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SMARCA4/BRG1 is a novel prognostic biomarker predictive of cisplatin-based chemotherapy outcomes in resected non-small cell lung cancer

Erica Hlavin Bell, PhD[1,#,*], **Arup R. Chakraborty, PhD**[1,*], **Xiaokui Mo, PhD**[2], **Ziyan Liu, MS**[1], **Konstantin Shilo, MD**[3], **Simon Kirste, MD**[1,4], **Petra Stegmaier, MD**[1,4], **Maureen McNulty, BS**[1], **Niki Karachaliou, MD**[5], **Rafael Rosell, MD, PhD**[5,6], **Gerold Bepler, MD, PhD**[7], **David P. Carbone, MD, PhD**[8], and **Arnab Chakravarti, MD**[1]

[1]Department of Radiation Oncology, Arthur G. James Hospital/Ohio State Comprehensive Cancer Center, Columbus, OH, United States of America

[2]Center for Biostatistics, The Ohio State University Wexner Medical Center, Columbus, OH, United States of America

[3]Department of Pathology, The Ohio State University Wexner Medical Center, Columbus, OH, United States of America

[4]Department of Radiation Oncology, University Medical Center Freiburg, Freiburg, Germany

[5]Translational Research Unit, Dr Rosell Oncology Institute, Quirón Dexeus University Hospital, Barcelona, Spain

[6]Catalan Institute of Oncology, Badalona, Barcelona, Spain

[7]Barbara Ann Karmanos Cancer Institute, Wayne State University, Detroit, MI, United States of America

[8]Department of Internal Medicine, The Ohio State University Wexner Medical Center, Columbus, OH, United States of America

[#]corresponding author: Erica Hlavin Bell, PhD, Department of Radiation Oncology, Arthur G. James Hospital/Ohio State Comprehensive Cancer Center, Wiseman 385G, Columbus, OH, 43210, United States of America, 614-366-0845, Erica.Bell@osumc.edu.

[*]denotes equal contribution

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AUTHORS' CONTRIBUTIONS:

Conception and design: E.H. Bell, A.R. Chakraborty, X. Mo, D.P. Carbone, A. Chakravarti

Development of methodology: E.H. Bell, X. Mo

Acquisition of data: E.H. Bell, A.R. Chakraborty, X. Mo

Analysis and interpretation of data: E.H. Bell, A.R. Chakraborty, X. Mo, Z. Liu, K. Shilo, S. Kirste, P. Stegmaier, M. McNulty, N. Karachaliou, R. Rosell, G. Bepler, D.P Carbone, A. Chakravarti

Writing, review, and/or revision of the manuscript: E.H. Bell, A.R. Chakraborty, X. Mo, Z. Liu, K. Shilo, S. Kirste, P. Stegmaier, M. McNulty, N. Karachaliou, R. Rosell, G. Bepler, D.P Carbone, A. Chakravarti

Administrative, technical, or material support: E.H. Bell, A.R. Chakraborty, X. Mo, Z. Liu, K. Shilo, S. Kirste, P. Stegmaier, M. McNulty, N. Karachaliou, R. Rosell, G. Bepler, D.P Carbone, A. Chakravarti

Study supervision: E.H. Bell

Abstract

Purpose—Identification of predictive biomarkers is critically needed to improve selection of patients who derive the most benefit from platinum-based chemotherapy. We hypothesized that decreased expression of *SMARCA4*/BRG1, a known regulator of transcription and DNA repair, is a novel predictive biomarker of increased sensitivity to adjuvant platinum-based therapies in NSCLC.

Experimental Design—The prognostic value was tested using a gene expression microarray from the Director's Challenge Lung Study (n=440). The predictive significance of *SMARCA4* was determined using a gene expression microarray (n=133) from control and treatment arms of the JBR.10 trial of adjuvant cisplatin/vinorelbine. Kaplan-Meier method and log-rank tests were used to estimate and test the differences of probabilities in overall survival (OS) and disease-specific survival (DSS) between expression groups and treatment arms. Multivariate Cox regression models were used while adjusting for other clinical covariates.

Results—In the Director's Challenge Study, reduced expression of *SMARCA4* was associated with poor OS compared to high and intermediate expression (P<0.001 and P=0.009, respectively). In multivariate analysis, compared to low, high *SMARCA4* expression predicted a decrease in risk of death (HR=0.6, 95% CI: 0.4–0.8, P=0.002). In the JBR.10 trial, improved five-year DSS was noted only in patients with low *SMARCA4* expression when treated with adjuvant cisplatin/vinorelbine (HR=0.1, 95% CI: 0.0–0.5, P=0.002 [low]; HR 1.0, 95% CI: 0.5–2.3, P=0.92 [high]). An interaction test was highly significant (P=0.01).

Conclusions—Low expression of *SMARCA4*/BRG1 is significantly associated with worse prognosis; however, it is a novel significant predictive biomarker for increased sensitivity to platinum-based chemotherapy in NSCLC.

