

Original Article

Aryl hydrocarbon receptor activated by benzo (a) pyrene promotes SMARCA6 expression in NSCLC

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Abstract: Recent studies suggest that individual subunits of chromatin-remodeling complexes generate epigenetically specific signaling in tumorigenicity. The impact of environmental factors on the chromatin-remodeling factor has not been thoroughly elucidated to date. We detected the expression level of SMARCA6 (SWI/SNF2-Related, Matrix-Associated, Actin-Dependent Regulator of Chromatin, Subfamily A, Member 6) in NSCLC (Non-small-cell lung carcinoma) and measured it through quantitative real-time PCR (qRT-PCR) and immunohistochemistry. The effects of BaP on proliferation and cell cycle progression were evaluated using MTT, colony formation and FACS analyses. Tumor growth in vivo was observed in a xenograft model. ChIP and qPCR were performed to validate that SMARCA6 was a potential target of AhR in NSCLC. As a result, BaP increased SMARCA6 expression. Smoking was linked with elevated SMARCA6 expression in NSCLC. BaP promoted cancer progression in vitro and in vivo. ChIP assay confirmed that BaP increases SMARCA6 expression via recruitment of AhR and induces SMARCA6 expression by facilitating AhR translocation to the nucleus. Furthermore, inhibition of AhR expression decreases SMARCA6 expression in NSCLC. Finally, knockdown of SMARCA6 attenuates BaP-induced cancer progression. This study demonstrates that BaP promotes proliferation by activation of AhR, which promotes SMARCA6 expression, and may identify new diagnostic and therapeutic targets in lung cancer.

Keywords: SMARCA6, BaP, AhR, smoking, lung cancer

Introduction

Lung cancer is the leading cause of cancer-related deaths, and the most common cancer worldwide, including China. Lung cancer is classified into small cell lung cancer and non-small cell lung cancer (NSCLC), including adenocarcinoma (ADC) and squamous cell carcinoma (SCC), which accounts for 80 to 85% of all cases of lung cancer [1, 2]. Lung cancer is associated with environmental factors such as cigarette smoke and air pollution. Smoke and air pollution contain chemicals which calls polycyclic aromatic hydrocarbons (PAHs). PAHs are reported as major carcinogens in regions where

air is polluted by PM2.5/PM10 particulate [3]. These chemicals cause epigenetic changes in the lung, which are major risk for developing inflammation, oncogene senescence, and lung cancer progression [4, 5]. Combination epigenetic therapy induces depletion of MYC and can increase responsiveness to interferon, potentiate T-cell attraction, and exert direct antiproliferative activity [6]. However, the link between PAHs, epigenetics and gene expression in lung cancer is unclear.

The SWI/SNF-related, matrix-associated, actin-dependent regulators of chromatin (SMARCA6), also called BRG1-associated factors, are com-

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ponents of human SWI/SNF-like chromatin-remodeling protein complexes. SMARCA6, also called lymphoid-specific helicase (LSH), is critical for the normal development of plants and mammals, because it maintains optimum levels of DNA methylation and acts as a critical chromatin modifier [7-11]. SMARCA6 maintains genome stability in mammalian somatic cells [12]. SMARCA6 also contributes to the malignant progression of prostate cancer, melanoma, nasopharyngeal carcinoma, and glioma [13-16]; however, the correlation between PAHs and SMARCA6 has not been thoroughly elucidated to date.

The aryl hydrocarbon receptor (AhR), a ligand-operated transcription factor, is a xenosensor traditionally associated with xenobiotic metabolism [17]. AhR facilitates tumor progression, disease tolerance defense, intestinal immunity and B-cell proliferation [18-21]. Interestingly, AhR influences the major stages of tumorigenesis, and studies of aggressive tumors and tumor cell lines have shown increased levels of AhR protein and constitutive nuclear localization in cancer tissue, whereas in normal tissues, AhR is primarily inactive and resides in the cytoplasm [22, 23].

In this study, we show that BaP promotes proliferation by activation of AhR, which promotes SMARCA6 expression, and may identify new diagnostic and therapeutic targets in lung cancer.

Materials and methods

Animal study

Four-week-old male BALB/C-NU mice were purchased from Hunan SJA Laboratory Animal Co., Ltd. (Changsha, China). The animal study protocol was approved by the Institutional Animal Care and Use Committee of the Central South University of Xiangya School of Medicine and conformed to the legal mandates and federal guidelines for the care and maintenance of laboratory animals. For protein overexpression or knockdown, cells were transfected with lentivirus in 10 mg/ml polybrene, for 24 hours. Media was changed, and 2 mg/ml puromycin was supplemented for 7 days to promote selection. Cells were counted, and 5×10^6 cells were subcutaneously injected into the bilateral regions of the nude mice. Tumor volumes were later

measured every 3 days until sacrifice at 31 days. Tumors were weighed and fixed in 10% formalin, followed by paraffin embedding or extraction of RNA with Trizol reagent.

Ethics approval and consent to participate

This study was conducted at the Cancer Research Institute, Central South University, Hunan, China. All of the protocols were reviewed and approved by the Joint Ethics Committee of the Central South University Health Authority and performed in accordance with national guidelines.

Cell lines and plasmids

The human lung bronchus cell line HBE (CRL-2741) and lung carcinomatous cell lines H358 (CRL-5807) and A549 (CCL-185) were obtained from American Type Culture Collection (University Boulevard, Manassas, VA, USA). The human lung carcinomatous cell lines SPC-A-1 and PC9 was stored in the Department of Molecular Biology, Cancer Research Institute (Central South University, Hunan, China). The cell lines were characterized by mycoplasma detection, DNA fingerprinting, isozyme detection, and cell viability by the provider; no further authentication of the cell lines was conducted. HBE, H358, PC9 and SPC-A-1 cells were cultured in Roswell Park Memorial Institute 1640 Media (Gibco) supplemented with 10% FBS, penicillin, and streptomycin. A549 cells were cultured in Dulbecco's Modified Eagle's Medium/Nutrient Mixture F-12 (Gibco) supplemented with 10% FBS, penicillin, and streptomycin.

