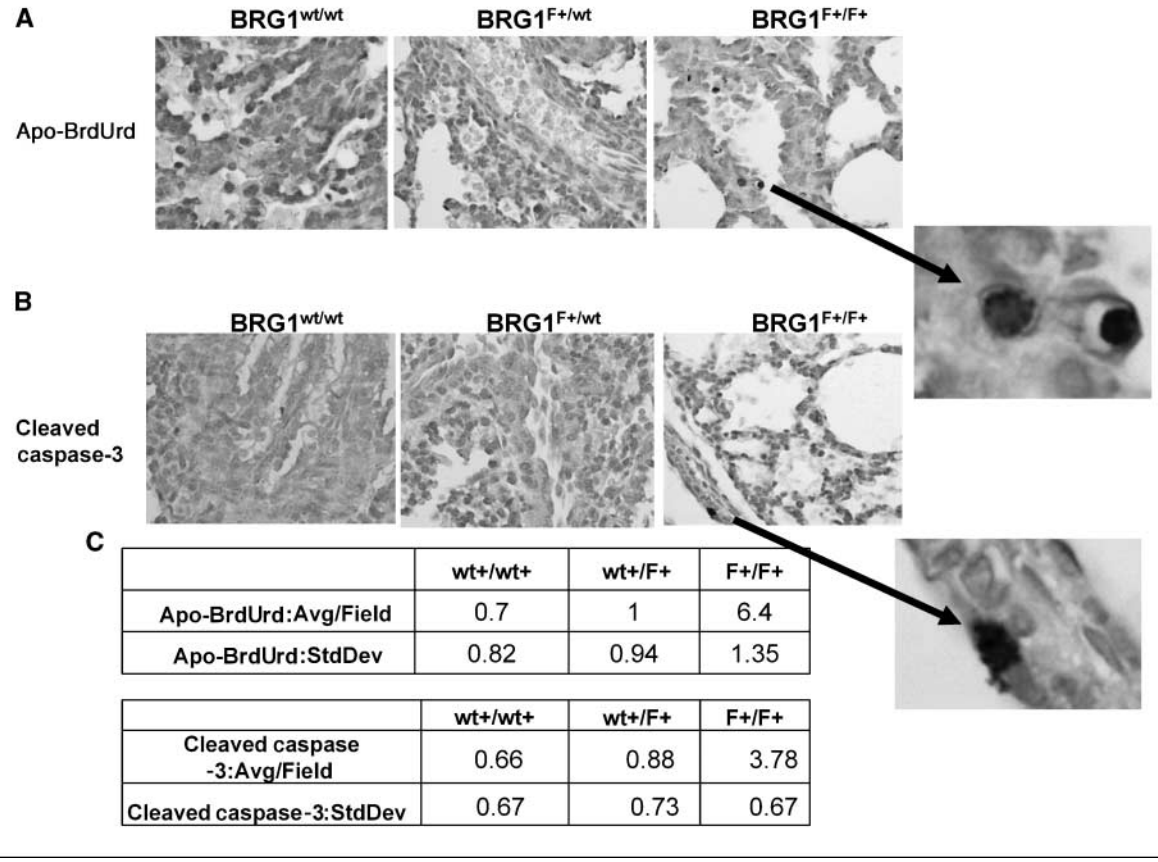
To test this hypothesis, we compared the relative amount of *BRG1* recombination that occurs in $BRG1^{F+/wt}$ (heterozygous) versus $BRG1^{F+/F+}$ (homozygous) pups. Cre-mediated rearrangement of floxed BRG1 should be 1:2 in $BRG1^{F+/wt}$ versus $BRG1^{F+/F+}$






| CT (BRG1 rearrangement) - CT (GAPDH)=ΔCT | | | |
|---|---|---|---|
| BRG1 -SKO | 32 | 27 | 5 |
| BRG1-DKO | 35 | 27 | 8 |

**Figure 3.** *A,* the breeding scheme to determine the effect of biallelic BRG1 inactivation. Pups were given tetracycline at birth and then assayed for BRG1 rearrangement. *B,* BRG1 recombination was compared between heterozygous and homozygous floxed BRG1 pups by quantitative PCR. *C,* the effect on BRG1 recombination was compared when Tet was given either prenatal (*pre*) or postnatally (*post*); in both cases heterozygous BRG1 pups show more robust rearrangement compared with the mice carrying the homozygous floxed BRG1 alleles. Ten mice were used per group for each experiments. *Columns,* mean; *bars,* SE; experiments were done in triplicate.