Keywords

SMARCA4; BRG1; NSCLC; predictive; cisplatin

INTRODUCTION

Lung cancer is the most deadly cancer in the world and 85% of lung cancers are non-small cell lung cancer (NSCLC) (1). Chemotherapy remains a major treatment modality and the only therapy proven to prolong survival of early stage patients after surgery. Although in recent years there have been major advancements in early detection and targeted therapies, the five-year survival gains have remained relatively small (2). High mortality is due to advanced stage detection of the disease together with the absence of targetable driver mutations in most tumors leaving systemic chemotherapy as the only first line therapeutic option. These therapies are toxic, and while biomarkers can inform the selection of targeted therapies, biomarkers that enable the identification of patients who would benefit from chemotherapy have remained elusive.

The mechanisms responsible for drug resistance include increased efflux and/or inactivation of drugs, defects in apoptosis, and activation of DNA repair pathways (3). Studies on DNA repair pathways to date have been disappointing. *ERCC1*, a critical component of nucleotide

excision repair (NER), has been one of the most well-studied genes in NSCLC in regards to cisplatin sensitivity, albeit with conflicting results attributed to issues regarding detection techniques in clinical tissues (4). The majority of studies have shown that low ERCC1 levels are associated with cisplatin sensitivity (5). However, effect sizes have been small, prospective studies have failed to confirm this association, and these markers are not used in clinical practice.

Overall, the predictive effect of driver mutations with drug sensitivity in tumors with "oncogene addiction", such as *ALK* and *EGFR* is now clear. Numerous studies have attempted to evaluate the impact of tumor suppressors on clinical outcome in lung cancer, but none have produced clinically impactful results. For example, the effect of *TP53* mutations on prognosis and chemotherapy sensitivity is unclear and inconsistent. Similarly *RB* and *LKB1* mutations have not had clinical utility. However, little has been done to fully understand how loss of other tumor suppressors, such as *SMARCA4*/BRG1, affects treatment sensitivities to drugs in standard of care regimens, such as platinum-based therapies as well as emerging therapeutics.

The SWItch/Sucrose NonFermentable (SWI/SNF) chromatin remodeling complex, which functions as a fundamental regulatory component of transcription, plays a critical role in DNA repair (8). SWI/SNF is frequently abnormal in lung cancer, but has not been previously studied for chemotherapy prediction in resected NSCLC. Notably, BRG1 (*SMARCA4*), one of two catalytic subunits of SWI/SNF, is a tumor suppressor and mutations have been identified in approximately 10% of NSCLC (9–11). *SMARCA4* mutations and/or decreased expression have also been identified in other tumor cell lines and tissues (12, 13). Furthermore, alterations in other components of SWI/SNF, including the other catalytic subunit BRM (*SMARCA2*) and *ARID1A*, have been recently identified in cancer (8, 14). Even though somatic missense mutations appear to be the most common mutations, other mechanisms such as insertions, partial and complete deletions, and promoter methylation may have been less well studied but also contribute to the loss of BRG1 in lung cancer (11, 15). Interestingly, although SNF5-deficient rhabdoid tumors and SMARCA4/SMARCA2-deficient small cell carcinoma of the ovary, hypercalcemic type (SCCOHT) tumors do not exhibit genomic instability (16, 17), loss of *SMARCA4*/BRG1 function in lung cancer may lead to genomic instability as evidenced by a recent publication (18).

The SWI/SNF chromatin remodeling complex has recently been implicated in double-strand break (DSB) repair and NER, two DNA repair pathways inherently involved in resistance toward DNA-damaging agents (19–22). Recently, multiple *in vitro* studies have shown that reduced expression of BRG1 can enhance sensitivity to cisplatin (23), radiation (24), and the combination of EZH2/TopoII inhibitors (25). Thus, more studies are required not only to validate *SMARCA4*/BRG1 as a prognostic factor for overall survival (OS), but also as a potential predictive factor in well controlled patient populations with complete clinical treatment data.

Due to BRG1's apparent role as a tumor suppressor in lung cancer, it has been demonstrated that loss of BRG1 is associated with poor prognosis; however, these studies lack treatment

data and have small sample sizes (26, 27). The goal of this study was to characterize the predictive effect of *SMARCA4*/BRG1 expression on adjuvant cisplatin therapy using patient specimens from a clinical trial (JBR.10; NCT00002583) (28). Specifically, the decreased DNA repair capacity in lung cancer that SMARCA4- and SMARCA2-deficient tumors harbor may in fact be an "Achilles heel" if this type of repair deficiency can be exploited using specific DNA-damaging or targeted agents (8). Therefore, based on *in vitro* data on the regulation of drug sensitivity by BRG1 and its involvement in DNA repair, we hypothesized that decreased expression of *SMARCA4*/BRG1 is a predictive biomarker that promotes sensitivity to platinum-based therapies in NSCLC. To address this, we evaluated the association between gene expression and clinical outcomes of both the Director's Challenge Lung Study (29) (prognostic effect) and the JBR.10 trial (28) (predictive effect). In addition, using the JBR.10 trial we also evaluated the predictive role of *SMARCA2*. Importantly, herein, we are the first to report that both *SMARCA4* and *SMARCA2* are predictive biomarkers of cisplatin-based chemotherapy using NSCLC patient specimens.

