Atractyloside inhibits gefitinib-resistant non-small-cell lung cancer cell proliferation

WANXIN YAO[1,2*], CHEN LIU[3*], NINGYIN ZHANG[2], YANMEI ZHANG[1,2] and YONG QIAN[1]

[1]Affiliated Yongkang First People's Hospital and School of Pharmacy, Hangzhou Medical College, Hangzhou, Zhejiang 310014; [2]School of Laboratory Medicine and Bioengineering, Hangzhou Medical College, Hangzhou, Zhejiang 310013; [3]The First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, Zhejiang 310000, P.R. China

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Abstract. Atractyloside is a traditional Chinese medicine used to treat nasal congestion, and allergic rhinitis; however, its effects on cancer are unknown. Non-small cell lung cancer (NSCLC) is associated with high mortality rates worldwide, and relapse due to epidermal growth factor receptor mutations is a problem in clinical therapy. Therefore, novel biomarkers are required for the diagnosis and treatment of NSCLC. Brother of the regulator of imprinted sites (*BORIS*; also known as *CTCFL*) is a potential therapeutic target in NSCLC. *BORIS* promotes cisplatin resistance and it has been suggested that it may account for multidrug resistance. The present study examined *BORIS* expression in tyrosine kinase inhibitor (TKI)-resistant NSCLC cells. Subsequently, small interfering RNA was used to knock down *BORIS* expression, and the effects of this knockdown were assessed on TKI-resistant NSCLC cell viability. The present study also investigated the effect of atractyloside on the proliferation of NSCLC cells using MTT assay. The results of the present study indicated that the inhibition of BORIS or its related downstream pathways may have potential for the treatment of TKI-resistant NSCLC. In addition, atractyloside mimicked *BORIS* knockdown, regulated its downstream genes and inhibited the proliferation of TKI-resistant NSCLC cells. In conclusion, the findings of the present study supported the potential application of atractyloside in TKI-resistant NSCLC therapy.

Correspondence to: Professor Yong Qian or Professor Yanmei Zhang, Affiliated Yongkang First People's Hospital and School of Pharmacy, Hangzhou Medical College, 599 Jinshanxi Road, Hangzhou, Zhejiang 310014, P.R. China
E-mail: qian13758975007@126.com
E-mail: yanmeizhang@hmc.edu.cn

*Contributed equally

Abbreviations: NSCLC, non-small cell lung cancer; BORIS, brother of the regulator of imprinted sites; ATP, adenosine triphosphate; TKI, tyrosine kinase inhibitor

Key words: atractyloside, NSCLC, TKI-resistant, *BORIS*

Introduction

Atractyloside, a diterpenoid glycoside, is a naturally occurring active component common in medicines and animal feed, particularly in plants (Asteraceae and *Atractylis*) native to east Asia. Atractyloside has been used in traditional Chinese medicine to treat nasal congestion and allergic rhinitis (1). Numerous studies (2-4) have demonstrated the biological functions of atractyloside, indicating that it is a strong candidate for the treatment of illnesses, including digestive disorders, liver injury and diabetes. According to a pharmacological clinical study in humans (1), atractyloside has been demonstrated to inhibit the mitochondrial ATP translocase, also known as the adenine nucleotide translocator, and to effectively reduce fat accumulation in the liver (steatosis) and improve insulin sensitivity, which protects the liver. Cho *et al* (5) revealed that atractyloside is a modest hypoglycemic agent in splenocytes, thus suggesting that it may be used to treat diabetes. Previous research has demonstrated that atractyloside inhibits mitochondrial ATP transporters, leading to cancer cell death (6).

The incidence and mortality rates of cancer are rapidly increasing worldwide. In both sexes, lung cancer is the most commonly diagnosed type of cancer (11.6% of total cases) and the leading cause of cancer-related deaths (18.4% of total cancer deaths) worldwide (7). Lung cancer is histologically classified as small-cell lung cancer (SCLC) and non-SCLC (NSCLC), and ~85% of patients have NSCLC. Of these patients, lung squamous cell carcinoma and lung adenocarcinoma are the most common subtypes, accounting for 40 and 20-25% of global cases, respectively (8,9). Notably, the application of biomarkers for NS CLC is clinically beneficial. Epidermal growth factor receptor (EGFR) is a well-known biomarker for NSCLC management (10). Although small-molecule tyrosine kinase inhibitors (TKIs), such as gefitinib, have curative effects, relapse caused by EGFR mutations usually lead to patients succumbing to the disease 2 years after the first diagnosis (11). First-generation EGFR inhibitors, such as gefitinib and erlotinib, have significantly improved the survival of patients with NSCLC; however, the secondary EGFR-T790M mutation leads to clinical resistance to first-generation EGFR-TKIs (11-13). New biomarkers may improve the diagnosis and treatment of NSCLC.

Brother of the regulator of imprinted sites (*BORIS*, also known as *CTCFL*)which is a paralog of CCCTC-binding factor, is commonly expressed in most types of cancer, whereas it is not expressed in the corresponding normal tissues; therefore, it is considered a potential therapeutic target for lung cancer (14-16). In our previous study, it was revealed that *BORIS* suppressed apoptosis and enhanced 5-fluorouracil resistance in colorectal cancer (17), and *BORIS* has also been reported to increase resistance to cisplatin treatment in NSCLC (12). Debruyne *et al* (18) reported that *BORIS* may be associated with various tumor occurrences, including brain cancer and cervical cancer, drug resistance and the prognosis of patients with cancer. Based on the ubiquitous expression of *BORIS* and the high incidence of EGFR resistance in NSCLC, it is worth studying whether *BORIS* influences targeted therapies for lung cancer.