AhR shRNA vectors and control (GV248) were purchased from GeneChem Co., Ltd., Shanghai, China. Transfection of plasmids was performed using Lipofectamine® 2000 according to the manufacturer's protocol. Stable expressing colonies were selected using 2 mg/ml puromycin.

Cell proliferation and colony formation

Cell proliferation assay was performed with MTT according to the manufacturer's protocol. First, 500 cells were cultured in a 96-well plate. OD₄₅₀ was measured 1 hour after adding MTS. For the cell colony formation assay, approximately 300 cells were seeded into each well of 6-well plates and cultured in media. After 2

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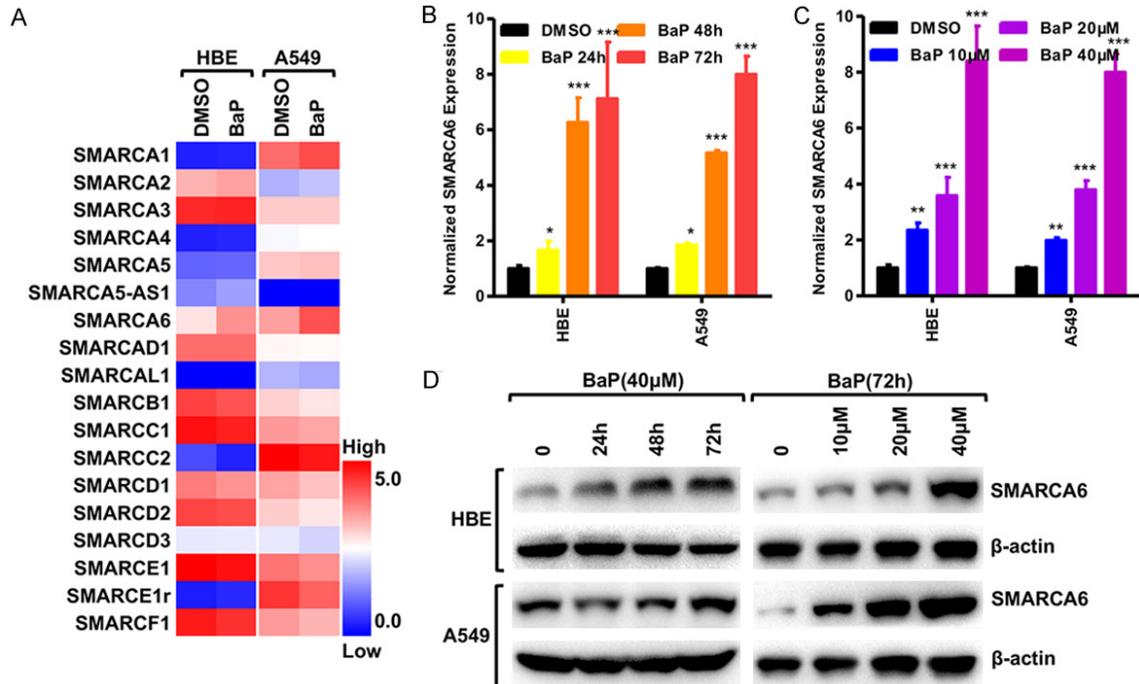


Figure 1. BaP increased SMARCA6 expression. (A) PCR array assessing the expression level of 18 SMARC genes in HBE and A549 cells after treatment with BaP. Log₂-fold-change in RNA expression over the DMSO control; blue to red color gradation is based on the ranking of each condition from minimum (blue) to maximum (red). (B, C) qRT-PCR was used to detect SMARCA6 mRNA levels in HBE and A549 cells, in a time- (B) and dose-dependent manner (C) Treated with BaP. (D) Western blot shows the levels of SMARCA6 protein in HBE and A549 cells in a time- and dose-dependent manner in response to BaP. *P < 0.05, **P < 0.01, ***P < 0.001.

weeks, cells were treated with methanol and stained with 0.1% crystal violet. The number of visible colonies with > 0.05 mm diameter were scored.

Cell cycle analysis

For cell cycle analysis assay, cells were washed twice with 1 × PBS, trypsinized, centrifuged at 4°C for 5 min, and washed with 1 × PBS twice. Cells were later stained with FxCycle PI/RNase Staining Solution in the dark for 30 min at room temperature. The events of cell cycle progression were analyzed using the BD FACSCanto Flow Cytometer (BD Biosciences). Flow cytometry data were analyzed using FlowJo to calculate cell-cycle distribution.

RT-qPCR

Total RNA was isolated using Trizol reagent. cDNA was generated using the PrimeScript™ RT reagent kit with gDNA Eraser (Perfect Real Time). Real-Time PCR was performed using ABI 7500 with FastStart Universal SYBR Green

Master (ROX). All gene expression levels were normalized by subtracting the Ct of the ACTB. The RT-qPCR primer sequences are listed below: SMARCA6-Forward: GATTTTGGATCGAATGCTGCCAG, SMARCA6-Reverse: ATGGACCCATCAAGCCTGCTGA; AhR-Forward: GTCGTCTAAGGTGTCTGCTGGA, AhR-Reverse: CGCAAACAAA-GCCAACTGAGGTG.

Subcellular fractionation

To determine the cellular localization of AhR, cell cytoplasm and nuclear fractions were isolated and collected with Nuclear and Cytoplasmic Extraction Reagents Kit (Thermo Fisher Scientific) according to the manufacturer's instructions.

Histology and immunohistochemistry

Lung and related diseases biopsies were validated and obtained from Department of Pathology in Xiangya Hospital. IHC analysis of paraffin sections, from lung cancer tissues was previously described. The images were ana-

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Table 1. Correlation between SMARCA6 expression and clinicopathological parameters of NSCLC (n = 70)

Clinical parameters	Factors	n	Relative high	Relative low	P values
Ages	≤ 60	36	12	24	0.4973
	> 60	34	14	20	
Gender	Male	51	22	29	0.3800
	Female	19	6	13	
Smoking history	Yes	31	20	11	0.0049
	No	39	12	27	
Type	ADC	35	9	26	0.0076
	SCC	35	20	15	
Differentiation	Poor	31	10	21	0.0261
	Well or Moderate	39	23	16	
T-classification	T1-T2	44	14	30	0.0337
	T3-T4	26	15	11	
N- classification	N0	31	15	16	0.0485
	N1-N3	39	10	29	
Clinical Stage	I-II	38	14	24	0.0324
	III-IV	32	20	12	

Chi-square test. *P < 0.05.

lyzed and captured using a CX41 microscope (OLYMPUS, Tokyo, Japan) equipped with a Microscope Digital Camera System DP-72 (OLYMPUS, Tokyo, Japan), and differentially quantified by two pathologists from the Xiangya Hospital, Changsha, China.