METHODS

Study Design and Patient Cohorts

The Director's Challenge Study (n=440) was the first large-scale study to combine high-throughput gene expression data with clinical outcomes in NSCLC from multiple institutions (University of Michigan, Memorial Sloan-Kettering Cancer Center, the H. Lee Moffitt Cancer Center and Research Institute, the Dana-Farber Cancer Institute, and the National Cancer Institute of Canada Clinical Trials Group) (29). Enrollment criteria included diagnosis of lung adenocarcinoma with stage I–III disease and frozen surgical specimen collection. Approximately 60% of the patients had stage I disease and a proportion of the patients were treated with a mixture of adjuvant therapies (chemotherapy and radiation). However, none of the patients received pre-operative chemotherapy or radiation and at least two years of follow-up information was required. The JBR.10 trial (NCT00002583) was a phase III randomized trial of observation (OBS) versus adjuvant cisplatin and vinorelbine (ACT) in completely resected stage IB (T2N) or II (T1-2N1) NSCLC. Patients were stratified by participating institution, nodal status (N0 vs N1), and *Ras* mutation status of the primary tumor. Four cycles of adjuvant cisplatin were given (cisplatin (50 mg/m2) on days 1 and 8 every 4 weeks and vinorelbine (25 mg/m2) weekly for 16 weeks. In addition, post-operative radiation was not permitted. A subset of patients enrolled on JBR.10 (n=133; 62 OBS, 71 ACT) had frozen surgical specimens collected for gene expression analysis (30). The Director's Challenge Lung Study (29) and JBR.10 (30) (GSE14814, latest update December 2014) gene expression profiling data were downloaded from the National Cancer Institute Center for Bioinformatics and the National Center for Biotechnology Information GEO database. Consent was obtained for all subjects as part of the clinical studies and the protocols were approved by each institution's respective Institutional Review Board.

Statistical Analysis

Microarray-based gene expression (Affymetrix U133A, Santa Clara, CA) data from both the Director's Challenge Lung Study and JBR.10 trial were normalized by the RMA method (31). All probe sets (n=8) for *SMARCA4* were tested for both studies. Each probe set was

treated individually due to prior recommendations and evidence that unique probe sets for the same gene can have different hybridization signals and sometimes opposite trends likely due to detection of different or multiple splice variants of the gene as shown previously (32–34). For the Director's Challenge study (n=440), patients were classified into three groups for each probe set based on their tertile expression levels, while for the JBR.10 study (n=133), patients were classified into two groups only for each probe set based on the median expression levels due to the small patient cohort. We used OS and disease-specific survival (DSS) as the time-to-event outcomes. Kaplan-Meier product-limit method and log-rank tests were used to estimate and test the differences of probabilities in OS and DSS between expression groups and treatment arms, and hazard ratios (HR) and 95% confidence intervals (CI) were generated by the univariate Cox regression model. Multivariate Cox regression models were used to validate the prognostic and predictive effects of probes on OS and DSS, respectively while adjusting for other baseline clinical covariates. The interaction test of treatment and *SMARCA4* expression group was performed to assess treatment effect differences (HR of ACT and OBS) between the high and low *SMARCA4* expression groups in the JBR.10 trial. All analyses were performed using SAS 9·4 (SAS, Inc; Cary, NC) and STATA 13 (StataCorp LP; College Station, Texas).

RESULTS

Prognostic Significance of *SMARCA4* Expression

To determine the prognostic significance of *SMARCA4*, we analyzed the gene expression microarray dataset from the Director's Challenge Study. This dataset contained 440 adenocarcinoma (NSCLC) samples with associated clinical data. Patients were classified into tertiles: (High (expression > 70%); Intermediate (30% ≤ expression ≤ 70%); and Low (expression < 30%)). Clinical characteristics of this dataset are shown in Table 1 using the most significant probe set (212520_s_at) in relation to survival. Poor OS was noted following low expression of *SMARCA4* compared to high and intermediate expression (P<0.001 and P=0.009, respectively) for the most significant probe set (212520_s_at) (Fig. 1A). However, no significant differences in OS was observed between high and intermediate levels of *SMARCA4* expression (P=0.47). Decreased OS was observed with low expression of *SMARCA4* both with stage I (High vs Low P=0.01) and stages II–III (High vs Low P=0.01) of the disease (Supplementary Fig. S1). Multivariate analysis suggested that patients with high *SMARCA4* expression had a decreased risk of death compared to patients with low *SMARCA4* expression (high vs low: HR=0.6; 95% CI: 0.4–0·8, P=0.002; intermediate vs low: HR=0.7, 95% CI 0.5–0.9, P=0.01; Table 2) independent of age, stage, gender and differentiation grade. Data utilizing an additional probe set (214360_at) demonstrated a similar trend and statistical significance between high vs low expression (P=0.03; Supplementary Fig. S2). Further, prognostic effects of *SMARCA4* were examined in patients who did not receive adjuvant chemotherapy or radiation in the Director's Challenge study, similar to the entire cohort, low expression of *SMARCA4* was significantly correlated with decreased OS (212520_s_at; high vs low P=0.001; intermediate vs low P=0.02; Fig. 1B). However, no significant differences in OS were observed between high and intermediate level of *SMARCA4* expression (P=0.58). Univariate analysis results are shown in the Supplement (212520_s_at; Supplementary Table S1). In addition, in the multivariate

analysis of patients who did not receive adjuvant treatment, high expression was also a significant independent prognostic marker and correlated with better prognosis (high vs low: HR=0.4; 95% CI: 0.2–0.8, P=0.01; intermediate vs low: HR=0.7, 95% CI 0.4–1.1, P=0.09; Table 2).

