BRM Promoter Polymorphisms and Survival of Advanced Non–Small Cell Lung Cancer Patients in the Princess Margaret Cohort and CCTG BR.24 Trial

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Abstract

Introduction: BRM, a key catalytic subunit of the SWI/SNF chromatin remodeling complex, is a putative tumor susceptibility gene that is silenced in 15% of non–small cell lung cancer (NSCLC). Two novel BRM promoter polymorphisms (BRM-741 and BRM-1321) are associated with reversible epigenetic silencing of BRM protein expression.

Experimental Design: Advanced NSCLC patients from the Princess Margaret (PM) cohort study and from the CCTG BR.24 clinical trial were genotyped for BRM promoter polymorphisms. Associations of BRM variants with survival were assessed using log-rank tests, the method of Kaplan and Meier, and Cox proportional hazards models. Promoter swap, luciferase assays, and chromatin immunoprecipitation (ChIP) experiments evaluated polymorphism function. In silico analysis of publicly available gene expression datasets with outcome were performed.

Results: Carrying the homozygous variants of both polymorphisms (“double homozygotes”, DH) when compared with those carrying the double wild-type was associated with worse overall survival, with an adjusted hazard ratios (aHR) of 2.74 (95% CI, 1.9–4.0). This was confirmed in the BR.24 trial (aHR, 8.97; 95% CI, 3.3–18.5). Lower BRM gene expression (by RNA-Seq or microarray) was associated with worse outcome (P < 0.04). ChIP and promoter swap experiments confirmed binding of MEF2D and HDAC9 only to homozygotes of each polymorphism, associated with reduced promoter activity in the DH.

Conclusions: Epigenetic regulatory molecules bind to two BRM promoter sequence variants but not to their wild-type sequences. These variants are associated with adverse overall and progression-free survival. Decreased BRM gene expression, seen with these variants, is also associated with worse overall survival.

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Introduction

Lung cancer is the leading cause of cancer deaths in the industrialized world, even in this new era of screening (1, 2). The majority of non–small cell lung cancer (NSCLC) patients present at an advanced stage for which treatment is met with limited success. However, with considerable interindividual variability in lung cancer development, outcomes, and treatment response, heritable polymorphisms may play roles in lung cancer susceptibility/risk (3, 4) and outcome (5).

The SWI/SNF (SWItch/sucrose non-fermentable) complex is a multimeric chromatin remodeling complex that plays a key role in regulating multiple cellular processes, including gene expression, differentiation, DNA repair, and cell-cycle control (6–9). The complex, consisting of a catalytic subunit with helicase ATPase activity [either Brahma (BRM) or BRM-related gene 1 (BRG1)] is required for the function of a variety of signal transduction pathways and anticancer protein activities [retinoblastoma (Rb), p53 and BRCA1, refs. 7, 9–11]. BRM regulates the expression of 4% to 7% of mammalian genes, many of which have anticancer roles, among them, vimentin, E-cadherin, N-cadherin, estrogen receptor, progesterone receptor, and CD44 (9, 12–16).

BRM protein expression is lost in 15% to 40% of many primary solid tumors, including in 17% to 30% of NSCLC (17, 18). Unlike most tumor suppressor genes, however, BRM is reversibly
epigenetically silenced (18–20). Initially, pan-histone deacetylase (HDAC) inhibitors were identified as compounds that could restore BRM, but these agents were also found to inactivate BRM by inducing its acetylation (21). As these compounds showed that BRM is regulated by HDACs, further analysis showed that BRM silencing is regulated specifically by class 1 HDAC (HDAC3) and class 2 HDAC (HDAC9) enzymes. Further, HDAC9 is overexpressed in both BRM-deficient (>500-fold) cancer cell lines and (>20-fold) primary tumors (22, 23).

