Int J Clin Exp Pathol 2019;12(8):2997-3004
www.ijcep.com /ISSN:1936-2625/IJCEP0097610

Original Article

HOTAIR contributes to chemoresistance by activating NF-κB signaling in small-cell lung cancer

Rui Chen[1*], Bin Chen[4*], Deyu Li[5], Qi Wang[2], Yaru Zhu[3], Man Li[1], Yu Wang[1], Shun Fang[1], Linlang Guo[1]

Departments of [1]Pathology, [2]Oncology, [3]Cardiothoracic Surgery, Zhujiang Hospital, Southern Medical University, Guangzhou, China; [4]Department of Hepatic Surgery, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China; [5]Provincial Clinical College, Fujian Medical University, Department of Medical Oncology, Fujian Provincial Hospital, Fuzhou, China. [*]Equal contributors.

Received May 28, 2019; Accepted June 25, 2019; Epub August 1, 2019; Published August 15, 2019

Abstract: Our previous study showed that lncRNA HOTAIR affects the chemoresistance of SCLC by regulating HOXA1 methylation. However, the downstream regulatory mechanism remains unknown. The article aimed to further explore the potential downstream mechanism. In this study, we demonstrate that the knockdown of HOTAIR inhibits the NF-κB pathway in SCLC cells. The overexpression of HOXA1, the downstream gene of HOTAIR, also suppresses the NF-κB pathway, but the downregulation of HOXA1 shows the opposite results. Notably, the knockdown of HOXA1 in HOTAIR downregulated cells can rescue the inhibition of the NF-κB pathway mediated by HOTAIR downregulation. Meanwhile, we found that the NF-κB pathway is activated in multidrug-resistant SCLC cells (H69AR, H446AR) compared with the parental cells (H69, H446). The inhibition of the NF-κB pathway with celastrol increases cell sensitivity to anticancer drugs, cell apoptosis, and cell cycle arrest. Collectively, these results revealed that the NF-κB pathway may be involved in the chemoresistance of SCLC caused by HOTAIR methylating HOXA1.

Keywords: HOTAIR, HOXA1, SCLC, the NF-κB pathway

Introduction

Small-cell lung cancer (SCLC) is a neuroendocrine malignancy and accounts for 15% to 17% of all patients with lung cancer [1]. There were about 130,000 patients diagnosed with SCLC and 100,000 died of the disease in China in 2013 [2, 3]. In clinical practice, SCLC is generally divided into limited disease and extensive disease. Platinum plus etoposide is the first-line chemotherapy regimen for patients with SCLC. Although most people have high percentages of response to chemotherapy initially, this rapidly turns into drug resistance during treatment [4]. Therefore, the molecular mechanism involved in SCLC chemotherapy resistance needs to be further explored.

Long noncoding RNAs (lncRNAs), whose length are over 200 nucleotides, have been shown to regulate every step of cell growth and death [5]. Furthermore, many studies have confirmed that lncRNAs can regulate the occurrence, development and metastasis of tumors through classic signaling pathways [6-9]. Our previous research confirmed that HOTAIR induced HOXA1 methylation leads to the chemoresistance of SCLC [10], and the purpose of the present research is to explore whether classic signals are involved in the HOTAIR/HOXA1 axis or not. We reviewed the related literature and found that HOTAIR can regulate the NF-κB pathway in some cancers [11-13]. Meanwhile, the result of the enrichment analysis about differentially expressed proteins from H69AR and H69 revealed that the NF-κB pathway was activated in H69AR [14]. Therefore, we sought to explore whether the NF-κB pathway is involved in the process in which HOTAIR induced HOXA1 methylation contributes to the chemoresistance of SCLC.

In this research, we showed that HOTAIR induced HOXA1 methylation leads to the chemoresistance of SCLC via the NF-κB pathway. The inhibition of NF-κB signaling increases cell sensitivity to anticancer drugs, cell apoptosis, and cell-cycle arrest. The study adds to our understanding of the chemoresistance of SCLC.

Materials and methods

Cell culture

The human SCLC cell lines H69, H69AR and H446 were obtained from the American Type Culture Collection, and H446AR was established by exposing H446 cells to a high dose of Adriamycin for at least 6 months. All the cells were cultured in a 10-20% fetal calf serum (Gibco, USA) medium. However, H69AR and H446AR were alternately cultured with a medium containing 0.8 μM of ADM and a drug-free medium.

To inhibit the NF-κB pathway, the cells were treated with 5 μM celastrol (InvivoGen, USA) for 48 h.