Predictive Significance of *SMARCA4* in Resectable NSCLC

To determine the predictive significance of *SMARCA4*, gene expression profiling microarray data from the JBR.10 trial were analyzed. Clinical and sample characteristics of these 133 patients have been previously reported (30) and are shown split by *SMARCA4* (213719_s_at) expression (Table 1). Two probe sets (208794_s_at and 213719_s_at) showed significantly greater five-year DSS in the *SMARCA4* low patient population after the treatment (both P<0.05) (Supplementary Tables S2–3). Kaplan-Meier curves are shown for the most significant probe set (213719_s_at) (Fig. 2–3). Patients with low (Fig. 2A,C) and high (Fig. 2B,D) *SMARCA4* expression are plotted comparing two treatment arms (OBS vs. ACT) in Figure 2. Patients with low *SMARCA4* expression demonstrated improved DSS with ACT suggesting this subgroup derives a significant benefit from adjuvant cisplatin-based therapy (5-YR DSS P=0.002; Fig. 2C), whereas patients with high *SMARCA4* expression did not show DSS advantage after treatment (Fig. 2B,D). In contrast to the low *SMARCA4* expression group, HRs were approximately 1 in the high *SMARCA4* expression group suggesting this subgroup derives minimal benefit from ACT. Similarly to DSS, patients at 5-YR OS with low *SMARCA4* expression levels derived significant benefit to ACT (5-YR OS P=0.001; Fig. 3). This benefit is also demonstrated at ten years and trended towards significance for both DSS (10-YR DSS P=0.07; Fig. 2A) and OS (10-YR OS P=0.08; Fig. 3A) and although the curves get closer together they are still split even after ten years. Five probe sets for *SMARCA4* are shown in the Supplementary Fig. S3–5 and although some of the probe sets did not reach significance, all data support the conclusion that patients expressing low levels of *SMARCA4* derive a large benefit from cisplatin-based adjuvant therapy, while the patients with high *SMARCA4* expression did not show this benefit.

Upon univariate analysis, two probe sets (213719_s_at and 208794_s_at) were statistically significant (P<0.05) (Supplementary Tables 2–3) and four other probe sets trended toward improved benefit for the low *SMARCA4* expression group with ACT (Supplementary Fig. S5). Upon multivariate analysis (Table 3), in the low *SMARCA4* patient subset, independent of age, stage, and histology, patients have improved five-year DSS after treatment (213719_s_at (ACT vs OBS HR=0.1, 95% CI: 0.0–0.5, P=0.002); 208794_s_at (HR=0.3, 95% CI: 0.1–0·9, P=0.03)). Thus, low expression of *SMARCA4* mRNA was statistically associated with improved disease-specific survival with adjuvant cisplatin/vinorelbine in completely resectable stage IB/II NSCLC patients. Importantly, multivariate analysis showed in the low *SMARCA4* expression patients that overall survival was also improved after treatment (Table 3). No probe sets approached significance in the high *SMARCA4* expression group demonstrating this subgroup did not associate with improved survival with cisplatin/vinorelbine. An interaction test was performed comparing HRs of ACT and OBS for five-year DSS and OS between the high and low *SMARCA4* expression groups. The testing results revealed that the ACT treatment effect was affected significantly by

SMARCA4 expression in one probe set and trended toward significance in another (5-YR DSS: 213719_s_at; P=0.01; 5-YR OS: 213719_s_at; P=0.007, Table 3).

Since *SMARCA2* is another catalytic subunit of SWI/SNF, it is of interest to determine if *SMARCA2* loss is also associated with improved survival with cisplatin-based chemotherapy and increases the predictive power of *SMARCA4* in the JBR.10 trial. The benefit of adjuvant chemotherapy was determined in patients with low expression values of *SMARCA2* (206543_at) individually and combined with *SMARCA4* (Supplementary Fig. S6 and Fig. 2E–F). As shown, patients with low levels of *SMARCA2* showed a trend toward improved survival with adjuvant chemotherapy, but did not demonstrate the same predictive significance of *SMARCA4* (Supplementary Fig. S6A). High *SMARCA2* did not show improvement (Supplementary Fig. S6B). Strikingly, patients with low levels of both *SMARCA2* and *SMARCA4* (Fig. 2E) seemed to achieve a dramatic benefit (HR 0.3, 95% CI: 0.1–0.9, log-rank P=0.02) in DSS upon treatment with adjuvant cisplatin/vinorelbine compared to observation after surgery. The patients with high expression of both probes did not show a difference (Fig. 2F).