To determine how BRM was silenced, we previously sequenced the BRM promoter in cancer cell lines and identified the presence of two insertion/deletional polymorphisms (BRM-741:TAAA) and (BRM-1321:TATTTTT) that correlated strongly with loss of BRM protein expression in cancer cell lines and confirmed in primary lung cancers. The variant insertion alleles of both polymorphisms produce sequence variants that are highly homologous to myocyte enhancer factor-2 (MEF2) transcription factor binding sites, where MEF2 is known to recruit HDACs and silence genes (24). shRNA knockdown of HDAC9, MEF2D in BRM-deficient cell lines results in the induction of BRM (24, 25). BRM demonstrates attributes of a tumor susceptibility gene, as the BRM-null mouse does not develop spontaneous tumors, but shows distinct abnormalities in cell-cycle control; when combined with a carcinogen, tumor development is potentiated (18). We reasoned, therefore, that the promoter insertion variants may be associated with cancer risk and have confirmed that individuals carrying both BRM homozygous promoter insertion variants have a 2-fold increase in the risk of lung cancer (24), particularly early-stage lung cancer (25), and in other cancers (26–28).

Prognostically, loss of BRM protein expression has been linked to adverse outcome across a varied mix of NSCLC stages (17, 29). However, the relationships between BRM promoter polymorphisms, BRM gene expression, and outcome have not been documented previously. Potential prognostic implications are important, as involvement of the BRM pathway in tumors may identify a subset of individuals with worse outcome that potentially could benefit from focused development of a class of drugs targeting BRM reexpression. That the BRM polymorphisms are associated with epigenetic factors is unique, but even more so when such epigenetic silencing has been reversed by a variety of compounds and drugs such as certain nonsteroidal anti-inflammatory drugs (indoprofen) and flavonoids (genistein; refs. 30, 31).

As cancer drug development focuses on advanced disease, we undertook this study to evaluate the role of these polymorphisms on survival outcomes in two independent patient cohorts. However, we first evaluated the functional significance of the polymorphisms through promoter swap and chromatin immunoprecipitation (ChIP) experiments; these analyses can provide important adjunctive evidence supporting a potential clinical role of this gene and specifically these two polymorphisms. Finally, we assessed the significance of BRM gene expression on clinical outcome, using publicly available databases, to provide the first evidence of a link between our putative biological mechanism and the associations found in this study.

Materials and Methods

Study cohorts

The study was approved by the institutional research ethics board and by the Lung Cancer Correlative Science and Tissue Banking Subcommittee of the Canadian Cancer Trials Group. Figure 1 shows the flow of patient specimens in the polymorphism-survival analyses.

Princess Margaret (PM) cohort study

Cases were incident (diagnosis ≤ 6 months prior to enrollment) stage III–IV NSCLC patients who participated in a prospective study evaluating the molecular epidemiology of lung cancer at the PM Cancer Centre, Toronto, between 2006 and 2010. Eligibility included histological confirmation of NSCLC and provision of written consent; exclusions were inability to communicate in English and cognitive deficits interfering with ability to understand consent. For cohort analysis of survival, cases were derived from the same underlying pool of advanced, incurable stage III–IV NSCLCs as in the case–control analysis, with the following exceptions: smoking status was no longer part of the eligibility criteria, and all adult patients (≥18 years old) were eligible. Available treatment, covariate, and survival data for at least 6 months after diagnosis were an additional inclusion criterion.

BR.24 clinical trial

The Canadian Cancer Trials Group (CTG) led the BR.24 clinical trial, a randomized double blinded, international phase II trial that evaluated carboplatin (area under the curve, AUC 6) and paclitaxel (200 mg/m²) every 3 weeks for 6 to 8 cycles in combination with either daily oral cediranib or placebo in the first-line treatment of advanced stage IIIb–IV NSCLC patients, conducted between 2005 and 2008. Cediranib/placebo monotherapy afterward in the absence of intolerable toxicity or disease progression was allowed. A preplanned interim analysis determined an imbalance in the number of investigator-designated cause of deaths, and the trial was terminated without entering the phase III portion.

DNA extraction and genotyping

Germline DNA was extracted from the lymphocytes of whole blood of all patient cohorts using a commercially available DNA isolation kit (5Primer, Cat#2300740). Genotyping for BRM-741 TATA...
and BRM-1321 was performed using two custom-designed Taqman assays (24, 25). For quality control, positive and negative controls and blinded duplicate samples were included.