Immunoblotting

Cells were harvested, washed with PBS, and lysed in IP lysis buffer with protease inhibitors cocktail. Cell lysates were separated on an SDS-polyacrylamide gel, transferred to a polyvinylidene fluoride membrane, and immunoblotted using the following primary antibodies. Protein detection was performed using mouse monoclonal anti-β-actin (Sigma Aldrich, A5441), mouse monoclonal anti-α-tubulin (Santa Cruz, sc-5286), rabbit polyclonal anti-histone-H3 (Proteintech, 17168-1-AP), rabbit polyclonal anti-AhR (Santa Cruz, sc-5579), mouse monoclonal anti-Flag (Santa Cruz, sc-166355), and mouse monoclonal anti-SMARCA6 (Santa Cruz, sc-46665).

Statistical analysis

The data in the figure legends are presented as the means ± SD. The exact values of the sample size (n) are presented in the figure legends

and reflect either the number of experimental replications with cells, biochemical model systems, or the number of animals used in *in vivo* experiments. For measurements of human samples, *n* represents the number of patients. Statistical analyses were performed by either Student's *t* test or one-way ANOVA for normally distributed data, and by Kruskal-Wallis one-way ANOVA on the ranks for data that were not normally distributed. When the overall ANOVA revealed a significant effect, the data were further analyzed with the Tukey/Dunn post hoc test to determine specific group differences. The statistical significance of differences was set at *P < 0.05, **P < 0.01, ***P < 0.001.

Results

BaP increases SMARCA6 expression

PAHs were reported to be the major carcinogens in PM2.5/PM10 particles [3]. To test the effects of PAHs on *SMARC* genes, bronchial epithelial cell line HBE and lung adenocarcinoma cell line A549 were exposed to a representative PAH compound, benzo (a) pyrene (BaP), at 1 mM for 3 days. PCR arrays showed that expression of SMARCA6 was significantly up-regulated among the 18 *SMARC* genes with BaP treatment (**Figure 1A**). We further confirmed that BaP up-regulated SMARCA6 at mRNA levels in dose- and time-dependent manners in HBE and A549 cells (**Figure 1B, 1C**). Finally, Western blot analysis confirmed that BaP up-regulated SMARCA6 protein levels with BaP treatment (**Figure 1D**).

Smoking promoted SMARCA6 expression in NSCLC

To explore the expression of SMARCA6 in NSCLC, we examined the mRNA level of SMARCA6 in NSCLC tissues compared with adjacent normal tissues (n = 70). We determined that SMARCA6 expression was significantly increased in NSCLC samples compared with the adjacent normal tissues. Patient characteristics and correlations with SMARCA6

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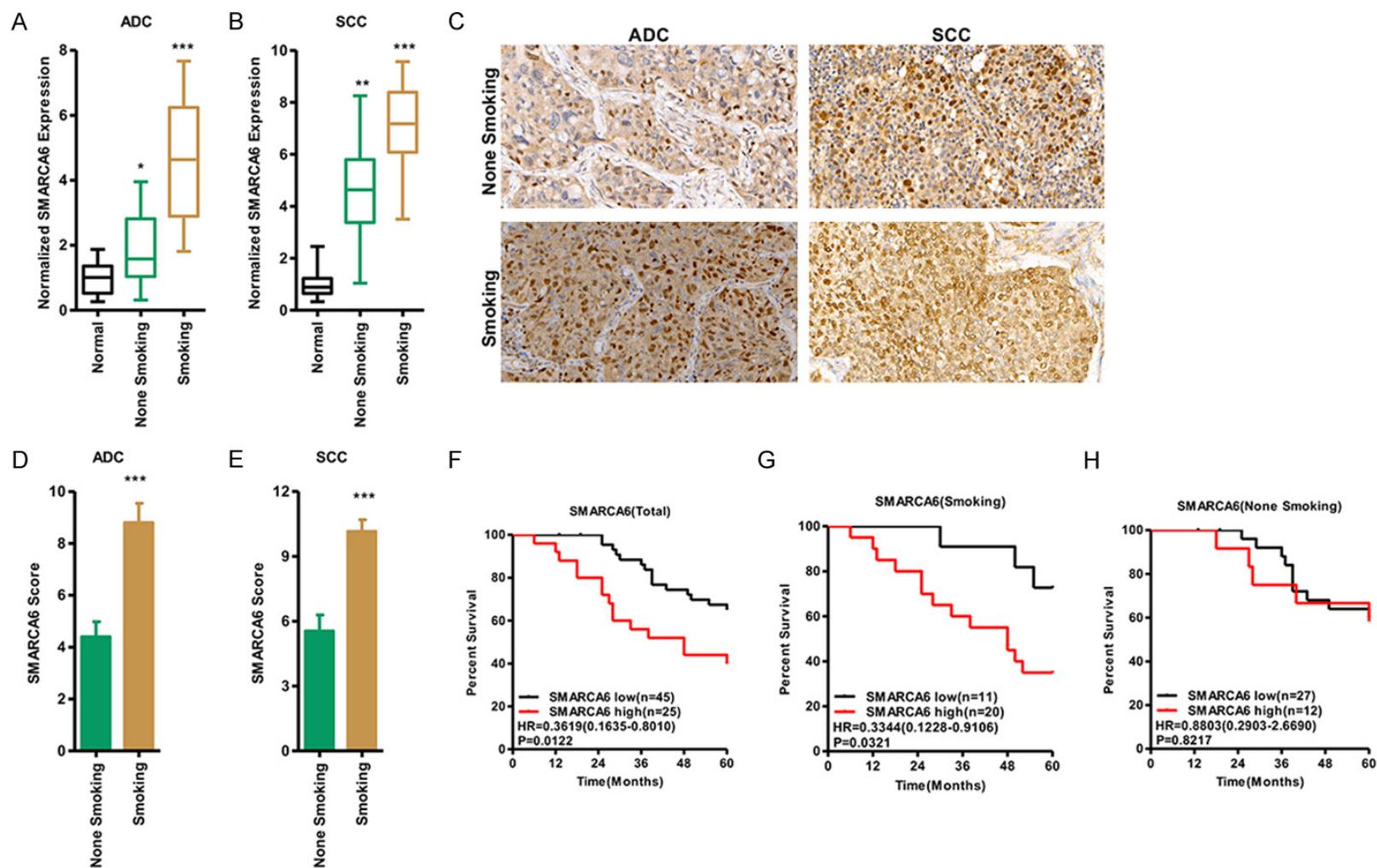