DISCUSSION

Adjuvant cisplatin-based chemotherapy in NSCLC patients reduces the risk of recurrence after complete resection in unselected stage IB, II, and IIIA patients; however, while all patients experience toxicity, not all receive benefit. Thus, predictive biomarkers of adjuvant chemotherapy in NSCLC are desperately needed to determine which patients derive the most benefit. Conversely, identification of those unlikely to benefit opens the opportunity for novel approaches to adjuvant therapy in these patients. Individualizing chemotherapy based on multiple candidate biomarkers in lung cancer has recently failed to demonstrate significant clinical benefit in several clinical trials (35, 36), underscoring the need for better markers.

Common alterations in SWI/SNF in NSCLC have only been recently elucidated. No predictive studies of *SMARCA4* using clinical tissues have been published to date and very few studies have been published analyzing the prognostic effect. Importantly, this study is the first to demonstrate the predictive effects of *SMARCA4*/BRG1 in NSCLC using patient samples from the JBR. 10 trial. In this study, we validated in a large cohort that decreased *SMARCA4* is associated with worse prognosis in patients harboring lung adenocarcinomas using the Director's Challenge Lung Study. Notably, this study also demonstrated for the first time that low *SMARCA4*/BRG1 expression is associated with increased benefit from cisplatin-based chemotherapy in resectable NSCLC using specimens from the JBR.10 trial.

The connections between DNA repair and chromatin remodeling have only recently begun to be explored. In particular, SWI/SNF remodeling complexes have also been implicated in NER, a critical pathway involved in cisplatin resistance. Recently, BRG1 has been shown to affect the stability of XPC protein as well as the recruitment of XPG and PCNA, which are all essential proteins within NER (22). In a recent paper, knockdown of BRG1 or BRM in H460 lung cancer cells increased cisplatin sensitivity and showed reduced repair of both intrastrand and interstrand adducts suggesting that the mechanism of sensitivity is primarily

due to defects in DNA repair (23). In addition, this previous study suggested that BRG1 is important for ERCC1 recruitment, a well known important mediator of NER (23). Of importance, the phenotype of cisplatin resistance was not as pronounced for the BRG1 or BRM knockdowns as previously shown for XPF and ERRC1 demonstrating the different roles of chromatin remodeling and repair proteins in cisplatin sensitivity (23). Given the complexity of the data which has arisen from ERCC1 as a potential biomarker and the known connection of ERCC1 and *SMARCA4*/BRG1, a panel of molecular biomarkers comprised of both epigenetic regulators and DNA repair/response genes to assess activity may be necessary to accurately select patients for platinum-based regimens in NSCLC and other cancers. In addition, an alternative mechanism of sensitivity to cisplatin in tumors that have loss of *SMARCA4* and/or *SMARCA2* is loss of Rb activity leading to inhibition of a DNA damage-induced cell cycle checkpoint. This could be of particular importance in patients that have concomitant loss of both *SMARCA4* and *SMARCA2* which is demonstrated by a previous *in vitro* study where cancer cells that have loss of both BRG1/*SMARCA4* and BRM/*SMARCA2* showed loss of the Rb-dependent cisplatin-induced cell cycle checkpoint (37). Due to the growing evidence of the role *SMARCA4* on DNA damage response, DNA repair, and drug sensitivity *in vitro*, it is imperative that the effects of *SMARCA4* as a predictive biomarker using clinical specimens is further investigated. Moreover, the best detection method for its predictive value still needs to be determined specifically in regards to mutation vs expression vs protein analysis. Even for expression analysis in this study it is clear that unique probe sets result in slightly different results likely due to hybridization to different areas of the gene (Supplementary Fig. S7) and expression of multiple transcripts. A limitation of this study was that only mRNA expression datasets were analyzed as these were publicly available from both the Director's Challenge Study and JBR.10. Although mutations are common in clinical specimens, there is evidence that some patients lack expression but have no mutations as we and others have previously found (11, 15). Therefore, a multi-platform approach for detection of *SMARCA4*/BRG1 along with other epigenetic regulators (including *SMARCA2*) and DNA repair/response proteins may be in order.

Importantly, our study is the first to show that *SMARCA4*/BRG1 can be used as a predictive biomarker in clinical specimens. Specifically, our results utilizing expression data from the JBR.10 trial demonstrated *SMARCA4* expression levels depict efficacy of cisplatin and vinorelbine in the setting of stage IB–II resectable NSCLC independent of age, stage, and histology. Thus, patients (even those older than 65) with low levels of *SMARCA4*/BRG1 expression appear to be excellent candidates for platinum-based chemotherapy regimens based on an overall survival advantage in the JBR.10 trial. Further research on the predictive effect of *SMARCA4*/BRG1 on cisplatin therapy and other DNA repair targeted therapies as well as other SWI/SNF components and methods of detection is warranted to assess their potential to serve as a companion diagnostic in NSCLC.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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TRANSLATIONAL RELEVANCE

Predictive biomarkers of chemotherapy response in NSCLC are needed to better characterize patients who derive the greatest benefit. We hypothesized that *SMARCA4*/BRG1 could be such a marker due to its established role in cisplatin sensitivity and DNA repair *in vitro*. No studies on the predictive effect of *SMARCA4*/BRG1 using clinical tissues have been published to date and very few prognostic studies with limited sample sizes have been published. We analyzed data available from both the Director's Challenge Lung Study and the JBR.10 phase III randomized trial in a hypothesis-driven manner to determine the prognostic and predictive role of *SMARCA4*/BRG1 from two prospective studies. Importantly, this study is the first to demonstrate the predictive effects with a highly significant interaction test of *SMARCA4*/BRG1 in NSCLC using patient samples from a randomized trial with an untreated control. We also validated in a large cohort that decreased *SMARCA4* is associated with worse prognosis.