In the present study, the association between *BORIS* and TKI-resistant NSCLC was assessed. In addition, atractyloside was used to mimic *BORIS* knockdown to study the therapeutic function of BORIS on the prevention of TKI resistance. The results revealed that atractyloside could facilitate TKIs to suppress NSCLC cell proliferation.

Materials and methods

Cell culture. NSCLC cancer cell lines H1299, PC-9, PC-9-IR and H1975, and the colorectal cancer cell line Caco2 were purchased from The Cell Bank of Type Culture Collection of The Chinese Academy of Sciences. All cells were cultured in RPMI 1640 medium (Nanjing BioChannel Biotechnology Co., Ltd.) containing 10% heat-inactivated fetal bovine serum (GeminiBio) at 37˚C in an incubator containing 5% CO_2.

Cell transfection and treatment. Lipofectamine® RNAiMAX reagent (Invitrogen; Thermo Fisher Scientific, Inc.) was used to transfect small interfering RNA (siRNA) into cells. According to the manufacturer's protocol, H1975, PC-9 and PC-9IR cells at 70% confluence were used for transfection. Briefly, 1 pmol siRNA/well was used for the transfection of cells on a 96-well plate, and 30 pmol siRNA/well was used for the transfection of cells on a 6-well plate. After a 5-min incubation at room temperature, the RNAiMAX-siRNA mixture was added to either 6-well or 96-well plates. The cells were incubated for 48 h without media replacement, after which subsequent experiments were performed. The 96-well plates were used for MTT and cell proliferation assays. The 6-well plates were used for the analysis of transcript or protein expression levels. The siRNA sequences used in the present study are listed in Table I. Negative control siRNA and si*BORIS* were synthesized by Xiangyin Biotechnology Co., Ltd. The cells were then incubated at 37˚C in an incubator containing 5% CO_2. After 48 h, the cells were used for subsequent experiments. Following transfection, cells were treated with their respective drug treatments, with the control group receiving an equal volume of DMSO. Atractyloside (cat. no. HY-N1462), the TKI inhibitor gefitinib (cat. no. HY-50895) and erlotinib (cat. no. HY-50896) were purchased from MedChemExpress. Cells were treated with gefitinib (50 μM) 4 h post-transfection at room temperature. Cells were subjected to experiments after 48 or 96 h of gefitinib treatment. In addition, cells were treated with gefitinib (1 μM), erlotinib (5 μM), or atractyloside (1, 2.5 or 5 μM) for 48 h at 37˚C prior to performing Cell Counting Kit (CCK)-8 assays, western blotting and reverse transcription-quantitative PCR (RT-qPCR).

Cell viability analysis. A total of 3,000 cells/well were seeded in a 96-well plate for transfection or drug treatment. Subsequently, MTT (500 μg/ml; cat. no. M2128; Sigma-Aldrich; Merck KGaA) was added to the cells and incubated for 4 h at 37˚C, and 100 μl dimethyl sulfoxide was added for 15 min at room temperature. Signals were recorded using a BioTek Synergy 2 plate reader at a wavelength of 490 nm (BioTek; Agilent Technologies, Inc.).

CCK-8. A total of 3,000 cells/well were seeded in a 96-well plate and underwent drug treatment. After treatment with drugs (gefitinib, 1 μM; erlotinib, 5 μM; atractyloside, 5 μM) for 48 h at 37°C, the cell culture medium was discarded, and 100 μl medium containing 10 μl CCK-8 (cat. no. K1018; APeXBIO Technology LLC) reagent was added. The cells were then incubated for 1 h at 37˚C and signals were recorded using a BioTek Synergy 2 plate reader at a wavelength of 450 nm.

Western blotting. H1299, Caco2, PC-9 and H1975 cells were cultured in a 6-well plate and were lysed using RIPA buffer (cat. no. 20188; MilliporeSigma) containing PMSF (1:100; cat. no. ST506; Beyotime Institute of Biotechnology) and Roche cOmplete™ Protease Inhibitor Cocktail (1:25; cat. no. 04693116001; Sigma-Aldrich; Merck KGaA). After centrifugation at 12,000 x g for 30 min at 4˚C, the supernatants were collected, and the total protein was quantified using a detergent-compatible Bradford protein assay kit (cat. no. P0006C ; Beyotime Institute of Biotechnology). Samples (30 μg/lane) were separated by SDS-PAGE on a 10% gel and were transferred onto a PVDF membrane (cat. no. ISEQ00010-PVDF; MilliporeSigma). The membrane was blocked with a protein-free rapid blocking buffer (cat. no. PS108P; New Cell & Molecular Biotech Co., Ltd.) for 15 min at room temperature and then incubated at 4˚C overnight with the following antibodies: Mouse anti-GAPDH (1:500,000; cat. no. 60004-1-Ig; Proteintech Group, Inc.), rabbit anti-XRCC4 (1:1,000; cat. no. 15817-1-AP; Proteintech Group, Inc.), mouse anti-BORIS (1:1,000; cat. no. sc-377085; Santa Cruz Biotechnology, Inc), rabbit anti-AKT (1:1,000; cat. no. 9272; Cell Signaling Technology, Inc.) and mouse anti-phosphorylated (p)-AKT (1:1,000; cat. no. 66444-1-Ig; Proteintech Group, Inc.). After washing with TBS-1% Tween (TBST) three times (10 min/wash), the membrane was incubated with HRP-conjugated secondary antibodies (anti-rabbit and anti-mouse; 1:5,000; cat. nos. DW-GAR007 and DW0990-100; Hangzhou Dawen Biological Co., Ltd.) for 2 h at room temperature. Signals were detected after washing with TBST three times (10 min/wash) using the Ultrasensitive ECL Kit (cat no. P2300; New Cell & Molecular Biotech Co., Ltd)and ChemiDoc XRS+ system (Bio-Rad Laboratories, Inc.). The relative expression of the protein bands was semi-quantified using ImageJ version 1.53 software (National Institutes of Health).