Cell transfection

For downregulation, we transiently transfected cells with siRNAs (GenePharma, Shanghai) using Lipofectamine 2000 (Invitrogen) and OPTI-MEM (Invitrogen). The targeting sequences were as follows: siHOTAIR-1: 5'-cccauggacucauaaacaatt-3' and 5'-uuguuuaugaguccaugggtt-3'; siHOTAIR-2: 5'-gccuuuggaagcucuugaatt-3' and 5'-uucaagagcuucaaaggctt-3'; siHOXA-1: 5'-accauaggauuacaacuuutt-3' and 5'-aaaguuguaaucccuauggutt-3'; siHOXA-2: 5'-uggaugaaagucaaaagaatt-3' and 5'-uucuuuugacuuucauccatt-3'.

For upregulation, the HOXA1 overexpression plasmid (HOXA1-eGFP) and the empty plasmid (eGFP) were purchased from Genepharma (Shanghai, China). The specific operational steps were the same as above.

Quantitative RT-PCR

TRIZOL reagent (TaKaRa) was used to obtain total RNA in the cell. The RNA concentrations were measured using NanoDrop 2000 (Thermo). And reverse transcription reactions were conducted using a PrimeScript RT Reagent Kit (TIANGEN Biotech, Beijing). Finally, qRT-PCR was run in an ABI Illumina instrument (San Diego, USA). The 2-ΔΔCT method was used to calculate the relative mRNA expression. The primer sequences designed by Shanghai Sangong Biotech were as follows: HOTAIR: F: 5'-ggtagaaaaagcaaccacgaagc-3', R: 5-acataaacctctgtctgtgagtgcc-3'; HOXA1: F: 5'-agttggagagtacggctactg-3', R: 5'-tgcagggatgcagcgatctccac-3'; GAPDH: F: 5'-gagtcaacggatttggtcgt-3', R: 5'-catgggtggaatcatattgga-3'.

Western blotting analysis

A RIPA lysate (Biyuntian, China) was used to obtain the total protein in the cells. Cell lysates with the same content were separated using 10% SDS-polyacrylamide gel electrophoresis and then transferred to a PVDF membrane (Millipore). The membranes were blocked with 5% skim milk for 1 h and then incubated with an anti-BCL-XL antibody (Santa Cruz, USA), anti-HOXA1 (Sigma, USA), and an anti-GAPDH antibody (Sigma, USA) overnight at 4°C. After they were washed three times with 1 × TBST, the membranes were incubated for 1 h with an HRP-linked anti-rabbit secondary antibody (EarthOx, San Francisco). Finally, the proteins were visualized using chemiluminescence (ECL).

Luciferase reporter assays

The cells were transfected with a pNF-κB lucreporter plasmid (NF-κB-dependent firefly luciferase reporter) and a pRL-CMV vector (a kind gift from Metastasis Research Laboratory, Peter MacCallum Cancer Centre, Melbourne, Australia) using Lipofectamine 2000 (Invitrogen) and OPTI-MEM (Invitrogen). After 48 h transfection, the luciferase activity was measured using the Dual-Luciferase Reporter Assay System (Promega).

Drug-resistance assay (CCK8)

Drug sensitivity was detected using a CCK-8 assay. Transfected cells were incubated with different concentrations of chemotherapy drugs (Adriamycin (ADM; Jiangsu), cisplatin (CDDP; Shandong), and etoposide (VP16; Jiangsu). After 24 h, 10 μl CCK-8 reagent (Dojindo, Japan) was added into cells and incubated for 3 h. Then, the OD value was measured at 450 nm. According to the OD value measured, the IC50 of each drug was calculated. Each group had five vice holes, and three parallel experiments were performed.

Flow cytometry

The cells were treated with anticancer drugs for 24-48 h and then collected for further experiments. For cell cycle assays, the cells were fixed for 4 h with 75% ethanol and stained with