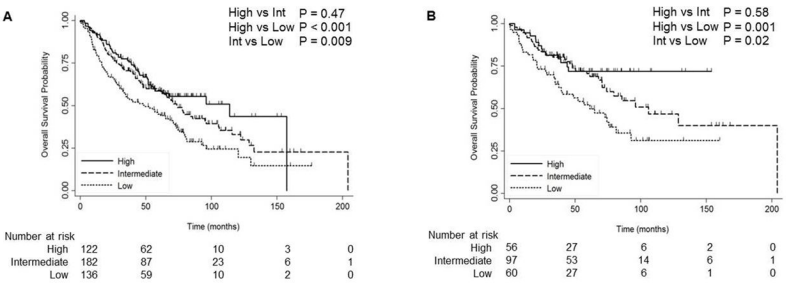

Figure 1.

Overall survival curves for patients with high, intermediate, and low levels of *SMARCA4* (212520_s_at) expression in the Director's Challenge Study. (A) all patients; (B) patients without adjuvant treatment. Log-rank P-values are shown.

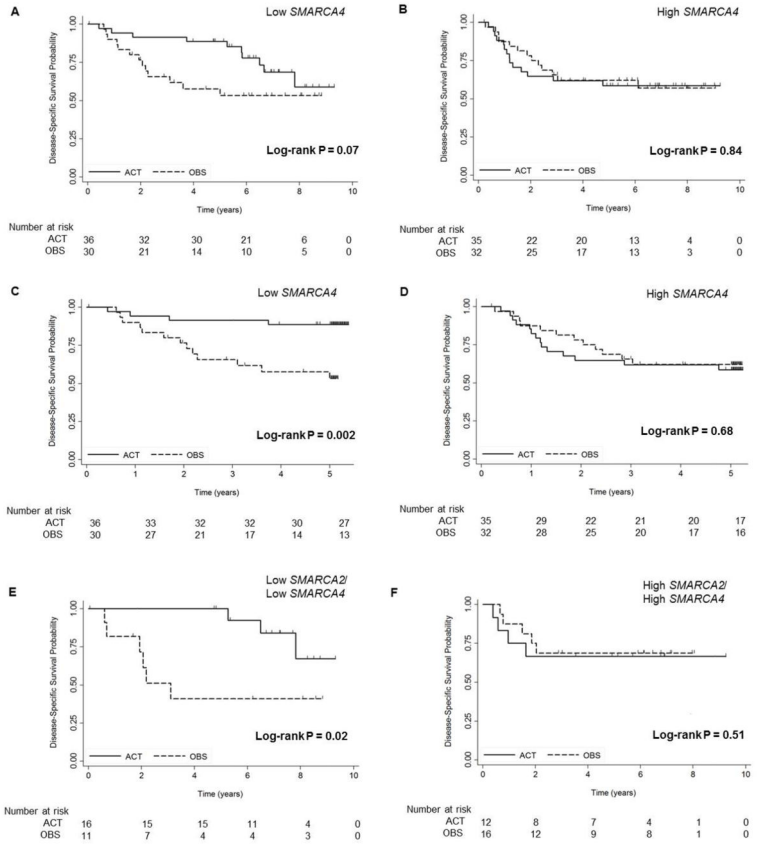

Figure 2.

Overall and five-year disease-specific survival curves by treatment arm (ACT or OBS) for patients with low (A and C) and high (B and D) levels of *SMARCA4* (213719_s_at) expression, and low (E) and high (F) levels of both *SMARCA4* (213719_s_at) and *SMARCA2* (206543_at) expression in the JBR.10 trial. OBS, observation. ACT, adjuvant chemotherapy.

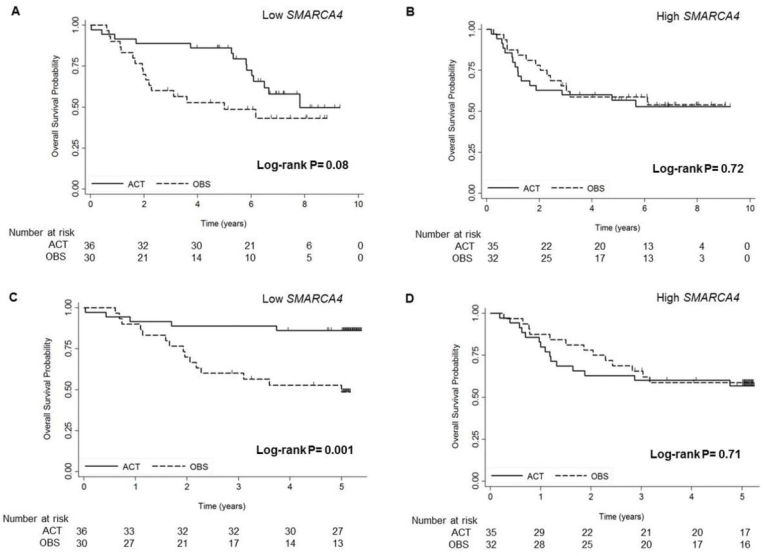

Figure 3.