C

| | wt+/wt+ | wt+/F+ | F+/F+ |
|---|---|---|---|
| Apo-BrdUrd:Avg/Field | 0.7 | 1 | 6.4 |
| Apo-BrdUrd:StdDev | 0.82 | 0.94 | 1.35 |

| | wt+/wt+ | wt+/F+ | F+/F+ |
|---|---|---|---|
| Cleaved caspase -3:Avg/Field | 0.66 | 0.88 | 3.78 |
| Cleaved caspase-3:StdDev | 0.67 | 0.73 | 0.67 |

**Figure 4.** Lungs derived from BRG1 wild-type (wt), heterozygous knockout, or homozygous knockout were immunostained for Apo-BrdUrd (*A*) and cleaved caspase-3 (*B*). Little or no staining is shown in lungs of the wild-type and heterozygous BRG1 knockout. Both Apo-BrdUrd (*A*) and cleaved caspase-3 (*B*) is shown in the lung of homozygous BRG1 knockout mice. *Black arrow,* high-power views that reveals the cellular staining. These data are quantitated and shown in *C*.

mice. However, if biallelic inactivation of *BRG1* adversely affects cells, we expected to observe a far greater degree of rearrangement in the heterozygous than in the homozygous floxed *BRG1*. We thus generated littermates that contained the CCSP/rtTA and Cre transgenes but that were either homozygous or heterozygous for the floxed BRG1 allele (Fig. 4*A*). The newborn pups were either sacrificed at birth after 48 to 72 hours of *in utero* treatment or were treated for 48 hours postbirth and then sacrificed. Their lungs were then removed and examined for the degree of *BRG1* recombination. By quantitative PCR, we consistently observed about a 3-cycle difference or about an 8-fold greater rearrangement in the heterozygous versus homozygous floxed *BRG1* mice (Fig. 3*B*). Similarly, inactivating *BRG1 in utero* versus postnatally yielded an 8-fold greater *BRG1* rearrangement in heterozygous floxed *BRG1* pups (see Fig. 3*C*). Even when the duration or timing of Tet exposure was varied, heterozygous floxed *BRG1* mice ($BRG1^{F+/wt}$) showed 4-fold greater *BRG1* rearrangement. Thus, biallelic inactivation of *BRG1* occurs at less than the expected frequency. To further investigate this phenomenon, we stained these lung tissues with markers of apoptosis—specifically, we stained for both Apo-BrdUrd and cleaved caspase-3, well-known markers of apoptosis. Both Apo-BrdUrd and cleaved caspase-3 staining was detected in cells in the homozygous *BRG1* floxed pups ($BRG1^{F+/F+}$) but much less in the heterozygous *BRG1* floxed pups ($BRG1^{F/wt}$) and the wild-type ($BRG1^{wt/wt}$; Fig. 5*A* and *B*). At high power, these stained cells show morphologic signs of apoptosis: nuclear fragmentation and cellular clumping. These data indicate that biallelic inactivation of *BRG1* can induce apoptosis in certain untransformed cell types.

**Inactivation of *BRG1* after carcinogen exposure induced lung adenomas.** Although homozygous inactivation of *BRG1* adversely affects nontransformed cells, complete loss of BRG1 activity is not uncommon in lung cancer cells. We reasoned that because tumor cell lines, such as SW13, H522, and A427, as well as primary lung tumors, seem to tolerate the absence of BRG1 expression, inactivation of *BRG1* may not be able to initiate tumor development. Instead, loss of *BRG1* may serve to accelerate tumor development in cells that have begun the transformation process.