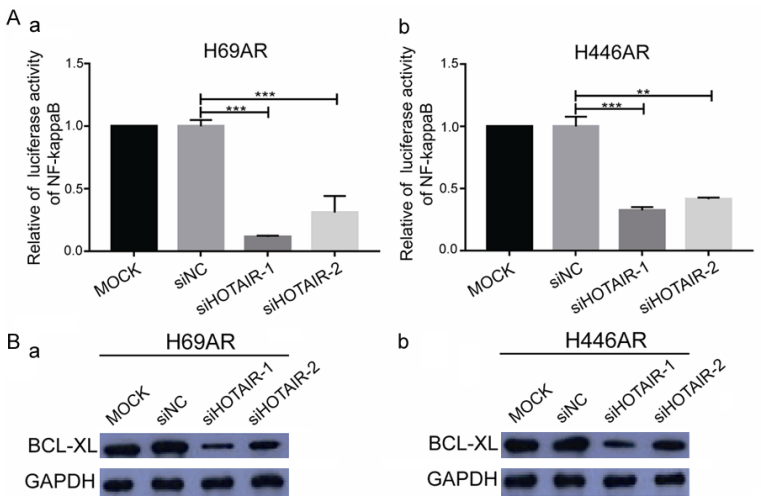


Figure 1. HOTAIR regulates the NF-κB pathway in SCLC cells. A. NF-κB induced luciferase activity in HOTAIR downregulated cells were measured by dual luciferase reporter assays. B. Western blot analysis of BCL-XL expression in HOTAIR downregulated cells. $^{**}P < 0.01$, $^{***}P < 0.001$.

50 mg/ml propidium iodide (Sigma, USA). For the apoptosis assays, Annexin V450/eff660 APC (eBioscience, USA) was used according the manufacturer's protocol. All samples were analyzed using a BD FACS-Verse flow cytometer.

Statistical analysis

The data were represented as the mean ± SD. An independent-samples *t* test or a one-way ANOVA were used to analyze the differences between groups. SPSS (Chicago, USA) was used for the statistical analysis, and $P < 0.05$ was considered statistically significant.

Results

HOTAIR regulates the NF-κB pathway in SCLC cells

Our team has confirmed that HOTAIR affects the chemoresistance of SCLC by regulating HOXA1 methylation. To understand the role of HOTAIR in the NF-κB pathway, we transfected HOTAIR siRNAs into SCLC cells. And the qRT-PCR results showed that the expression of HOTAIR was downregulated in the SCLC cells [10]. We found that the downregulation of HOTAIR in chemoresistant SCLC cells significantly decreased the NF-κB induced luciferase activity (**Figure 1A**). Meanwhile, western blot showed that the expression of BCL-XL (a classical NF-κB target gene) was downregulated in HOTAIR-silenced SCLC cells (**Figure 1B**). These results indicate that HOTAIR may regulate the NF-κB pathway in SCLC cells.

HOXA1 inhibits the NF-κB pathway in SCLC cells

After revealing the relationship between HOTAIR and the NF-κB pathway, we sought to explore whether HOTAIR downregulation inhibits the NF-κB pathway by increasing HOXA1 expression in SCLC. To further verify the negative regulatory effect of HOXA1 on the NF-κB pathway, we constructed HOXA1 downregulated and overexpressed SCLC cells (**Figure 2A**). We found that NF-κB induced luciferase activity and the expression of BCL-XL was markedly enhanced in HOXA1 downregulated SCLC cells. On the other hand, the luciferase activity and the expression of BCL-XL were downregulated in SCLC cells with HOXA1 overexpression (**Figure 2B**). Meanwhile, the knockdown of HOXA1 by siRNA in HOTAIR downregulated cells could rescue the effects of HOTAIR's silencing of luciferase activity (**Figure 2C**). A previous study confirmed that DNA methylation can reduce the expression of HOXA1 in SCLC [10]. Therefore, we treated SCLC cells with the DNA methyltransferase inhibitor 5-Aza-2-deoxycytidine (5-Aza-CdR) and found that the NF-κB pathway was inhibited in the treatment group compared to the control group (**Figure 2D**). Our results suggest that HOTAIR affects the NF-κB pathway partly by regulating HOXA1 in SCLC cells.

The NF-κB pathway affects the chemoresistance of SCLC

The above experiments have demonstrated that the NF-κB pathway can be regulated by the HOTAIR/HOXA1 axis in SCLC cells. In addition, we found that the NF-κB pathway is activated in chemoresistant SCLC cells (H69AR, H446AR) than in parental cells (H69, H446) (**Figure 3A**). In order to assess whether the NF-κB pathway was involved in the chemoresistance of SCLC, we treated cells with NF-κB inhibitors (celas-