**ChIP, promoter luciferase, and swap experiments**

To demonstrate the proteins that bind the BRM polymorphic sequences and altered promoter activity levels by these polymorphic variants, ChIP, promoter luciferase, and swap experiments were performed. The insertion variants of the BRM polymorphisms are MEF2 binding sites (with >92% homology; ref. 24). Upon binding, MEF2D recruits class II HDACs, specifically HDAC9, which results in targeted gene silencing (32, 33). To demonstrate this, ChIP experiments were performed to determine the specificity of MEF2D/HDAC9 binding to BRM insertion alleles, and not to wild-type deletion alleles. To further demonstrate that the specificity is polymorphism dependent and not cell-line dependent, isogenic BRM promoter constructs (forming double-homozygous and double-wild-type genotypes) were placed stably in the SW13 cell line via homologous recombination. In these constructs (Fig. 3), a luciferase reporter IRES-neomycin gene was inserted near exon 2 of the BRM gene, which effectively disrupted BRM expression, and allowed the inserted luciferase reporter gene to be under the control of the BRM promoter. These isogenic promoter swapped constructs were stably integrated into the SW13 cell line, and then assessed using ChIP assays. These same constructs were then used to determine the relative level of promoter activity through measurement of luciferase.

**Statistical analyses**

For patient cohorts, baseline demographic and clinicopathologic data were described and cross-tabulated. Additionally, for the BR.24 trial, baseline demographic, clinical information, and survival outcome were compared between individuals with data available for genetic analysis and those without. Departure from Hardy–Weinberg equilibrium (HWE) was tested using the Pearson $\chi^2$ test. All models assumed codominant genetic inheritance for both polymorphisms, and combined polymorphism analyses were performed using previously described categorizations (24, 25).

Survival was defined as the time from date of pathological diagnosis of stage III/IV (PM cohort) or randomization (BR.24 cohort) to either the date of death from any cause (overall survival, OS), or date of first disease progression/recurrence or death from any cause (progression-free survival, PFS). Patients were censored when they were last known to be alive (for OS) and last assessed for progression/recurrence (for PFS).

Survival rates and median survival times were estimated using the Kaplan–Meier method. Cox proportional hazards models and the log-rank test were used to test associations between the sequence variants and survival. Multivariate models were constructed with genetic markers after adjusting for individual covariates found to be associated with survival (at the $P < 0.10$ level). $P$ values, adjusted hazard ratios (aHR) and their 95% confidence intervals (95% CI) for survival were reported. All statistical tests were two-sided and were conducted using the SAS software.
(version 9.2, SAS Institute Inc.) and the R software (version 2.11.0, R Development Core Team). The Wald test was used for all the genetic models of inheritance. A two-sided \( P \) value of less than 0.05 was considered statistically significant.

For ChIP experiments, protein–DNA sequence binding affinity results were reported as specific anti-HDAC9 and anti-MEF2D antibody binding, normalized against background, non-specific IgG, which resulted in a measurement of fold change (against the background). Log-transformed triplicate fold change data were analyzed using Student \( t \) tests. Similar fold-change data were analyzed for the promoter luciferase activity, comparing different promoter constructs.

*Gene expression analysis of publicly available databases:* we used a publicly available tool (www.kmplot.com/lung) to evaluate the prognostic effect of low BRM (gene symbol SMARCA2) gene expression on OS in patients with NSCLC, using a lung cancer dataset encompassing 1,715 samples (1,405 NSCLC) with gene expression and survival data from 10 independent studies (January, 2015); analyses compared the bottom quartile of BRM expression with the top three quartiles. RNA-Seq lung cancer data were available from TCGA (\( n = 431 \) adenocarcinomas; \( n = 323 \) squamous cell carcinomas), and survival analysis by quartiles was performed. In all cases, the method of Kaplan–Meier was used, and log-rank tests were performed.