To test this hypothesis, we bred test and control mice as before but reversed the order in which we administered Tet and ethyl carbamate to the mice (Fig. 5*A*). Specifically, we first administered ethyl carbamate to the mice at 8 weeks and waited 10 weeks until adenomas were just detectible in these mice. At this juncture, we administered Tet to the mice to homozygously inactivate *BRG1*. After an additional 12 weeks, the control mice showed only a slight increase in the number of tumors visible on the surface of the lungs, from an average of 2 per mouse to 4 per mouse. In contrast, there was a significant increase in adenomas ($n = 10$) observed in the Cre-positive mice; ~20 adenomas were visible on the surface the lungs of the Cre-positive test mice (Fig. 5*B*).

To confirm that BRG1 expression had been extinguished, we immunostained for BRG1 in a subset of these lungs. Using a

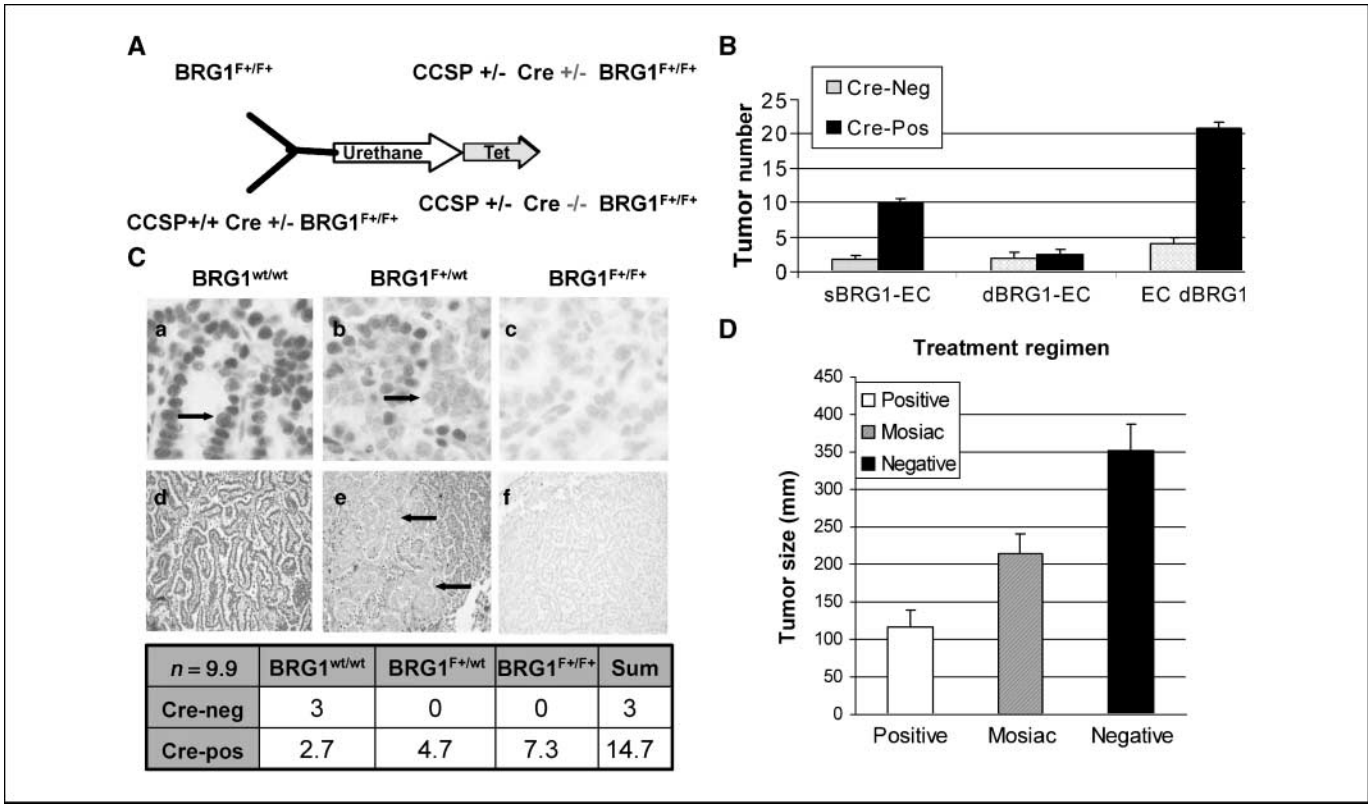
BRG1-specific antibody, we observed a differential pattern of BRG1 staining in many tumors in the Cre-positive mice (Fig. 5*C*). Although ~18% of tumors in the Cre-positive mice were uniformly positive for BRG1, the majority of adenomas lacked BRG1 expression, either in a subset of cells within the resultant adenoma (mosaic; 32%) or nearly all of the cells within the adenoma (50%; Fig. 5*C*). When comparing tumors from the Cre-positive and Cre-negative mice, we noted a similar number of tumors that stained uniformly positive for BRG1. Thus, the increase in the number of tumors in the test group seemed to be due to those tumors that were either partially or completely devoid of BRG1 expression. In comparison, BRG1 staining in the lung of these mice is abundantly seen (Supplementary Fig. S2). We also noted size differences among subgroups (Fig. 5*D*). The adenomas mosaic for BRG1 expression were statistically ($P$ = 0.0042) larger than adenomas that were uniformly positive for BRG1 expression. Similarly, adenomas that were essentially devoid of BRG1 expression were statistically larger than either the mosaic ($P$ = 1.58E-6) or positive BRG1 ($P$ = 0.0017) expression. Interestingly, however, differences in histology among the various adenoma subgroups were not observed.