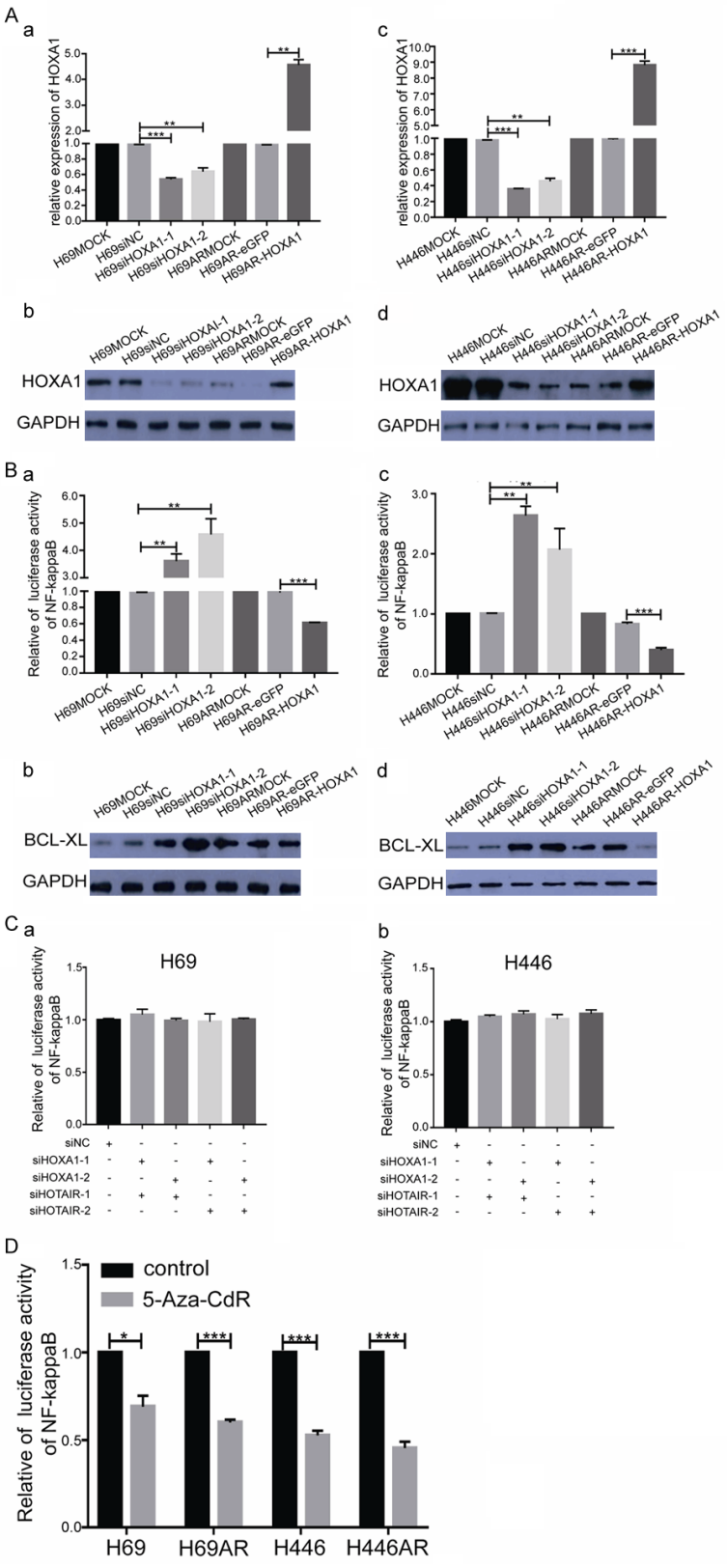


Figure 2. HOXA1 inhibits the NF-κB pathway in SCLC cells. A. Inhibition or overexpression of HOXA1 by transfection of HOXA1 siRNA or plasmid in SCLC cells. B. NF-κB induced luciferase activity and BCL-XL expression were measured by dual luciferase reporter assays and western blot analysis in HOXA1 downregulated and overexpressed cells. C. After the cells were co-transfected with HOXA1 siRNA and HOTAIR siRNA, NF-κB-induced luciferase activity was examined using dual luciferase reporter assays. D. After SCLC cells are treated with 5-Aza-CdR, the NF-κB induced luciferase activity was measured by dual luciferase reporter assays. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

trol). Dual luciferase reporter assays and a western blot analysis showed that both the NF-κB induced luciferase activity and the expression of BCL-XL were decreased after treatment with celastrol in chemoresistant SCLC cells (**Figure 3B**). CCK8 assays were conducted to evaluate the chemosensitivity of the SCLC cells to various drugs (ADM, CDDP, and VP-16). The results showed that the IC50 values were significantly decreased after treatment with celastrol in the H69AR and H446AR cells (**Figure 3C**). These results suggest that the NF-κB pathway may be involved in the multidrug resistance of SCLC.