**Results**

**Patient cohort demographics**

Patient demographics for each study cohort are shown in Table 1. Patients from BR.24 included only those suitable for first-line chemotherapy, rather than surgery or combined chemotherapy and radiation. Patients from the PM cohort included primarily unresectable patients. Compared with BR.24 participants, PM cases were more likely to have adenocarcinoma and have a higher fraction of the stage III patients. When comparing BR.24 patients analyzed with those not analyzed due to lack of sample, excluded patients were more likely of later stage, have adenocarcinoma, and have unknown weight loss prior to randomization, leading to decisions to adjust for these variables in the final BR.24 model. Despite these differences, the analyzed population had similar survival outcomes and derived similar benefit from treatment compared with the total BR.24 cohort (test of homogeneity between treatment arm and availability of biospecimens \( P = 0.93 \)). In comparison, the analyzed and non-analyzed PM cohorts, however, were very similar (Supplementary Table 1).

**BRM polymorphisms and survival analyses**

Both polymorphisms were in the Hardy–Weinberg equilibrium (\( P > 0.05 \)), and in mild linkage disequilibrium (\( D' = 0.48 \) for PM cohort, and 0.39 for BR.24 trial). Genotyping was successful in >99.8% of cases. Although \( ~20\% \) carried at least one homozygous variant of these two polymorphisms, 11% to 15% of patients carried homozygous variants of both polymorphisms (termed the “double homozygote” or DH). Because of a higher proportion of stage III patients and the inclusion of unresectable stage IIIA patients, the median OS for the PM cohort is substantially higher than that of BR.24 (Table 1).

Table 2 (OS), Table 3 (PFS), and Fig 2 (Kaplan–Meier curves) report strongly significant survival relationships with the individual polymorphisms, and further specifically with the DH variants of these two BRM polymorphisms (when compared with the double-wild-type polymorphisms). The strongest survival relationship was found in the placebo (conventional therapy) arm of BR.24, where there was uniform treatment of all patients with carboplatin–paclitaxel, and adjusted hazard ratios exceeded 8 (PFS) and 16 (OS). Subgroup prognostic analyses (Supplementary Table S2) showed similar results in never-smokers and smokers, in patients with adenocarcinomas versus squamous cell carcinomas, by age and gender, for both the PM cohort and the

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**Table 1. Clinicodemographic and genotyping information**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Category</th>
<th>PM cohort</th>
<th>BR.24 cohort analyzed</th>
<th>BR.24 not analyzed</th>
<th>Analyzed vs. not analyzed ( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number</td>
<td></td>
<td>548</td>
<td>219</td>
<td>77</td>
<td>0.10</td>
</tr>
<tr>
<td>Age (years)</td>
<td>Median (range)</td>
<td>63 (31–90)</td>
<td>60 (38–82)</td>
<td>57 (36–72)</td>
<td>0.10</td>
</tr>
<tr>
<td>Sex</td>
<td>Male/female</td>
<td>52%/48%</td>
<td>59%/41%</td>
<td>56%/44%</td>
<td>0.74</td>
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<tr>
<td>Ethnicity</td>
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<td>67%/15%/18%</td>
<td>Not reported</td>
<td>Not reported</td>
<td></td>
</tr>
<tr>
<td>Smoking status</td>
<td>Current/former/never-smoker</td>
<td>30%/44%/26%</td>
<td>Not reported</td>
<td>Not reported</td>
<td></td>
</tr>
<tr>
<td>Pack-years</td>
<td>Median (smokers)</td>
<td>38 (1–216)</td>
<td>Not reported</td>
<td>Not reported</td>
<td></td>
</tr>
<tr>
<td>Weight loss</td>
<td>≥ 5%/&lt; 5%/unknown</td>
<td>Not reported</td>
<td>28%/58%/3%</td>
<td>18%/59%/43%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Histological</td>
<td>Adenocarcinoma</td>
<td>67%</td>
<td>46%</td>
<td>64%</td>
<td>0.01</td>
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<tr>
<td>Subtype</td>
<td>Squamous cell</td>
<td>16%</td>
<td>26%</td>
<td>12%</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>17%</td>
<td>28%</td>
<td>25%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage</td>
<td>III/IV</td>
<td>46%/54%</td>
<td>17%/83%</td>
<td>6%/94%</td>
<td>0.02</td>
</tr>
<tr>
<td>Platinum agent</td>
<td>Cisplatin/carboplatin based therapy</td>
<td>82%</td>
<td>100%</td>
<td>100%</td>
<td></td>
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<tr>
<td>No-platinum chemotherapy</td>
<td></td>
<td>10%</td>
<td>0%</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>Neither/unknown</td>
<td>8%</td>
<td>0%</td>
<td>0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Follow-up time</td>
<td>Median (months)</td>
<td>42</td>
<td>18</td>
<td>17</td>
<td>0.91</td>
</tr>
<tr>
<td>Overall survival</td>
<td>Median (months)</td>
<td>19</td>
<td>9</td>
<td>10</td>
<td>0.64</td>
</tr>
<tr>
<td>Time to randomization¹</td>
<td>&lt;6 months/≥6 months</td>
<td>89%/11%</td>
<td>91%/9%</td>
<td>0.89</td>
<td></td>
</tr>
<tr>
<td>Dose cohort</td>
<td>30 mg/45 mg of cediranib/placebo</td>
<td>84%/76%</td>
<td>86%/74%</td>
<td>0.94</td>
<td></td>
</tr>
<tr>
<td>Treatment arm</td>
<td>Conventional (placebo) arm/ experimental (cediranib) arm</td>
<td>50%/50%</td>
<td>50%/50%</td>
<td>50%/50%</td>
<td>0.99</td>
</tr>
</tbody>
</table>