Next, to characterize the proliferative status of these cells, we stained BRG1-positive adenomas and BRG1-negative adenomas for the expression of PNCA and Ki67, markers of cell proliferation (6A). In BRG1-positive adenomas, compared with BRG1-negative adenomas, we observed a 7-fold increase ($P$ = 0.0004) in the number of both Ki67 and PCNA staining cells (Fig. 6*B*). Little or no staining for KI67 or PCNA was detected in the adult tissues from wild-type, heterozygous *BRG1* knockout, or homozygous *BRG1* knockout (Supplementary Fig. S3).These data show that loss of BRG1 promotes early tumogr development by increasing the number and size of the resultant tumors and enhancing the proliferative status of the cells within these adenomas.

## Discussion

Our results give new insight into the role of BRG1 in tumorigenesis—specifically, these results indicate that inactivation of *BRG1* can enhance the tumorigenic effects of carcinogens. In particular, we found that the loss of even a single allele is sufficient to enhance the transforming effect of ethyl carbamate, indicating that the haplo-sufficiency of *BRG1* may be a contributing factor in human cancer development. This notion is supported by the findings that in a number of cancer types, loss of heterozygosity occurs at chromosome 19p13.2 locus, the BRG1 locus (31, 32). We also found that homozygous inactivation of *BRG1* causes apoptosis in a subset of lung cells, which, in turn, precludes the development






| $n$ = 9.9 | BRG1wt/wt | BRG1F+/wt | BRG1F+/F+ | Sum |
|---|---|---|---|---|
| Cre-neg | 3 | 0 | 0 | 3 |
| Cre-pos | 2.7 | 4.7 | 7.3 | 14.7 |

**Figure 5.** *A,* to determine the effect of homozygous BRG1 loss on carcinogen-induced lung tumors, mice at age 8 wk were treated with ethyl carbamate first, and then, after adenomas had developed (10 wk later), Tet for 48 h was administered. *B,* to show the ability of BRG1 to potentiate tumor development, we first inactivated a single BRG1 allele and then administered ethyl carbamate. This regimen is denoted as sBRG1-EC. Compared with Cre-mice, single allelic inactivation followed by ethyl carbamate lead to 5-fold increase in tumor formation ($n$ = 10; $P$ = 7.67E-8). To examine the effect of knocking out both BRG1 alleles, BRG1 was biallelically inactivated and then ethyl carbamate was administered. This regimen is denoted as dBRG1-EC. Compared with Cre-mice, there was no observed difference in tumor formation ($n$ = 10; $P$ = 0.221). Ethyl carbamate was also given before biallelic BRG1 inactivation. This regimen is denoted as EC-dBRG1. Compared with Cre-negative mice, which retain BRG1, homozygous inactivation of Brg1 given after carbamate exposure increased tumor formation 5-fold ($n$ = 10; $P$ = 4.84E-8). *C,* high (*a–c*) and low (*d–f*) power views of BRG1 staining of adenomas that developed after BRG1 was homozygously inactivated. These adenomas show three patterns of staining: BRG1-positive (*a* and *d*), mosaic (*b* and *e*), and negative (*c* and *f*). The average number of stained tumors for each group is provided in the table below. *D,* tumor size was compared among BRG1-positive, mosaic, and negative staining tumors. $n$ = 13; $P$ values comparing negative to mosaic, negative to positive, and mosaic to positive are 0.0042, 1.58E-6, and 0.0018, respectively. *Columns,* mean; *bars,* SE.