Figure 2. Smoking links to elevated SMARCA6 expression in NSCLC. (A, B) qRT-PCR detected SMARCA6 mRNA level in lung ADC (A) and SCC (B) of smokers versus non-smokers. (C-E) IHC was performed using antibodies against SMARCA6 in human lung ADC and SCC of smokers and non-smokers (C); the mean values of quantitative immunohistochemistry are shown (D for ADC; E for SCC). (F-H) Kaplan-Meier curve, showing the overall survival rates associated with samples measured for the expression of SMARCA6, in the NSCLC tissues of smokers in lung cancer (F), smoking (G), and non-smoking (H). *P < 0.05, **P < 0.01, ***P < 0.001.

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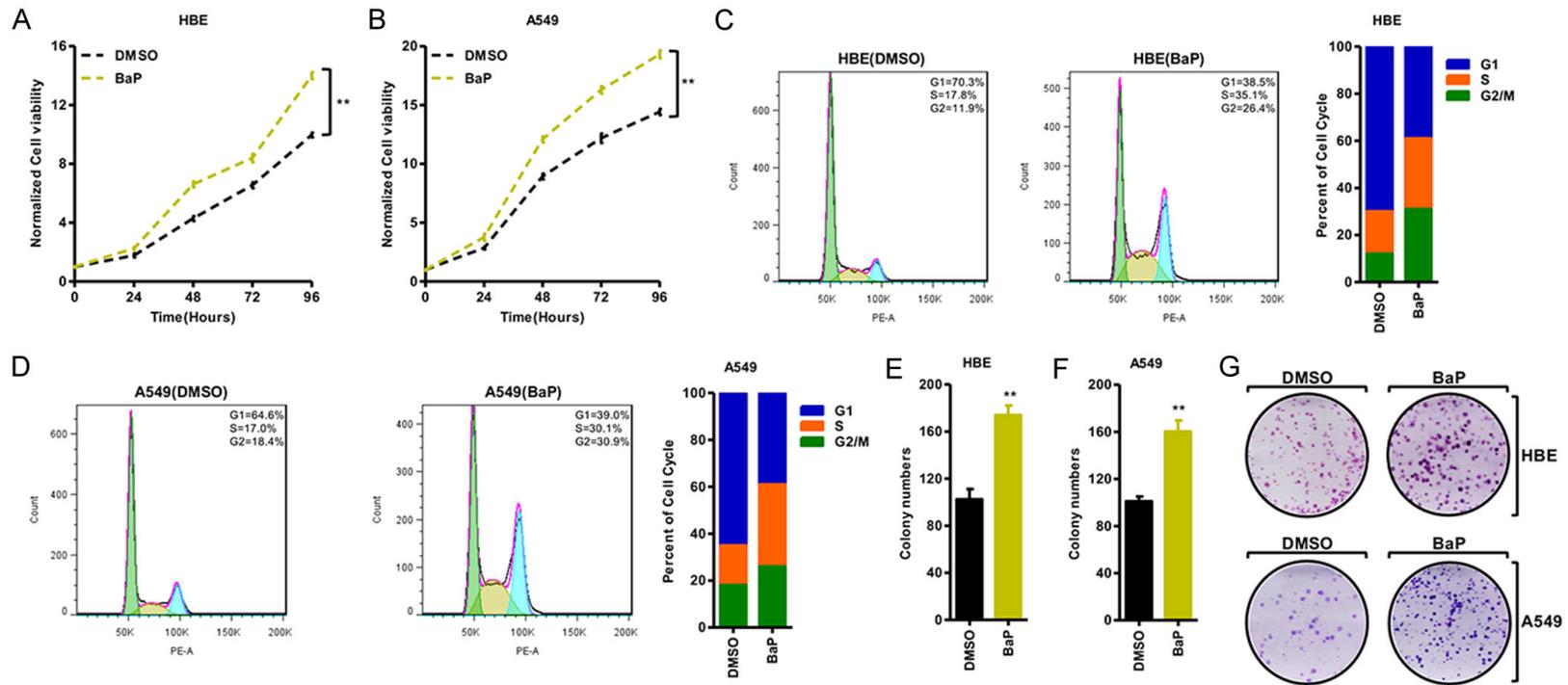


Figure 3. BaP promoted cancer progression in NSCLC in vitro. (A, B) MTT assay was applied to assess cell viability in HBE (A) and A549 (B) treated with BaP, and the results indicate that BaP promotes cell growth. (C, D) BaP promotes the transition of G1/S to G2/M cell cycle phases in HBE (C) and A549 (D) that were treated with BaP, the representative images are shown in the left. (E-G) Growth in the plate was assessed with colony formation assay in HBE (E) and A549 (F) cells; the representative images are shown (G), and the results show that BaP promotes colony formation. *P < 0.05, **P < 0.01, ***P < 0.001.