Inhibiting the NF-κB pathway increases cell sensitivity to chemotherapy drugs by enhancing cell apoptosis and cell cycle arrest

We further evaluated the effect of celastrol on cell apoptosis and the cell cycle upon exposure of the cells to chemotherapeutic drugs. Flow cytometry results demonstrated that cell apoptosis increased and the cell cycle was arrested in H69AR and H446AR cells treated with celastrol after anticancer drug exposure (**Figure 4A** and **4B**). These observations showed that the inhibition of the

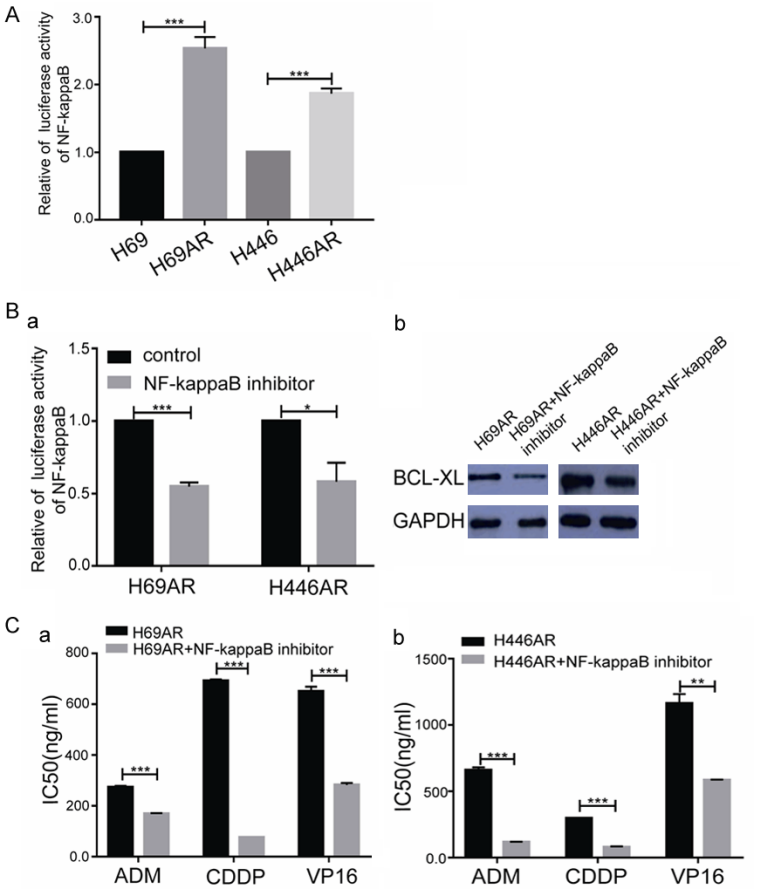


Figure 3. The NF-κB pathway affects the chemoresistance of SCLC. A. Dual luciferase reporter assays were used to evaluate the activity of the NF-κB pathway in SCLC cells. B. The inhibitory effect of celastrol on the NF-κB pathway was determined by dual luciferase reporter assays and a western blot analysis in chemoresistant SCLC cells. C. Celastrol treated cells were exposed to chemotherapy drugs, and the IC50 values were assessed using CCK8 assays. $^{**}P < 0.01$, $^{***}P < 0.001$.