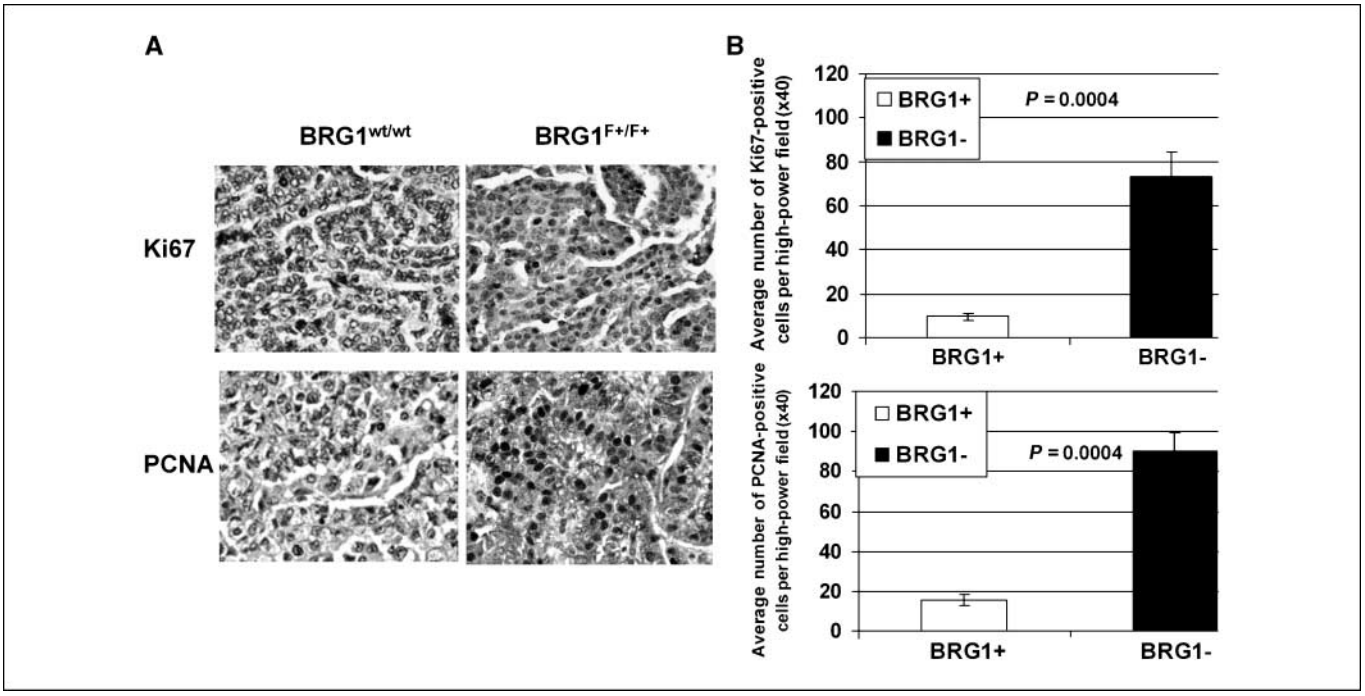



**Figure 6.** *A,* increased staining for Ki67 and PCNA is noted in Cre-positive but not in the Cre-negative adenomas. *B,* the relative increase in staining is 7-fold ($P$ = 0.0004; $n$ = 10); *columns,* mean; *bars,* SD.

of tumors. This effect can be bypassed by pretreating with ethyl carbamate. This may occur in part because ethyl carbamate exposure activates K-ras, which inhibits apoptosis in a variety of cell types. In the absence of carcinogens, the induction of apoptosis observed with *BRG1* inactivation may occur because BRG1 is required for TOPB1 suppression of E2F-mediated apoptosis (33).