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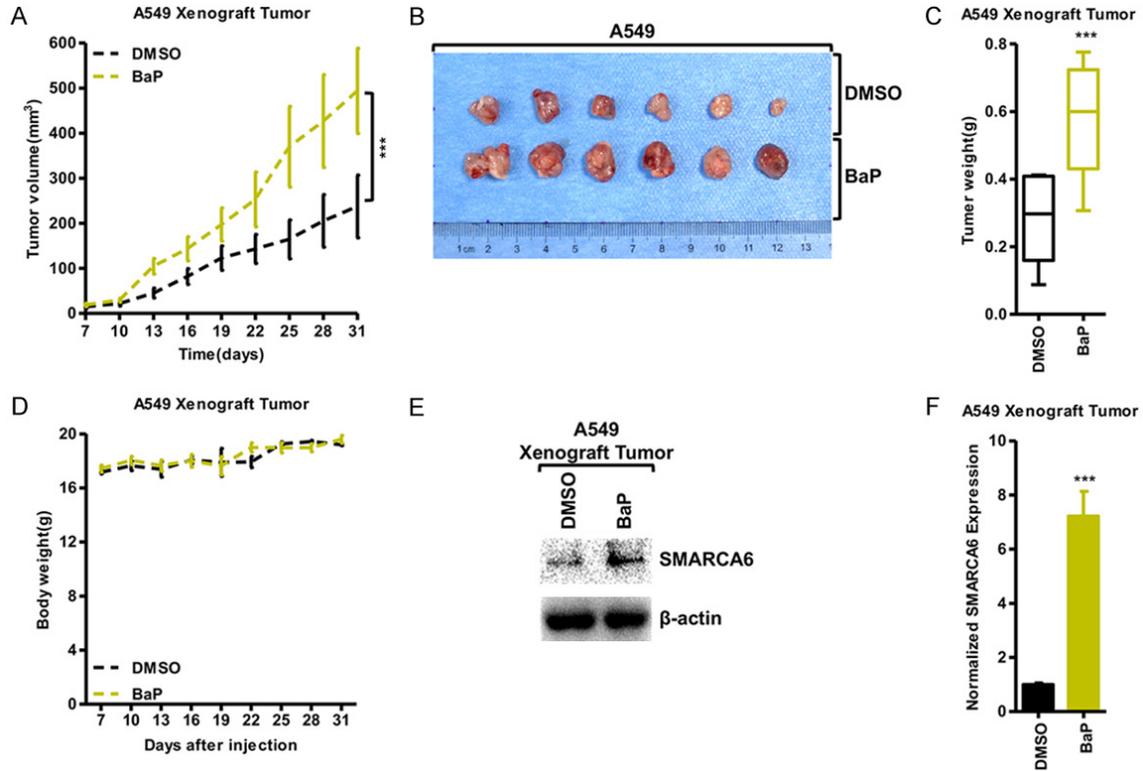


Figure 4. BaP promoted cancer progression in vivo. (A-D) Tumor volumes in nude mice after injection of A549 cells treated with BaP for 90 days at the indicated times (A); tumor formation (B) and weights (C) were recorded. (D) Nude mice after injection of A549 cells treated with BaP for 90 days; the weights of the mice were recorded at the indicated time points (n = 6). *P < 0.05, **P < 0.01, ***P < 0.001. (E, F) Western blot (E) and qRT-PCR (F) demonstrated that BaP treatment in the A549 xenograft tumor increased the expression of SMARCA6.

expression level are summarized in **Table 1**. SMARCA6 was significantly correlated with smoking history (P < 0.01), tumor type (P < 0.01), tumor differentiation (P < 0.05), classification (P < 0.05), clinical stage (TNM, P < 0.05), depth of invasion (P < 0.05) and relapse (P < 0.01). No statistically significant correlations were obtained between SMARCA6 and other clinicopathological characteristics, such as age or gender (P > 0.05) (**Table 1**). Importantly, the expression of SMARCA6 was significantly higher in smokers than in non-smokers in both SCC and ADC, suggesting a potential association between smoking and SMARCA6 expression level (**Figure 2A, 2B**). A total of 56 archived paraffin-embedded NSCLC specimens with extensive clinical follow-up, including 30 cases of ADC and 26 cases of SCC were analyzed by IHC staining with antibody against human SMARCA6. We found that SMARCA6 was up-regulated in all smoking tissues of NSCLC specimens (15 for ADC, 13 for SCC) compared with that in non-smoking tissues (**Figure 2C-E**). A Kaplan-Meier analysis was used to evaluate

the relationship between SMARCA6 expression level and patient survival in NSCLC, and the results showed that high SMARCA6 expression was also associated with poor survival. The survival time of patients with low SMARCA6 expression (n = 45) was longer than that in patients with high SMARCA6 expression (n = 25) (P < 0.05, **Figure 2F**). Among patients who were smokers, the survival time of patients with low SMARCA6 (n = 11) was longer than in patients with high SMARCA6 (n = 20) (P < 0.05, **Figure 2G**), whereas the survival time of patients showed no significant changes among patients who were not smokers (P > 0.05, **Figure 2H**), indicating that smoking and SMARCA6 expression were associated with overall survival in lung cancer.

BaP increases cancer progression in vitro and in vivo

To further uncover the physiological role of BaP in cancer, we treated HBE and A549 cells with 1 mM of BaP. We determined that BaP signifi-