RT-qPCR. The RNA of treated cells was extracted using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) through ethanol precipitation. cDNA was reverse transcribed using the Hifair® II 1st Strand cDNA Synthesis Kit

Table I. siRNA sequences using for *BORIS* knockdown.

siRNA	Forward, 5'-3'	Reverse, 5'-3'
Negative control siRNA	UUCUCCGAACGUGUCACGUdTdT	ACGUGACACGUUCGGAGAAdTdT
BORIS siRNA	GGAAAUACCACGAUGCAAATT	UUUGCAUCGUGGUAUUUCCtt

BORIS, brother of the regulator of imprinted sites; siRNA, small interfering RNA.

Table II. Primer sequences used for reverse transcription-quantitative PCR.

Gene name	Forward, 5'-3'	Reverse, 5'-3'
BORIS (*CTCFL*)	CAGGCCCTACAAGTGTAACGACTGCAA	GCATTCGTAAGGCTTCTCACCTGAGTG
GAPDH	CCCACTCCTCCACCTTTGAC	TGTTGCTGTAGCCAAATTCGT
XRCC4	ATGGCTCCTCAGGAGAATCAGC	GAGGTCTTCTGGGCTGCTGTTT
MSH6	CCAAGGCGAAGAACCTCAAC	ACCAGGGGTAACCCTCCATC
BRCA-1	ACTCTGAGGACAAAGCAGCG	CATCCCTGGTTCCTTGAGGG
c-myc	AAGCCAAGGACTGTCTGAACG	GGGACGAGTAATTCTTTCCCCT

BORIS, brother of the regulator of imprinted sites.

(gDNA digester plus) (cat. no. 11121ES60; Shanghai Yeasen Biotechnology Co., Ltd.), and was used for qPCR analysis. For RT, the temperature settings were as follows: 25˚C for 5 min, 42˚C for 30 min and 85˚C for 5 min. qPCR was performed using the 2X T5 Fast qPCR Mix (SYBR Green; cat. no. 11201ES08; Shanghai Yeasen Biotechnology Co., Ltd.) and a CFX connect real-time PCR detection system (Bio-Rad Laboratories, Inc.). According to the manufacturer's protocol, the thermocycling conditions were as follows: Initial denaturation at 95˚C for 5 min, followed by 40 cycles of denaturation at 95˚C for 15 sec and annealing/extension at 60˚C for 30 sec. GAPDH was used as an internal reference for normalization. The primers used for qPCR are listed in Table II. The qPCR results were analyzed using the $2^{-\Delta\Delta Cq}$ method (19).

Bioinformatics analysis. *BORIS* expression in NSCLC was determined using the R2 Genomics Analysis and Visualization Platform (http://r2.amc.nl). The Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/geo/) datasets GSE19188 (20) and GSE63074 (21) were utilized for bioinformatics analysis.

Statistical analysis. GraphPad Prism 8 software (Dotmatics) was used for all statistical analyses. All experiments were performed in triplicate. Data are presented as the mean ± standard deviation. Statistical differences were calculated using one-way or two-way ANOVA followed by Tukey's multiple comparisons test, paired Student's t-test or unpaired Student's t-test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Bioinformatics analysis. Bioinformatics analysis was performed using the R2 Genomics Analysis and Visualization Platform. Based on the GSE19188 dataset, *BORIS* expression was elevated in primary NSCLC tissues compared with in normal lung tissues (Fig. 1A). From the GSE63074 dataset, survival rate data were used to perform a risk stratification analysis, categorizing patients into high-risk and low-risk groups according to a previously described method (22); the analysis indicated a significant upregulation of BORIS expression in the high-risk NSCLC group (Fig. 1B). Given that EGFR upregulation or mutation was prevalent in high-risk NSCLC cases, further investigation into the potential association between BORIS and EGFR expression/mutation is warranted.

BORIS knockdown inhibits H1975 cell viability. The H1975 cell line comprises NSCLC cells resistant to gefitinib due to the T790M mutation. The present study knocked down *BORIS* in H1975 cells and the results demonstrated that the cell viability was significantly decreased in response to successful transfection with si*BORIS* (Fig. 1D). This finding indicated that the presence of *BORIS* may maintain the stability of H1975 cells and that its knockdown could be beneficial for treating drug-resistant lung cancer.

BORIS knockdown, alongside gefitinib treatment, inhibits NSCLC cell viability. To further explore the function of *BORIS* in TKI resistance, PC-9, PC-9IR and H1975 cells underwent *BORIS* knockdown and gefitinib treatment. PC-9 is an EGFR wild-type NSCLC cell line, whereas PC-9IR and H1975 are EGFR-mutant cells that are resistant to TKIs.

si*BORIS* effectively reduced the viability of NSCLC cells (Fig. 1D-F). All cell transfections were successful. When si*BORIS* was combined with gefitinib treatment, cell viability was significantly reduced compared with gefitinib treatment alone, with a more obvious effect observed on the drug-resistant cell lines H1975 and PC9-IR