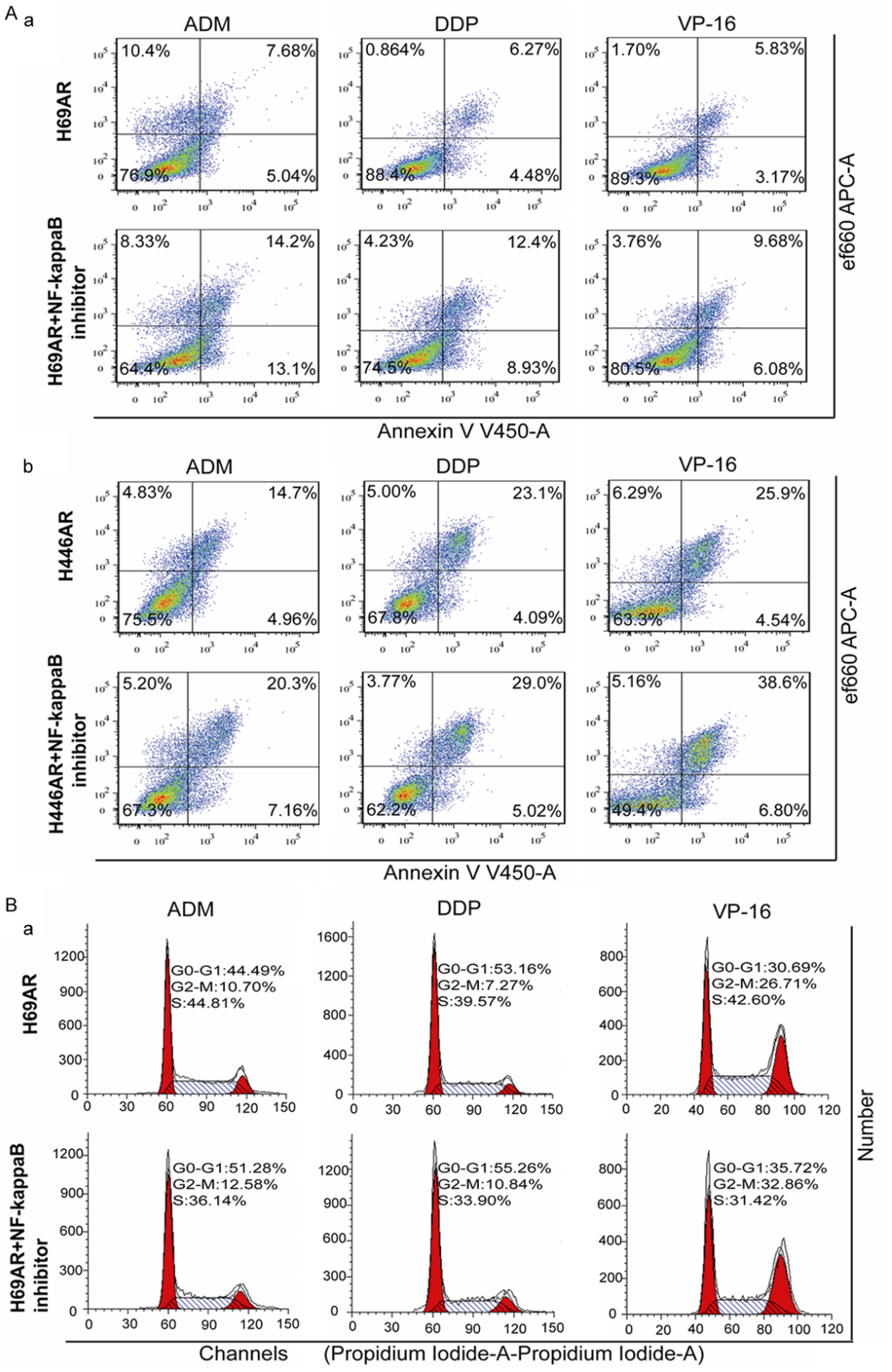
NF-κB pathway increased chemosensitivity by enhancing cell apoptosis and cell cycle arrest.

Discussion

Patients with SCLC usually have a poor prognosis, which is closely related to the occurrence of chemoresistance. But the precise mechanism of the chemoresistance remains unknown. Many recent studies have reported that lncRNAs are involved in tumor chemotherapy resistance. Zhang et al. demonstrated that lncRNA foxc2-as1 promotes Adriamycin resistance in osteosarcoma [15]. Xiong et al. showed that lncRNA HULC attenuates the chemosensitivity of HCC cells by increasing autophagy [16], and so on.

Our laboratory also confirmed that lncRNA HOTAIR affects the chemoresistance of SCLC by regulating HOXA1 methylation [10]. However, the downstream regulatory mechanism is still unclear. Through a literature review, we found that HOTAIR can regulate the NF-κB pathway in many cancers [11-13], and an enrichment analysis of mass spectrometry resulting from H69AR and H69 also revealed that the NF-κB pathway was activated in H69AR [14]. Thus, we hypothesized that the HOTAIR/HOXA1 axis modulated the chemoresistance of SCLC by the NF-κB pathway. To confirm this hypothesis, we changed the expressions of HOTAIR and HOXA1 in SCLC cells and found that HOTAIR downregulation and HOXA1 overexpression inhibited the NF-κB pathway. In contrast, the downregulation of HOXA1 produced the opposite results. To further confirm the results, we conducted a rescue experiment and found that HOXA1 downregulation could rescue the effects of HOTAIR silencing on the NF-κB pathway. As we previously reported, HOXA1 methylation induced by HOTAIR contributes to the chemoresistance of SCLC. We found that NF-κB-induced luciferase activity decreased in cells treated with 5-Aza-CdR compared with the control group. Therefore, our studies showed that the HOTAIR/HOXA1 axis can regulate the NF-κB pathway in SCLC cells.