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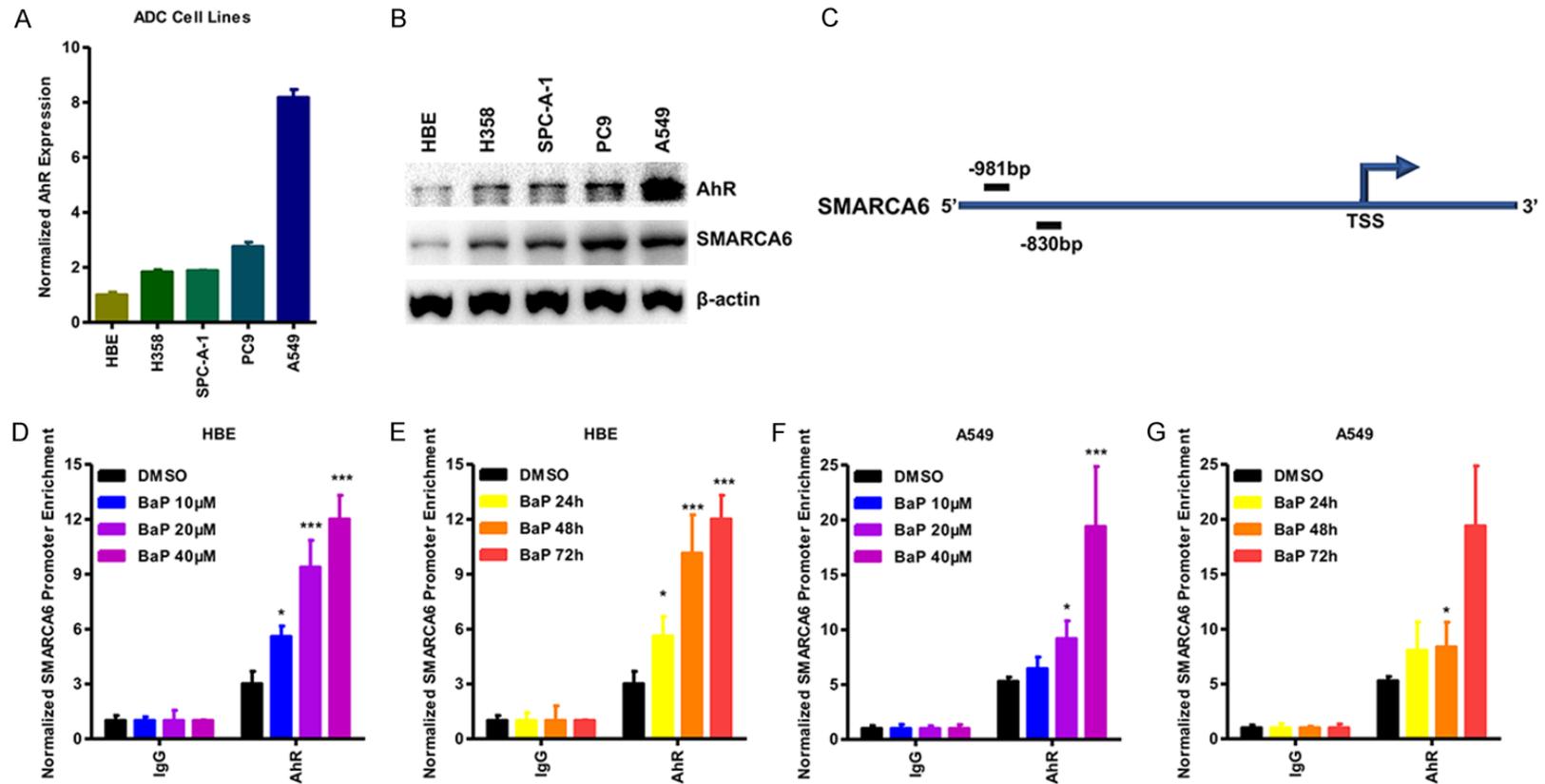


Figure 5. BaP increases SMARCA6 expression via recruitment of AhR. (A, B) qPCR analysis (A) and Western blot (B) were used to detect the expression of AhR and SMARCA6 in a panel of lung cancer cells. (C) The AhR binding site is located at ~0.9 kb upstream of the SMARCA6 transcription start site (TSS). (D-G) ChIP assay was performed using BaP-treated or untreated HBE (D, F) and A549 (E, G) cells. The SMARCA6 promoter, enriched for AhR, was detected by qPCR in a dose- (D, F) and time-dependent (E, G) manner. *P < 0.05, **P < 0.01, ***P < 0.001.