The targeted knockout of the SWI/SNF subunit BAF47, while highly transforming, induces apoptosis in the majority of targeted cells. In contrast, knockout of *BRG1* does not. Targeted *BRG1* inactivation has been used in a number of systems. In F9 EC cells, homozygous *BRG1* inactivation inhibits proliferation and has been reported to be lethal to these cells (25). In contrast, targeted biallelic *BRG1* inactivation in mouse embryo fibroblasts does not seem to affect cell viability (23). In the developing embryonic epidermis, the targeted homozygous knockout of *BRG1* has also been reported not to affect viability, but rather to impair normal differentiation, such that the epidermis fails to develop properly and the interactions between epithelium and mesenchyme are abnormal, resulting in hind limb defects (29). In the thymus, loss of BRG1 expression arrests lymphocytic development without affecting viability. Yet, our results indicate that homozygous *BRG1* inactivation in CCSP/rtTA-expressing lung cells induce apoptosis. Thus, *BRG1* inactivation seems to negatively effect target cells in general, but its loss leads to apoptosis only in certain cell types.

Our results also reveal that inactivation of *BRG1* not only increases the number of adenomas that arise but also enhances the size and proliferative status of these adenomas—an effect seen when either one or both alleles are inactivated. Curiously, although SWI/SNF is known to have a potential role in cellular adhesion and differentiation, the histologic characteristics of BRG1-positive and BRG1-negative cells do not seem to differ significantly. One potential explanation for these histologic findings may lie in the fact that the BRG1 homologue, BRM, shares functional properties with BRG1. In this regard, certain pathways linked to SWI/SNF may not be adversely affected unless the function of BRM or both BRG1 and BRM is abrogated. Although ethyl carbamate is sufficient to promote the transformation of certain cells into adenomas, our data indicate that other cells seem to be primed to progress into adenomas but do not unless additional molecular changes occur, such as the inactivation of *BRG1*. Because a subset of adenomas in the TET-treated Cre-positive mice completely lacked BRG1 expression, it is clear that *BRG1* is sufficient to facilitate the transformation of a subset of cells into adenomas.

Our observations indicate that the loss of BRG1 affects growth control within these cells. This is not surprising, given that SWI/SNF and BRG1 are required for the function of the retinoic acid receptor as well as the glucocorticoid receptor (25, 34, 35), both of which are known to thwart lung cancer development. BRG1 is also known to bind to and to be functionally linked to retinoblastoma, as well as its homologues p130 and p107 (22, 36). In particular, restoring BRG1 in cells lacking its expression causes these cells to differentiate and undergo growth arrest, processes that can block variants of the protein E1A, which selectively binds to retinoblastoma, p130, and p107 (22). Thus, the loss of BRG1 seems to inevitably impair one or more growth control or differentiation regulating pathways.

The interplay of molecular changes leading to cancer is clearly complex. Of the many molecular changes involved, some are sufficient in and of themselves to transform cells, whereas others have more subtle roles and only come into play once transformation has been initiated. Our results support this latter notion for BRG1, as it seems that, for most cells types, homozygous loss of *BRG1* is nontransforming and could be lethal to some cell types. However, biallelic loss of *BRG1* not only occurs in transformed cancer cell lines and malignant primary tumors, but we have also found that it can also occur early in transformed cells of adenomas. Further

studies will be necessary to determine just when loss of BRG1 comes into play and if this loss occurs before or after BRM loss. This latter finding suggests that even the partial loss of *BRG1* may play a role in cancer progression, and hence, our previous finding that 10% to 20% of lung tumors show loss of *BRG1* may be an underestimate. It is clear that loss of *BRG1* and SWI/SNF affect cancer development; however, just which pathways are most important to this process has yet to be determined. To this end, the conditional lung-specific murine knockout model presented will allow for exploration into the role of *BRG1* in lung cancer and provide insight to how its loss affects the development of other human tumors.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

## Acknowledgments

Received 12/14/2007; revised 2/21/2008; accepted 3/18/2008.

**Grant support:** University of Michigan Diabetes Core grant DK20572, University of Michigan Cancer Center grant 5-P30-CA46592, and K08 grant CA092149-03.

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We thank Rachel Dresbeck for diligent efforts in editing this manuscript and Jeff Whittset for his generous donation of the CCSP/rtTA/Cre mice.

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