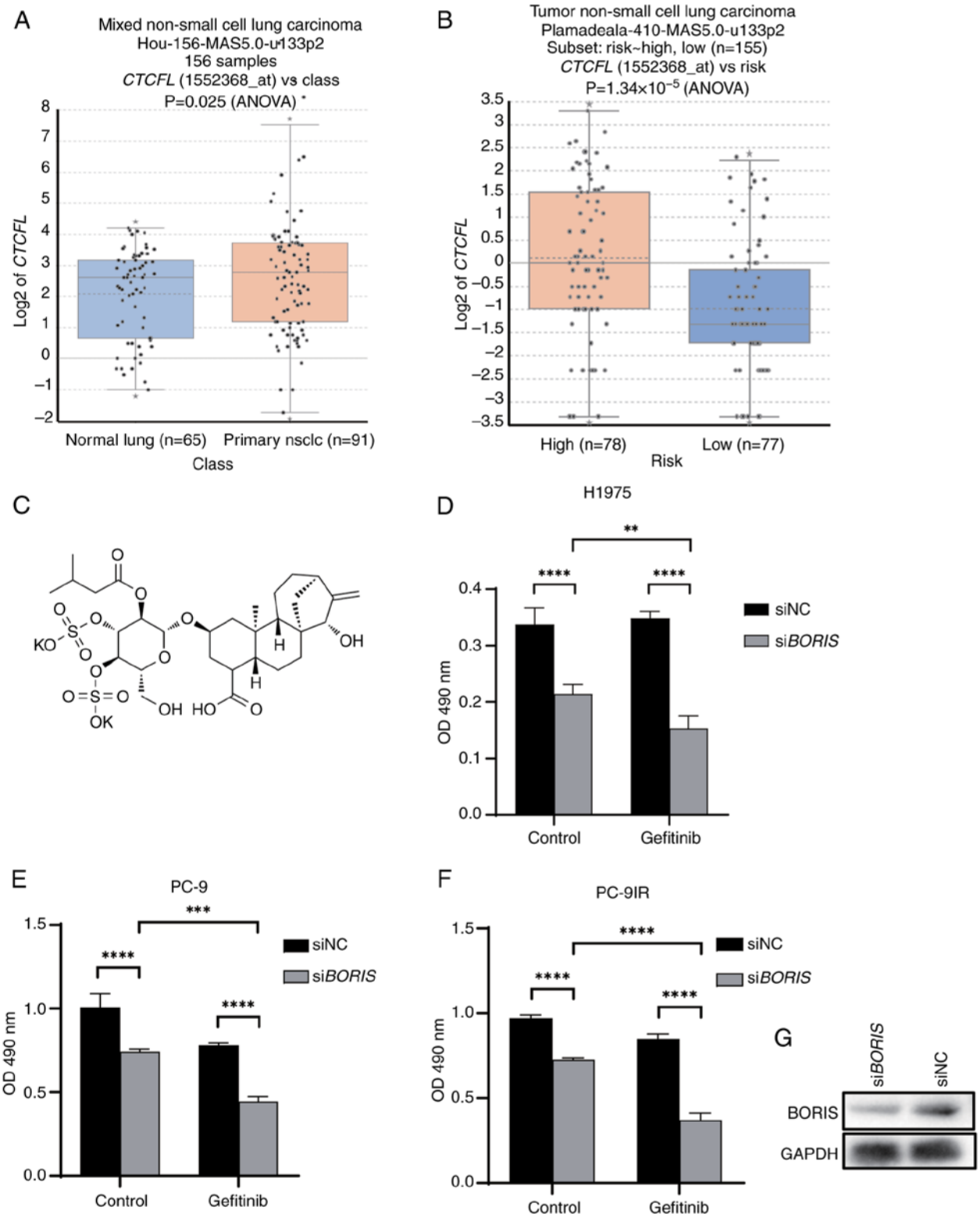

Figure 1. BORIS knockdown decreases the viability of NSCLC cell lines. (A and B) According to the R2 website, *BORIS* (also known as *CTCFL*) expression increases in NSCLC, and the higher its expression, the higher the risk of cancer. (C) Atractyloside chemical structure. The combination of *BORIS* knockdown and gefitinib effectively inhibited the viability of tyrosine kinase inhibitor-resistant NSCLC cells. (D) si*BORIS* and gefitinib (50 μM) suppressed H1975 cell viability. (E) si*BORIS* and gefitinib (50 μM) suppressed PC-9 cell viability. (F) si*BORIS* and gefitinib (50 μM) suppressed PC-9-IR cell viability. (G) Transfection efficiency of siBORIS in PC-9IR cells. **P<0.01, ***P<0.001, ****P<0.0001. BORIS, brother of the regulator of imprinted sites; NC, negative control; NSCLC, non-small cell lung cancer; si, small interfering.

(Fig. 1D and 1F). Verification of the knockdown efficiency of siBORIS is presented in Fig. 1G. These findings suggested that the knockdown of *BORIS* may be beneficial for lung cancer resistance. Western blot analysis confirmed that the expression of *BORIS* was decreased in response to siBORIS.