¹Calculated in ever-smokers only.
²From date of diagnosis.
³Majority are expected to be of Caucasian descent.
BRM polymorphisms and OS in advanced-stage NSCLC.

Table 2. BRM polymorphisms and OS in advanced-stage NSCLC.

Table 3. BRM polymorphisms and PFS in advanced-stage NSCLC.

BRM trial participants. For the PM cohort, subset analysis of the 82% of patients treated with platinum-based chemotherapy found virtually identical survival associations with these two BRM polymorphisms as the entire PM cohort; further subset analyses found consistent associations regardless of whether the therapy was cisplatin- or carboplatin-based doublet therapy.

ChIP and promoter swap experiments

Figure 3 shows ChIP experiments of representative cell lines (including several lung cancer cell lines) carrying different BRM polymorphism combinations. In brief, of the cell lines evaluated, all BRM wild-type genotypes resulted in little to no binding of MEF2/HDAC9, while there was significant binding observed in all homozygous variants (P < 0.0001, all comparisons), including homozygous variants created through the promoter construct swap experiment of the SW13 cell line. In addition, the same promoter constructs demonstrated a 7.8-fold increased luciferase expression when these insertion alleles are absent as compared with when they are present (Fig. 3), thereby demonstrating that these BRM polymorphic variants bind specific proteins and are functionally involved in regulating BRM expression.

BRM gene expression and survival

Gene expression data from publicly available sources of expression microarrays (Fig. 2, middle) and RNA-Seq (Fig. 2, right) of
mainly early and locally advanced stage NSCLC are presented. Significant association was observed between lower BRM expression levels and poorer survival in expression microarray data, with a HR of 0.42 (95% CI, 0.32–0.84; \( P = 0.02 \)). Lower BRM expression was associated inversely with prognosis consistently in adenocarcinomas regardless of the method of assessing BRM gene expression: from RNA-Seq data, \( P = 0.04 \) and expression microarray data, \( P = 0.01 \). In contrast, two different sets of data obtained through two different assessments of BRM gene expression differed on the relationships with survival in squamous cell carcinoma, where there were significant associations in the RNA-Seq data (\( P = 0.04 \)) but not in expression microarray data.
Figure 3.
Functional assays. Top, ChIP experiments demonstrate that binding of MEF2D and HDAC9 occurs only in the presence of the homozygous variants ($P < 0.001$; Student t test) and not the wild-types ($P > 0.05$). Vertical axes show the binding affinity relative to background binding by nonspecific IgG in the C33A (cervical carcinoma), H522 (lung adenocarcinoma), and A547 (lung squamous carcinoma) cell lines (log-transformed ratio $\pm$ standard error). (Continued on the following page.)
Discussion