Comparison of overall survival and five-year overall survival by treatment arm for patients with low (A and C) and high (B and D) level of *SMARCA4* (213719_s_at) in the JBR.10 trial. OBS, observation. ACT, adjuvant chemotherapy.

Table 1

Clinical Characteristics of Patients Analyzed for *SMARCA4* from the Director's Challenge Study & JBR.10 Trial

	Director's Challenge Study *SMARCA4* Expression					JBR.10 *SMARCA4* Expression		
Clinical Factor	**Low**	**Int**	**High**	**P-value**	**Clinical Factor**	**Low**	**High**	**P-value**
Age				0.52	**Age**			0.76
<=65 (n=230)	66 (29%)	100 (43%)	64 (28%)		<=65 (n=87)	44 (51%)	43 (49%)	
>65 (n=210)	70 (33%)	82 (39%)	58 (28%)		>65 (n=66)	22 (48%)	24 (52%)	
Gender				0.02	**Gender**			0.42
Female (n=219)	57 (26%)	90 (41%)	72 (33%)		Female (n=42)	23 (55%)	19 (45%)	
Male (n=221)	79 (36%)	92 (42%)	50 (22%)		Male (n=91)	43 (47%)	48 (53%)	
Treatment				0.19	**Treatment**			0.78
Adjuvant treatment (n=45)	17 (38%)	20 (44%)	8 (18%)		Adjuvant treatment (n=71)	36 (51%)	35 (49%)	
No Adjuvant treatment (n=213)	60 (28%)	97 (46%)	56 (26%)		No adjuvant treatment (n=62)	30 (48%)	32 (52%)	
Stage				0.46	**Stage**			0.53
IA (n=113)	33 (29%)	48 (42%)	32 (28%)					
IB (n=162)	49 (30%)	67 (41%)	46 (29%)		IB (n=73)	38 (52%)	35 (48%)	
IIA+IIB (n=94)	26 (28%)	39 (41%)	29 (31%)		IIA+IIB (n=60)	28 (47%)	32 (53%)	
IIIA+IIIB (n=68)	28 (41%)	26 (38%)	14 (21%)					
Histology					**Histology**			**0.22**
ADC (n=440)	**136 (31%)**	**182 (41%)**	**122 (28%)**		**ADC (n=71)** **SQCC (n=52)** **LCUC (n=10)**	**40 (56.3%)** **21 (40.4%)** **5 (50%)**	**31 (43.7%)** **31 (59.6%)** **5 (50%)**	

*P-values were calculated using X^2 Test

The probe sets that were the most statistically significant in correlation with clinical outcomes are shown for each study. Probe set 212520_s_at was used for the Director's Challenge Study and probe set (213719_s_at) was used for the JBR.10 study.

ADC = adenocarcinoma, SQCC = squamous cell carcinoma, LCUC = large cell carcinoma

Table 2

Multivariate analysis of *SMARCA4* expression in the Director's Challenge Study

	Multivariate Analysis			Multivariate Analysis (no adjuvant treatment)		
Variable	**HR**	**95% CI**	**P-value**	**HR**	**95% CI**	**P-value**
***SMARCA4* (212520_s_at)**						
Low expression	1.0			1.0		
Intermediate expression	0.7	(0.5, 0.9)	0.01	0.7	(0.4, 1.1)	0.09
High expression	0.6	(0.4, 0.8)	0.002	0.4	(0.2, 0.8)	0.01
Age						
<65 years	1.0			1.0		
≥65 years	1.6	(1.2, 2.1)	<0.001	1.8	(1.1, 2.9)	0.01
Stage						
I	1.0			1.0		
II–III	3.1	(2.4, 4.1)	<0.001	3.8	(2.5, 5.9)	<0.001
Gender						
Female	1.0			1.0		
Male	1.3	(1.0, 1.7)	0.05	1.2	(0.8, 1.9)	0.40
Differentiation						
Well	1.0			1.0		
Moderate	0.9	(0.6, 1.3)	0.51	0.9	(0.5, 1.6)	0.60
Poorly	1.1	(0.7, 1.6)	0.84	1.0	(0.5, 1.9)	0.90

Table 3

Multivariate analysis of *SMARCA4* expression and treatment arm (Observation vs Cisplatin/Vinerolbine) in the JBR.10 Trial