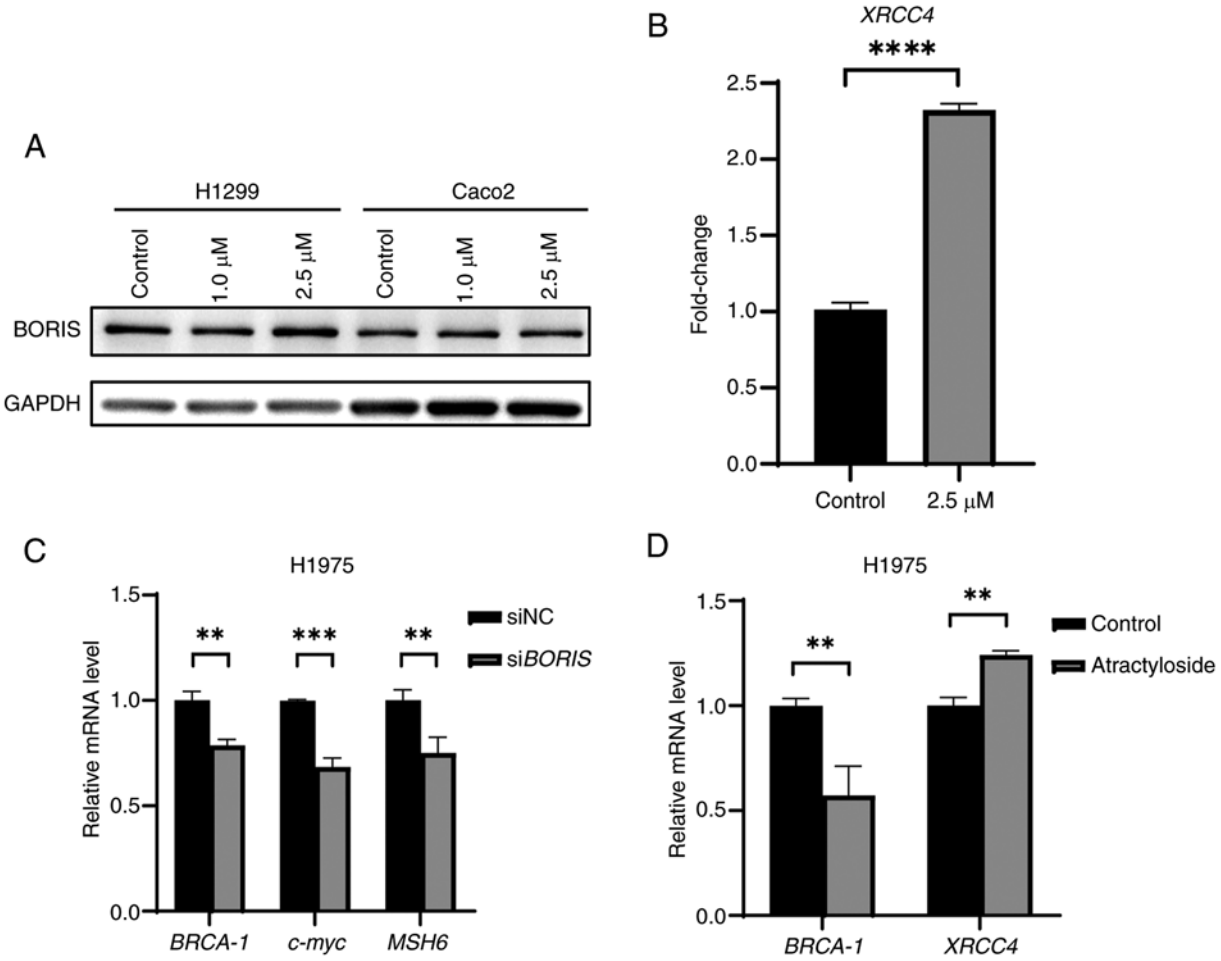

Figure 2. Atractyloside mimics the function of si*BORIS*, influencing DNA repair-related genes. (A) Expression of *BORIS* was not affected by atractyloside in Caco2 and H1299 cells. (B) In H1299, atractyloside can mimic the role of si*BORIS* in cells and affect DNA repair. *XRCC4* expression was detected by reverse transcription-quantitative PCR following treatment with atractyloside (2.5 μM) in H1299 cells. (C) Expression levels of genes related to DNA repair were significantly decreased after *BORIS* knockdown. (D) After treatment with atractyloside, the expression of a homologous recombination-related gene was decreased in H1975, and that of a non-homologous end-joining-related gene was compensatively upregulated. **P<0.01, ***P<0.001, ****P<0.0001. BORIS, brother of the regulator of imprinted sites; si, small interfering.

Atractyloside mimics BORIS knockdown to suppress NSCLC viability. In a previous study, a drug that could mimic *BORIS* knockdown was identified (23). To identify bioactive drugs that might mimic the effects of *BORIS* knockdown, genes regulated in *BORIS*-silenced Caco2 cells were analyzed using microarray and a connectivity map database was screened for associated drugs in our previous study (23). Based on gene expression patterns and drug correlation analysis, metronidazole and atractyloside were identified as promising candidates for further study (23). These previous findings using the Caco2 cell line demonstrated that atractyloside (Fig. 1C) inhibited cell viability (23).

The H1299 (wild-type EGFR) and H1975 (EGFR mutation) NSCLC cells were used for assessing the response of siBORIS or atractyloside treatment. The results demonstrated that siBORIS treatment downregulated the expression of DNA repair-related genes, including BRCA-1, MSH6 and c-myc (Fig. 2B). Atractyloside treatment, on the other hand, increased XRCC4 expression while downregulating BRCA-1 (Fig. 2D), which is consistent with our previous observations (23). In H1299 and H1975 cells, atractyloside treatment resulted in DNA damage and upregulation of XRCC4 expression (Fig. 2B and D). These results indicated that atractyloside could mimic the effects of siBORIS to regulate the downstream genes (Fig. 2C and D); however, atractyloside treatment did not influence BORIS expression (Fig. 2A). In the present study atractyloside was used instead of siBORIS in subsequent experiments to regulate BORIS-related downstream genes.