AhR activated by BaP promotes SMARCA6 expression

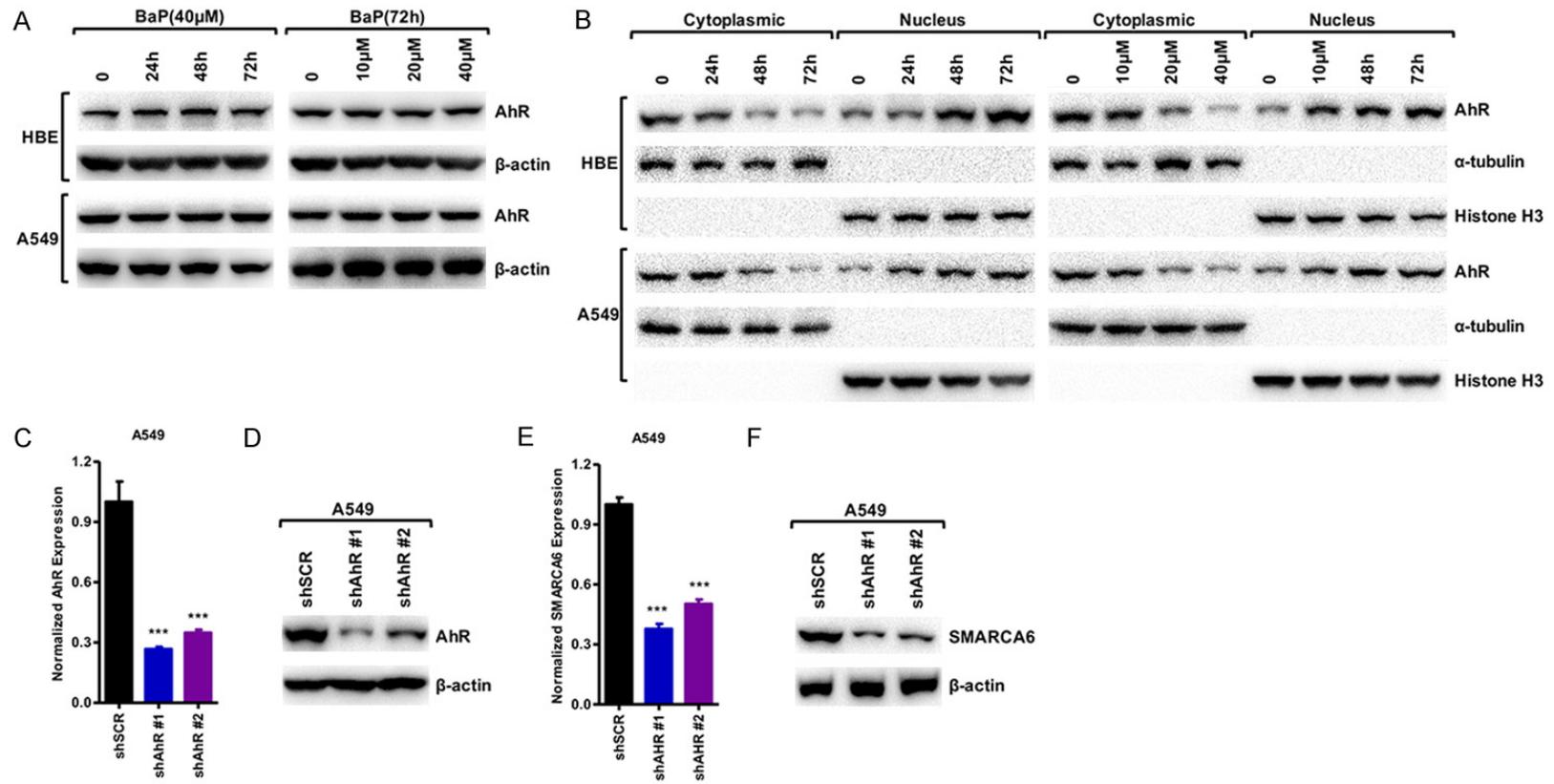


Figure 6. BaP induced SMARCA6 expression by facilitating AhR translocation to the nucleus. (A) Western blot shows the protein level of AhR does not change in HBE and A549 after treatment with BaP in a time- and dose-dependent manner. (B) Activation of the AhR level in the nuclear and cytosolic fractions derived from HBE cells (Top) and A549 cells (Bottom) after treatment with BaP in a time- (left) and dose-dependent manner (right). (C, D) qPCR analysis (C) and Western blot (D) were used to detect the expression of AhR levels in A549 cells after depletion of AhR. (E, F) qPCR analysis (E) and Western blot (F) were used to detect the expression of SMARCA6 level in A549 cells after depletion of AhR. *P < 0.05, **P < 0.01, ***P < 0.001.

cantly promoted the cell growth ability (**Figure 3A, 3B**). Moreover, BaP induced G0-G1 cell cycle progression, resulting in a considerable decrease of cell percentage in G1/S phases and an increase of G2/M-phase compared with the DMSO group (**Figure 3C, 3D**). Furthermore, the number of colonies formed was markedly increased in the BaP treatment of HBE and A549 cells (**Figure 3E-G**).

Next, we assessed the phenotype changes induced by BaP *in vivo* using xenograft model. Using A549 cells, treated with BaP for 90 days, significantly generated larger tumors after 1 month of tumor growth compared with those generated by treatment with DMSO (**Figure 4A**); BaP increased tumor formation and weight (**Figure 4B, 4C**), while whole-body weight remained unchanged (**Figure 4D**). Finally, we determined that SMARCA6 was significantly up-regulated in the tumor samples with BaP treatment compared with their counterparts at both the protein and mRNA levels in the xenograft tumor (**Figure 4E, 4F**).

BaP increased SMARCA6 expression via recruitment of AhR

Next, we screened for transcription factors that can regulate SMARCA6, and located potential aryl hydrocarbon receptor (AhR)-binding sites in the SMARCA6 promoter region (**Figure 5C**). AhR is a ligand-activated transcription factor that binds the xenobiotic-responsive element (XRE), or aryl hydrocarbon response element (AHRE) to regulate genes which respond to BaP [24]. Using qRT-PCR and Western blotting, we confirmed that the expression levels of AhR were significantly up-regulated in lung cancer cell lines compared with those of HBE. Consistent with our previous findings, SMARCA6 was up-regulated in a panel of lung cancer cells (**Figure 5A, 5B**). This finding indicates a strong correlation between AhR and SMARCA6. The potential interaction between AhR and SMARCA6 was analyzed by ChIP assay. The results showed that AhR can bind to the SMARCA6 promoter region in HBE and A549 cells, and BaP treatment promoted the enrichment of AhR binding in a dose- and time-dependent manner (**Figure 5D-G**), indicating that BaP increases SMARCA6 expression via recruitment of AhR to its promoter.

BaP induced SMARCA6 expression by facilitating AhR translocation to the nucleus

AhR influences the major stages of tumorigenesis, and studies of aggressive tumors and tumor cell lines have shown increased levels of AhR protein and constitutive nuclear localization in cancer tissue, whereas in normal tissues AhR is mainly inactive and resides in the cytoplasm [22, 23]. We found that BaP did not change AhR expression (**Figure 6A**). Interestingly, BaP induced AHR translocation from the cytoplasm to the nucleus in a dose and time-dependent manner indicating that BaP triggered the activation of AhR via nuclear relocation of AhR (**Figure 6B**). To further examine the regulation of AhR in SMARCA6, we knocked down AhR with two separate short hairpin RNAs (shRNAs) in A549 cell lines. The AhR expression was detected to confirm the efficiency of silencing by qRT-PCR and Western blot analysis (**Figure 6C, 6D**). The results demonstrated that the shRNA could efficiently knock down AhR expression by at least 35%. Knockdown of AhR in A549 cells decreased SMARCA6 expression at protein and mRNA levels (**Figure 6E, 6F**). Taken together, our results indicate that BaP increases SMARCA6 expression via the recruitment of AhR.

Knockdown of SMARCA6 attenuates BaP-induced lung cancer

To further uncover the critical role of SMARCA6 in BaP promoted lung cancer, we knocked down SMARCA6 expression in A549 cells using two separate shRNA sequences; we did not find the changes of AhR expression in the depletion of SMARCA6, but knockdown of SMARCA6 impaired SMARCA6 expression in response to BaP (**Figure 7A, 7B**). Moreover, knockdown of SMARCA6 decreased cell growth ability whereas BaP rescued the cell growth after knockdown of SMARCA6 in A549 cells (**Figure 7C**). Moreover, the number of colonies formed was markedly rescued after the BaP treatment in A549 cells after knockdown of SMARCA6 (**Figure 7D, 7E**). Taken together, SMARCA6 plays critical role of BaP-induced lung cancer progression.