			Multivariate Analysis (DSS)			Multivariate Analysis (5YR DSS)				Multivariate Analysis (OS)			Multivariate Analysis (5YR OS)			
Probe	**Variable**	**Comparison**	**HR**	**95% CI**	**P-value**	**HR**	**95% CI**	**P-value**	**Interaction P-value**	**HR**	**95% CI**	**P-value**	**HR**	**95% CI**	**P-value**	**Interaction P-value***
213719_s_at	Low *SMARCA4*	**ACT vs OBS**	**0.3**	**(0.1, 0.8)**	**0.02**	**0.1**	**(0.0, 0.5)**	**0.002**	**0.01**	**0.4**	**(0.2, 0.9)**	**0.02**	**0.1**	**(0.0, 0.5)**	**<0.001**	**0.007**
		Stage: II vs I	5.3	(2.0, 14.1)	<0.001	5.9	(1.8, 18.7)	0.003		3.1	(1.4, 6.9)	0.005	5.0	(1.8, 14.4)	0.003	
		Age: ≥65 vs <65	2.6	(1.0, 7.0)	0.05	4.2	(1.3, 13.8)	0.02		2.3	(1.0, 5.2)	0.05	4.2	(1.5, 12.1)	0.008	
		Histology: SQCC vs ADC	0.6	(0.2, 1.6)	0.29	0.7	(0.2,2.3)	0.60		0.7	(0.3,1.6)	0.43	0.9	(0.3,2.5)	0.83	
		Histology: LCUC vs ADC	4.3	(1.1,17.5)	0.04	8.1	(1.8,37.0)	0.007		2.6	(0.7,9.5)	0.16	6.8	(1.6,29.3)	0.01	
	High *SMARCA4*	ACT vs OBS	1.0	(0.4, 2.1)	0.91	1.0	(0.5, 2.3)	0.92		1.0	(0.5, 2.1)	0.99	1	(0.5, 2.2)	0.96	
		Stage: II vs I	1.5	(0.7, 3.2)	0.33	1.4	(0.6, 3.0)	0.42		1.4	(0.7, 2.8)	0.40	1.4	(0.6, 2.9)	0.41	
		Age: ≥65 vs <65	2.5	(1.1, 5.4)	0.03	2.5	(1.1,5.7)	0.02		2.7	(1.3, 5.7)	0.01	2.6	(1.2,5.5)	0.02	
		Histology: SQCC vs ADC	0.3	(0.1,0.6)	0.003	0.3	(0.1,0.7)	0.005		0.3	(0.1,0.7)	0.004	0.3	(0.1,0.7)	0.005	
		Histology: LCUC vs ADC	1.1	(0.3,4.0)	0.83	1.2	(0.3,4.2)	0.78		1.1	(0.3,3.7)	0.93	1.1	(0.3,4)	0.84	
208794_s_at	Low *SMARCA4*	**ACT vs OBS**	**0.4**	**(0.2, 0.9)**	**0.03**	**0.3**	**(0.1, 0.9)**	**0.03**	**0.22**	**0.5**	**(0.2, 1.2)**	**0.13**	**0.4**	**(0.2, 1.0)**	**0.04**	**0.37**
		Stage: II vs I	3.6	(1.4, 8.9)	0.007	2.8	(1.0, 7.6)	0.05		2.7	(1.2, 6.0)	0.02	3.0	(1.1, 8.1)	0.03	
		Age: ≥65 vs <65	2.3	(0.9, 5.5)	0.07	2.4	(0.9, 6.4)	0.09		1.8	(0.8, 1.0)	0.17	2.1	(0.8, 5.4)	0.14	
		Histology: SQCC vs ADC	0.4	(0.1,1.0)	0.05	0.5	(0.2,1.4)	0.18		0.3	(0.1,0.7)	0.01	0.4	(0.2,1.3)	0.13	
		Histology: LCUC vs ADC	4.9	(0.9,26.0)	0.06	6.6	(1.2,37.0)	0.03		2.8	(0.6,13.5)	0.21	5.9	(1.1,32.4)	0.04	
	High *SMARCA4*	ACT vs OBS	0.9	(0.4, 1.9)	0.70	0.7	(0.3, 1.7)	0.46		0.7	(0.4, 1.5)	0.43	0.7	(0.3, 1.4)	0.29	
		Stage: II vs I	2.0	(0.9, 4.4)	0.07	2.1	(0.9, 4.7)	0.08		1.6	(0.8, 3.2)	0.17	1.9	(0.9, 4.0)	0.09	
		Age: ≥65 vs <65	2.0	(0.9, 4.6)	0.09	2.3	(1.0, 5.4)	0.05		2.8	(1.3, 5.8)	0.007	2.8	(1.3, 6.1)	0.01	
		Histology: SQCC vs ADC	0.5	(0.2, 1.3)	0.15	0.6	(0.2, 1.4)	0.23		0.7	(0.3, 1.6)	0.45	0.7	(0.3,1.6)	0.39	
		Histology: LCUC vs ADC	1.7	(0.6,5.3)	0.34	2.0	(0.7,6.3)	0.22		1.7	(0.6,5.1)	0.36	1.9	(0.6,6.0)	0.25	

*interaction P-values displayed are for 5-YR disease-specific survival (DSS) or overall survival (OS) ADC = adenocarcinoma, SQCC = squamous cell carcinoma, LCUC = large cell carcinoma