Two novel BRM promoter insertion deletion polymorphisms, BRM-741 and BRM-1321, are linked to epigenetic silencing of BRM expression and have been linked to lung cancer risk (24, 25). The presence of both homozygous variants strongly correlates with loss of BRM expression in lung cancer, a loss that, in turn, has been linked to adverse clinical outcomes (17, 29, 34). While previously we had documented these polymorphisms as being associated with risk of early-stage NSCLC (27), in the present analysis, we further describe that individuals carrying the homozygous variants of one or both polymorphisms had substantially worse survival outcomes compared with their wild-type counterparts in two independent datasets, one an observational cohort, and the other, a clinical trial. We performed molecular experiments to explain the functional significance of these polymorphisms, demonstrating that the presence of these polymorphic variants is physically connected to binding of regulatory proteins important in BRM expression. Finally, we reported that BRM gene expression was also prognostic for NSCLC survival, through analysis of publicly available data. Each of these findings incrementally builds on our understanding of these BRM polymorphisms and its clinical relevance. These findings include the mechanism of polymorphism function, and consistent findings across DNA and RNA levels of BRM with prognosis, compatible with proposed mechanisms of BRM function. Such cumulative evidence supports the importance of BRM in NSCLC outcome.

BRM germline polymorphisms may be useful not only for risk stratification but also for prognostication. This is of importance because our prior research suggests that BRM is a potentially druggable target (21). In one clinical setting, CT screening of lung cancer may benefit from further refinement by incorporation of molecular risk markers (35). Yet in a contrasting set of circumstances, other markers such as somatic ALK translocations already help identify patients with aggressive (poor prognosis) tumors that respond well to drugs that target the rearranged ALK protein (36). If a somatic genomic alteration such as ALK can lead to drug targets, perhaps the concept of a germline epigenetic target may not be so far-fetched. In the past, a barrier to adoption of polymorphic variants in risk/prognostic models has been a lack of clear biological or functional significance of such polymorphisms, a flaw that is addressed in this study. In fact, putting all the available evidence from the literature and the results of this study together, truly functional consequences of these polymorphisms may affect tumor biology and drug sensitivity, while our prior research suggested BRM is a druggable target (21, 24).

To better understand why these polymorphisms might underlie clinically relevant associations, we examined potential roles of BRM in cancer. Loss of BRM expression has been implicated in the progression of lung adenocarcinoma into solid predominant tumors with features of epithelial–mesenchymal transition (EMT), which is consistent the fact the SWI/SNF regulates key genes involved in EMT changes, such as E-cadherin, N-cadherin, and vimentin, and loss of bronchial epithelial phenotype (34). Similarly, a link between BRM loss and cancer progression has been suggested among patients with gastric (37) and skin cancer (38). Loss of BRM protein expression has been linked to poorer outcome among patients with NSCLC (17, 29), and other cancers (39).

BRM can impact cancer evolution in a number of ways. Its functional linkage to such proteins as Rb and p53 may in part explain how BRM silencing can result in the loss of cellular growth control, a central mechanism by which BRM is thought to potentiate the risk of cancer (12, 40, 41). However, BRM and SWI/SNF are also involved in DNA repair, and the aberration of DNA repair proteins, such as GADD45, BRCA1, and p53, functionally linked to BRM and SWI/SNF, are known to potentiate cancer development (10, 42). Loss of BRG1 and BRM has been linked to the EMT, thought to be a major step toward more aggressive disease; BRM and SWI/SNF have been linked to E-cadherin, N-cadherin, vimentin, CD44, CEACAM1, and integrin expression, which are also linked to EMT (12, 34, 43, 44). In addition, SWI/SNF in general has been shown to play roles in cellular differentiation and organ development (45–49). Interestingly, the SWI/SNF complex may also increase sensitivity to cisplatin (50) but increase resistance to EGFR inhibitors. Indeed, there is no shortage of feasible mechanisms by which BRM loss could impact cancer development and clinical outcomes.