Combination of atractyloside and gefitinib treatment reduces the proliferation of NSCLC cells. To avoid using a high concentration of atractyloside, which would induce mitochondrial permeability transition and cause apoptosis (4), 5 μM atractyloside was selected for application in two lung cancer cell lines, the wild-type cell line PC-9 and the T790M mutant drug-resistant cell line H1975.

In PC-9 cells, it was observed that, after 2 days of administration, gefitinib inhibited cell proliferation, and after 4 days administration, the proliferation of PC-9 cells was significantly inhibited by gefitinib (Fig. 3A). Atractyloside was shown to suppress PC-9 cell proliferation, but was less effective than gefitinib. In addition, the effect of the two-drug combination on cell proliferation was not significant, thus indicating that atractyloside had little effect on wild-type lung cancer cells. In H1975 cells, gefitinib at a concentration of 1 μM did not

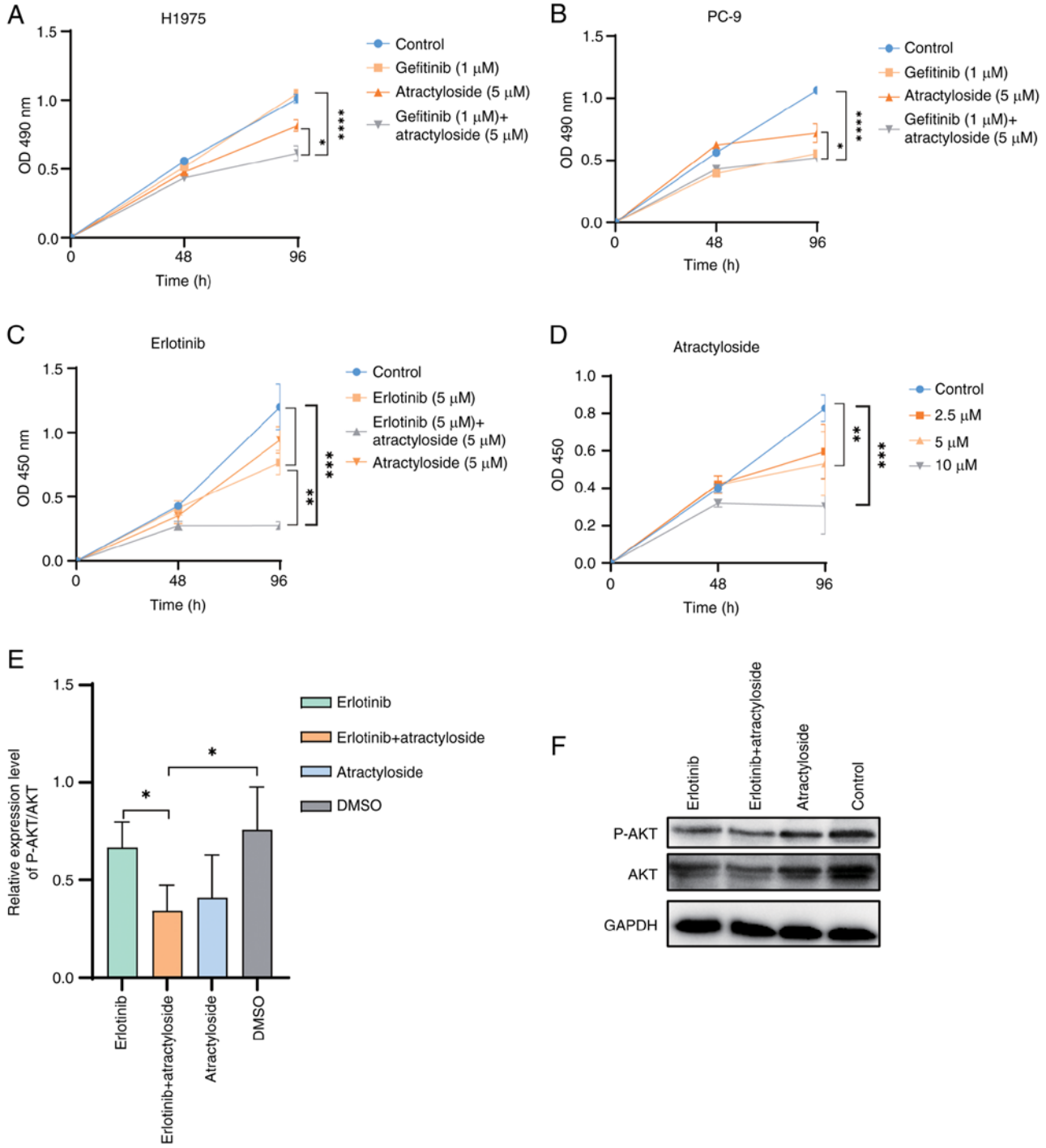

Figure 3. Combination of atractyloside and a TKI-inhibitor can effectively suppress the proliferation of non-small cell lung cancer cells. (A and B) MTT assay was used to detect cell proliferation. (A) Gefitinib did not significantly inhibit the proliferation of H1975 cells; however, when combined with atractyloside, H1975 cell proliferation was suppressed. (B) Gefitinib effectively suppressed PC-9 cell proliferation, however, atractyloside did not show any synergistic effect when used in combination. (C and D) CCK-8 assay was used to detect cell proliferation. (C) Erlotinib (5 μM) alone had a modest effect on H1975 cell proliferation, but combining it with atractyloside (5 μM) resulted in a significant enhancement of its suppressive effect. (D) Increasing the concentration of atractyloside effectively suppressed H1975 cell proliferation. (E) Semi-quantification of P-AKT/AKT relative to GAPDH. (F) Atractyloside suppressed the AKT pathway. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001. P-, phosphorylated.

affect cell proliferation (Fig. 3B). However, when used in combination with atractyloside, cell proliferation was significantly decreased, indicating that the combined administration of atractyloside and gefitinib may affect the proliferation of TKI-resistant cells.