The NF-κB pathway has been reported to be closely related to the occurrence and development of many tumors [17-19]. In addition, some studies have shown a relationship between the NF-κB pathway and treatment resistance in cancers. For example, Jacamo et al. demonstrated that reciprocal leukemia-stroma VCAM-1/VLA-4 mediates chemoresistance by activating NF-κB signaling [20]. And Zhang et al. showed that icariin increases the radiosensitivity of colorectal cancer by inhibiting NF-κB activity [21]. In our study, we showed that the NF-κB pathway was activated in multidrug-


A
a
ADM
DDP
VP-16
H69AR
10.4%
7.68%
76.9%
5.04%
0.864%
6.27%
88.4%
4.48%
1.70%
5.83%
89.3%
3.17%
H69AR+NF-kappaB inhibitor
8.33%
14.2%
64.4%
13.1%
4.23%
12.4%
74.5%
8.93%
3.76%
9.68%
80.5%
6.08%
ef660 APC-A
Annexin V V450-A
b
ADM
DDP
VP-16
H446AR
4.83%
14.7%
75.5%
4.96%
5.00%
23.1%
67.8%
4.09%
6.29%
25.9%
63.3%
4.54%
H446AR+NF-kappaB inhibitor
5.20%
20.3%
67.3%
7.16%
3.77%
29.0%
62.2%
5.02%
5.16%
38.6%
49.4%
6.80%
ef660 APC-A
Annexin V V450-A
B
a
ADM
DDP
VP-16
H69AR
G0-G1:44.49%
G2-M:10.70%
S:44.81%
G0-G1:53.16%
G2-M:7.27%
S:39.57%
G0-G1:30.69%
G2-M:26.71%
S:42.60%
H69AR+NF-kappaB inhibitor
G0-G1:51.28%
G2-M:12.58%
S:36.14%
G0-G1:55.26%
G2-M:10.84%
S:33.90%
G0-G1:35.72%
G2-M:32.86%
S:31.42%
Number
Channels (Propidium Iodide-A-Propidium Iodide-A)

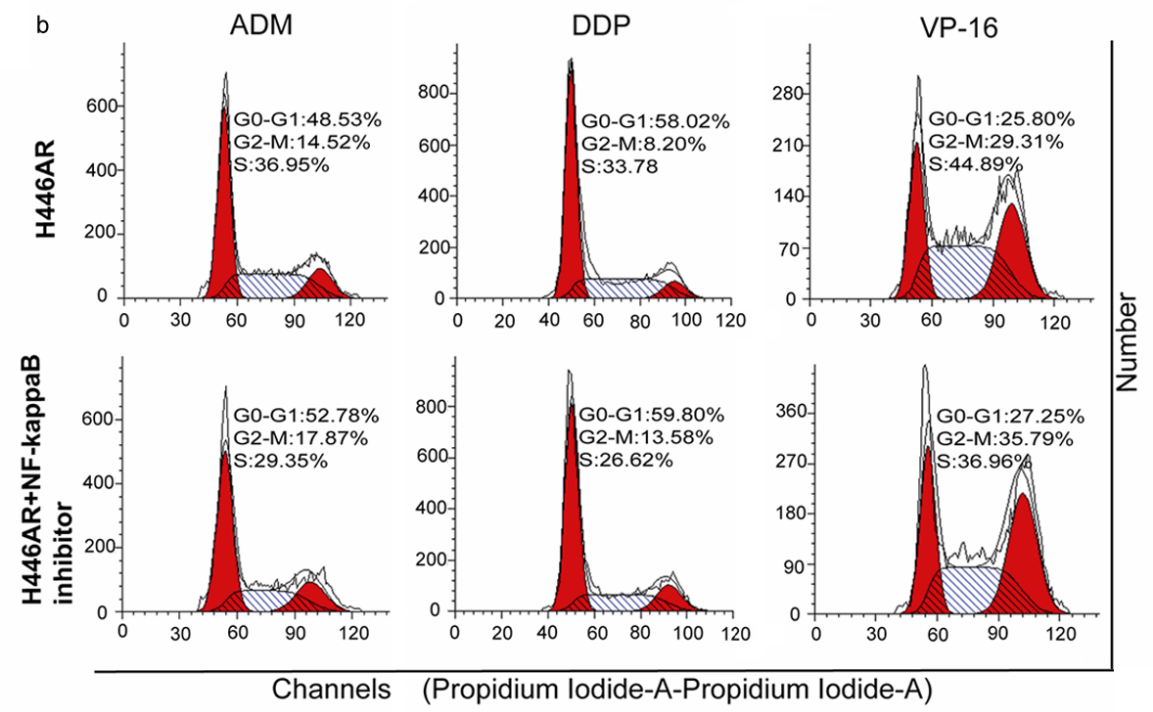


Figure 4. Inhibiting the NF-κB pathway increases cell sensitivity to chemotherapy drugs by enhancing cell apoptosis and cell cycle arrest. A and B. Cell apoptosis and cell cycles were measured by flow cytometry analysis in celastrol-treated cells after drug exposure.

resistant cell lines compared with the parental cell lines. Moreover, we found that the inhibition of the NF-κB pathway would increase cell sensitivity to ADM, CDDP, and VP-16 by enhancing cell apoptosis and cell cycle arrest. These results suggested that inhibiting the NF-κB pathway improved the drug sensitivity of SCLC in vitro.