Studies to evaluate lung cancer risk or prognosis as a function of a given germline polymorphism have been pursued vigorously. Prototypical polymorphisms that lie within the coding region of a protein are surmised to alter the protein’s function by changing its primary sequence. This change in protein sequence alters the function of the protein, the cell, and the organism, thus yielding a significant clinical association. When these polymorphisms are not specifically related to a protein sequence, the value of a given polymorphism is strengthened by evidence showing that it affects gene function. To this end, the ChiP and luciferase data presented herein show that two key
BRM-regulating proteins, MEF2D and HDAC9, bind in or near the polymorphisms, in the presence of the variant insertion allele. Luciferase assays suggest that the BRM variants are also associated with lower BRM expression.

In our study design, the consistency of our BRM findings across different study populations of risk (24, 25) and prognosis (herein) is a strength of our findings. Limitations are present, though. First, because we lacked sufficient quantities of research tumor specimens in our advanced stage cohorts, we were unable to perform matched polymorphism–gene expression–protein expression assessments of BRM. Previously published protein expression-outcome analyses (17, 29) and the current gene expression-outcome analyses confirm a consistent association with clinical outcome, but utilized samples from earlier stage NSCLC patients. Gene expression analysis of public databases may be confounded by differences in stage and other prognostic variables, possibly explaining the significant relationship in squamous cell carcinoma by RNA-Seq but not by expression microarray. Second, we are pursuing a parallel analysis of these polymorphisms in a randomized clinical trial of adjuvant cisplatin chemotherapy in early-stage NSCLC, where we will be able to answer questions of potential BRM–platinum drug interactions (50; all BR.24 patients were treated with carboplatin) and to evaluate a uniformly cisplatin-treated patient cohort. Nonetheless, the PM cohort was treated with a platinum doublet in 82% of patients, of which 61% were cisplatin-based doublets (primarily cisplatin–etoposide for stage III and cisplatin–vinorelbine for stage IV), where subset analyses found similar results regardless of use of platinum agents or the specific platinum agent itself. Third, the interplay between EGFR, ALK, and other somatic changes with BRM on prognosis is unknown; we had too few patients with these molecular alterations to perform molecular-specific analyses of BRM relationships. We also acknowledge that the dual roles of BRM polymorphisms on risk and prognosis may not be consistent: in other tumors and settings, a risk association may not translate into a survival association, and vice versa; evaluation in each specific cancer disease site is necessary (51). We did not have the power or tumor samples to explore the effect of these polymorphisms in different subgroups (e.g., different BRG expression patterns, modifying effect of other chromatin remodeling members). In the TCGA analysis, survival data could have been confounded by other known prognostic variables (e.g., stage); however, TCGA clinical data quality is heterogeneous, and thus these data are offered only as weak supporting evidence. Nonetheless, the totality of our findings has opened additional routes of exploration. In summary, the same two BRM promoter polymorphisms that were previously associated with increased risk of developing NSCLC are also strongly associated with adverse survival in two different advanced NSCLC patient samples. This is a clinically important discovery given the high magnitude of prognostic association found, with values that could conceivably lead to clinically meaningful germline molecular prognostication. Further, pharmacologic reversal of the epigenetic silencing of BRM has been shown to be a potentially viable therapeutic strategy (18, 21). With one in five patients homoygous for either BRM-741 or BRM-1321, and one in ten homozygous for both, a sizeable population of NSCLC may benefit either from future targeted therapy or prognostic stratification to allow decision-making on utilizing more aggressive or different therapeutic strategies. Additional studies are needed to further validate our study findings in other lung cancer populations, specifically the relationships between these polymorphisms and somatic lung cancer molecular alterations, and between these polymorphisms and other members of these chromatin remodeling complexes.

Disclosure of Potential Conflicts of Interest
G. Liu is a consultant/advisory board member for AstraZeneca, Novartis, and Pfizer. G. Goss reports receiving speakers bureau honoraria from AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb, Celgene, and Pfizer. D.N. Reisman holds ownership interest (including patents) in Zenagen. No potential conflicts of interest were disclosed by the other authors.

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