To further verify the inhibitory effect of combination therapy on EGFR-mutant cells, the 2nd-generation EGFR-TKI inhibitor erlotinib was used. After treatment with the drugs for 96 h, a combination of erlotinib and atractyloside suppressed H1975 cell proliferation better than erlotinib alone (Fig. 3C). Atractyloside demonstrated cytotoxic effects on cancer cells, with increasing concentrations inhibiting H1975 cell proliferation (Fig. 3D). When combined with gefitinib, the effect of atractyloside on drug-resistant lung cancer cells was stronger

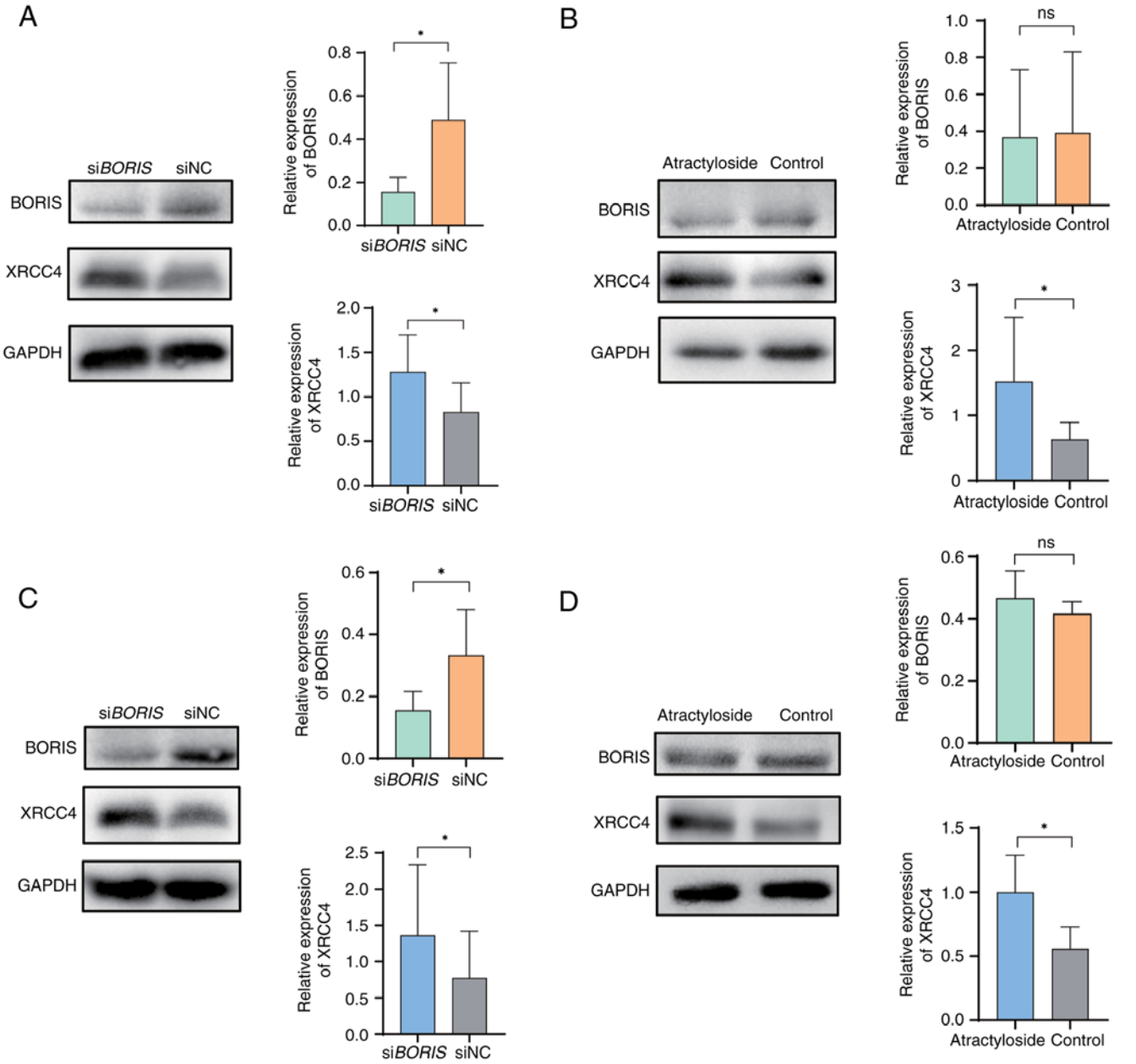

Figure 4. Both *BORIS* knockdown and atractyloside can induce cell DNA damage. In PC-9 cells, western blotting and grayscale analysis demonstrated that (A) *BORIS* knockdown and (B) can regulate the expression of XRCC4. In H1975 cells, western blotting and grayscale analysis demonstrated that (C) *BORIS* knockdown and (D) atractyloside can regulate the expression of XRCC4(D). *P<0.05. BORIS, brother of the regulator of imprinted sites; NC, negative control; ns, not significant; si, small interfering.

than that on wild-type cells, indicating that the *BORIS* pathway may be associated with lung cancer resistance. Western blot analysis indicated that atractyloside may suppress NSCLC cell proliferation by inhibiting AKT phosphorylation (Fig. 3E and F). This finding aligns with the results of a previous study demonstrating that AKT phosphorylation promotes lung cancer cell proliferation (24). AKT is a downstream factor of EGFR and can be used to examine the severity of cancer; therefore, the inhibition of AKT phosphorylation indicated that EGFR-related signaling was suppressed by atractyloside.