Discussion

Transcriptional regulation is a key regulatory mechanism determining cell fate; this regula-

AhR activated by BaP promotes SMARCA6 expression

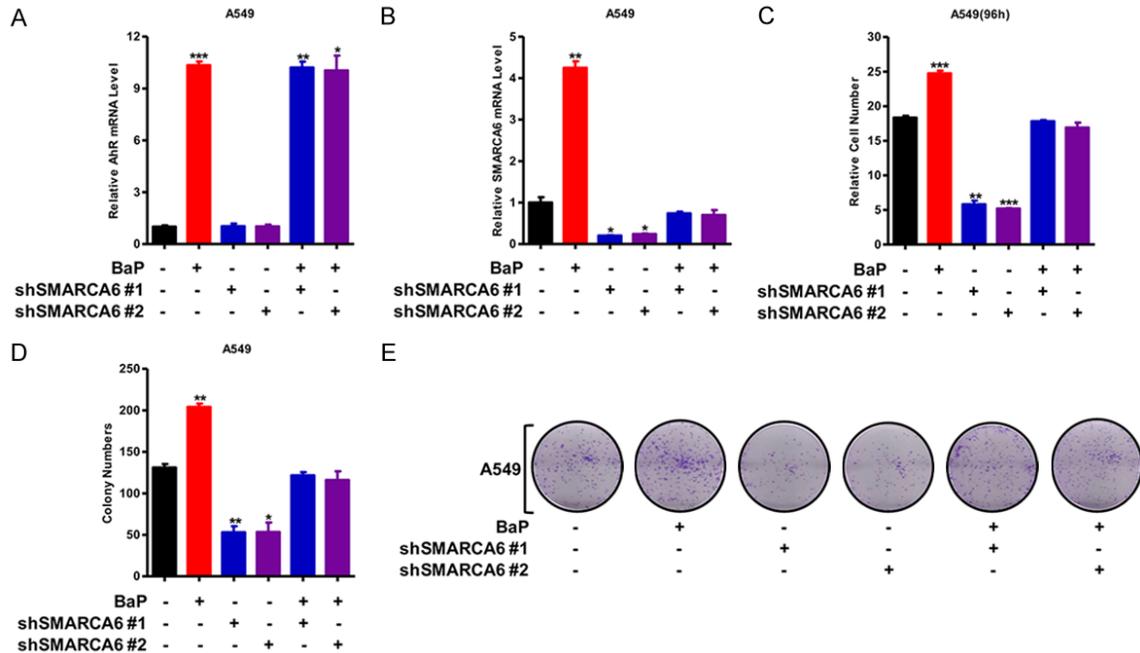


Figure 7. Knockdown of SMARCA6 attenuated BaP-induced lung cancer. (A, B) qRT-PCR detected AhR (A) and SMARCA6 (B) expression in A549 with BaP treatment and SMARCA6 knockdown. (C) MTT assay was applied to assess cell viability in A549 cells with BaP treatment and SMARCA6 knockdown, and the results indicate that knockdown of SMARCA6 impaired cell growth by BaP. (D, E) Growth in the plate was assessed with colony formation assay in A549 with BaP treatment and SMARCA6 knockdown (D), the representative images are shown (E), and the results indicate show that knockdown of SMARCA6 impaired colony formation and BaP rescued it. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

tion includes cis-regulatory elements and transacting factors. Among transacting factors, regulators of chromatin remodeling play a pivotal role in gene transcription and self-renewal [25-27]; these processes also rely on ATP-dependent chromatin remodeling complexes. The SWI/SNF complex is composed of 12-15 subunits, containing one of the two catalytic ATPase subunits, SMARCA4/BRG1 or SMARCA2/BRM, and several core components such as SMARCB1/SNF5, BAF170, and BAF155 [28, 29]. SMARCA6/LSH is critical for the normal development of plants and mammals because it establishes correct levels of DNA methylation and patterns as a critical chromatin modifier [7, 11]. SMARCA6/LSH maintains genome stability in mammalian somatic cells [12, 30]. SMARCA6/LSH, as an oncogene, contributes to the malignant progression of prostate cancer, melanoma, nasopharyngeal carcinoma, and glioma via specific functions of LSH in DNA damage repair, ferroptosis, epithelial-mesenchymal transition (EMT), and metastasis [13-16, 30-33]. Recent evidence indicates that SWI/SNF complexes, including SMARC genes, play a central role in human tumorigenesis.

SWI/SNF complexes are critical epigenetic regulators of tumorigenesis because of their pleiotropic roles in the regulation of the cell cycle, oncogenic pathways, and metabolism [29, 34-36]. However, the interplay of gene expression between environmental factors and SMARC genes remains unknown. In this study, we show that smoking and BaP affect the expression of SMARCA6, which promotes tumorigenesis, and AhR, a critical gene that responds to environmental factors, induces SMARCA6 expression.

AhR, a widely expressed nuclear receptor that senses environmental stimuli and modulates target gene expression, plays a critical role in such cells as embryonic stem cells [37], hematopoietic stem and progenitor cells [38], and breast cancer stem cells [39, 40]. AhR influences the major stages of tumorigenesis and chemoresistance [41, 43], and studies of aggressive tumors and tumor cell lines have shown increased levels of AhR protein and constitutive nuclear localization in cancer tissue, whereas in normal tissues AhR is mainly inactive and resides in the cytoplasm [22, 23]. In

this study, we found an elevated AhR level in the nucleus in response to BaP, indicating that environmental factors might induce the activation of AhR that contributes to cancer progression and tumorigenesis. Continued expression of endogenous AhR promotes centrosome amplification in breast cancer cells in a pathway that involves cyclin E [44], which is consistent with BaP promoting cell cycle progression.

AhR signaling plays a pivotal role in self-renewal of hematopoietic stem cells, differentiation, immunity and antibacterial defense [20, 45-47]. Aberrant activation of AhR signaling is implicated in breast cancer and leukemia [48, 49]. In this study, we have shown that AhR signaling is impaired after knockdown of SMARCA6, and depression of SMARCA6 inhibits the progression of lung cancer and tumorformation capacity in response to BaP. This result suggests that BaP-mediated AhR signaling is critical for promoting cancer progression and tumorigenicity in lung cells. Therefore, we consider that elevated SMARCA6 expression was activated by the signaling pathway of AhR in lung cancer cells. However, the specific mechanisms underlying the overexpression of SMARCA6 have not been thoroughly elucidated to date, and further investigations are needed.

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Disclosure of conflict of interest

None.

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