Atractyloside induces DNA damage in NSCLC. After knockdown of the expression of *BORIS* in the H1975 cell line, a decrease in the expression levels of the homologous recombination-related genes *c-myc*, *BRCA-1* and the mismatch repair gene *MSH6* (25), was detected (Fig. 2C). These findings were consistent with our previous results (12) and indicated the existence of *BORIS*-stabilized cell DNA. To further verify the effects of atractyloside on H1975 and PC-9 cells, XRCC4 protein expression was detected. The data demonstrated a consistent trend of XRCC4 upregulation, observed in response to both *BORIS* knockdown and atractyloside treatment (Fig. 4A-D). In PC-9 cells, *BORIS* knockdown elevated the expression of XRCC4 (Fig. 4A), as did atractyloside (Fig. 4B). In addition, in H1975 cells, *BORIS* knockdown elevated the expression of XRCC4 (Fig. 4C), as did atractyloside (Fig. 4D). In summary, atractyloside may disrupt DNA stability in H1975 cells and PC-9 cells.

Discussion

Gefitinib is a small-molecule EGFR-TKI that blocks the intracellular receptor binding site of adenosine triphosphate (ATP); this blocks downstream signal transduction, inhibits tumor cell proliferation and promotes apoptosis, all of which have a significant effect on the treatment of advanced NSCLC (13,26). However, the median survival time for patients from 61 centers across 11 European and Asia-Pacific countries with advanced NSCLC was revealed to be only 7-9 months, and drug resistance, frequently arising from secondary mutations, presents a significant obstacle to effective treatment (13). Among mutations, EGFR-T790M is considered the main cause of acquired drug resistance. This mutation competitively reduces binding with EGFR-TKIs to confer drug resistance by increasing the affinity between EGFR and ATP (27). Therefore, attention is required to identify novel ways to deal with this acquired drug resistance.

Through the analysis of *BORIS* expression in NSCLC in the present study, it was revealed that *BORIS* expression was increased in tissues from patients with high-risk NSCLC. As EGFR mutations are usually related with high-risk NSCLC, it may be hypothesized that *BORIS* is associated with EGFR mutations. In addition, *BORIS* knockdown or atractyloside treatment promoted TKI resistance in H1975 NSCLC cells with T790M mutation. These findings suggested that inhibiting BORIS could be a promising strategy for combination therapy with first-generation EGFR inhibitors in NSCLC. The prognosis of a number of patients with NSCLC is poor because of secondary drug-resistance gene mutations (27). However, the notable effect of *BORIS* knockdown provides a novel opportunity for treatment. In addition, atractyloside, a *BORIS* knockdown mimetic, holds promise as a means for treatment of NSCLC. However, the lack of *in vivo* experiments is a limitation of the present study. In future studies, we plan to conduct in-depth research on atractyloside and verify its medicinal value in xenograft models, since animal experiments may better reflect the occurrence and development of NSCLC. Future research may also construct stable drug-resistant H1975 and PC-9IR NSCLC cell lines with *BORIS* overexpression, and may assess treatment of drug resistance in lung carcinoma *in situ* and in a brain metastasis model of lung cancer.

In the present study, the knockdown of *BORIS* in H1975 cells or the administration of atractyloside decreased the expression of homologous recombination-related genes, such as *BRCA-1*. In addition, BORIS can influence DNA repair pathways, such as non-homologous end recombination, were compensatively upregulated, indicating that *BORIS* could stabilize the DNA of NSCLC cells. The H1299 cell line, expressing wild-type EGFR, was used to confirm that atractyloside treatment could induce *XRCC4*. Notably, the expression of DNA damage repair genes, including *BRCA-1*, *c-myc* and *MSH6*, was detected only in H1795 cells, which harbor an EGFR mutation. EGFR mutations or amplifications in NSCLC cells cause resistance to TKIs and induce downstream constitutive AKT phosphorylation, whereas inhibition of AKT reverses resistance to TKIs. In a study on neuroblastoma, ALK-mutated neuroblastoma cells were resistant to the ALK inhibitor TAE684 (18). Resistant cells exhibited upregulation of *BORIS*, which could lead to wide-ranging changes in chromatin interactions and transcriptional reprogramming. A 10-fold gain in genome-wide occupancy by *BORIS* was observed in resistant cells (22,891 vs. 2,211 in the sensitive cells) (18). We observed increased BORIS expression in TKI-resistant NSCLC cells and identified AKT as a potential downstream target of BORIS activation in these cells. The present study observed that atractyloside may inhibit AKT activity and suppress NSCLC cell proliferation, but did not affect the expression of *BORIS*. Inhibition of AKT by atractyloside suggests crosstalk between *BORIS* and factors downstream of EGFR.

In summary, *BORIS* expression was increased in patients with high-risk lung cancer, as determined by comparing groups with different survival rates. Notably, atractyloside is an inhibitor of the *BORIS* pathway and may be a potential therapeutic drug against TKI resistance.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

Conceptualization of the study was directed by YQ and YZ. The experiments were performed by WY, CL, NZ and YZ. The original draft was written by CL, WY and YZ. The review, editing and revisions were completed by YQ and YZ. The visualization of the data and the generation of the figures were performed by CL and NZ. The funding was provided by YQ and YZ. All authors read and approved the final version of the manuscript. WY and CL confirmed the authenticity of all the raw data.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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