In conclusion, our findings reveal that HOXA1 methylation induced by HOTAIR contributes to the chemoresistance of SCLC by regulating the NF-κB pathway. NF-κB inhibitors may be used in chemotherapy resistant patients with increased HOTAIR expression.

Acknowledgements

This work was supported by the Natural Science Foundation of Guangdong Province (2016A0-30310385) and the National Natural Science Foundation of China (81601986).

Disclosure of conflict of interest

None.

Address correspondence to: Shun Fang and Linlang Guo, Department of Pathology, Zhujiang Hospital, Southern Medical University, 253 Gongye Road, Guangzhou 510282, China. Tel: +86 20 627833-58; Fax: +86 20 84311872; E-mail: function19-45@163.com (SF); linlangg@yahoo.com (LLG)

References

[1] Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J and Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015; 65: 87-108.

[2] Zhao H, Ren D, Liu H and Chen J. Comparison and discussion of the treatment guidelines for small cell lung cancer. Thorac Cancer 2018; 9: 769-774.

[3] Chen W, Zheng R, Zhang S, Zeng H, Zuo T, Xia C, Yang Z and He J. Cancer incidence and mortality in China in 2013: an analysis based on urbanization level. Chin J Cancer Res 2017; 29: 1-10.

[4] van Meerbeeck JP, Fennell DA, De Ruysscher DK Small-cell lung cancer. Lancet 2011; 378: 1741-55.

[5] Guttman M and Rinn JL. Modular regulatory principles of large non-coding RNAs. Nature 2012; 482: 339-346.

[6] Jiang W, Zhang D, Xu B, Wu Z, Liu S, Zhang L, Tian Y, Han X and Tian D. Long non-coding RNA BANCR promotes proliferation and migration of lung carcinoma via MAPK pathways. Biomed Pharmacother 2015; 69: 90-95.

[7] Wu XS, Wang XA, Wu WG, Hu YP, Li ML, Ding Q, Weng H, Shu YJ, Liu TY, Jiang L, Cao Y, Bao RF, Mu JS, Tan ZJ, Tao F and Liu YB. MALAT1 promotes the proliferation and metastasis of gallbladder cancer cells by activating the ERK/MAPK pathway. Cancer Biol Ther 2014; 15: 806-814.

[8] Wang X, Sun W, Shen W, Xia M, Chen C, Xiang D, Ning B, Cui X, Li H, Li X, Ding J and Wang H. Long non-coding RNA DILC regulates liver cancer stem cells via IL-6/STAT3 axis. J Hepatol 2016; 64: 1283-1294.

[9] Zhang Z, Zhou C, Chang Y, Zhang Z, Hu Y, Zhang F, Lu Y, Zheng L, Zhang W, Li X and Li X. Long non-coding RNA CASC11 interacts with hnRNP-K and activates the WNT/beta-catenin pathway to promote growth and metastasis in colorectal cancer. Cancer Lett 2016; 376: 62-73.

[10] Fang S, Gao H, Tong Y, Yang J, Tang R, Niu Y, Li M and Guo L. Long noncoding RNA-HOTAIR affects chemoresistance by regulating HOXA1 methylation in small cell lung cancer cells. Lab Invest 2016; 96: 60-68.

[11] Li P, Zhang X, Wang L, Du L, Yang Y, Liu T, Li C and Wang C. lncRNA HOTAIR contributes to 5FU resistance through suppressing miR-218 and activating NF-κB/TS signaling in colorectal cancer. Mol Ther-Nucl Acids 2017; 8: 356-369.

[12] Ozes AR, Miller D, Guo C, Bhattral A, Liu Y and Nephew KP. The transcriptional regulation of the long non-coding RNA HOTAIR in ovarian cancer. Cancer Res 2013; 731.

[13] Ozes AR, Miller DF, Liu Y and Nephew KP. Non-coding RNA HOTAIR connects DNA damage signaling to NF-kappa B activation in cisplatin resistant ovarian cancer. Cancer Res 2014; 74S.

[14] Tripathi SC, Fahrmann JF, Celiktas M, Aguilar M, Marini KD, Jolly MK, Katayama H, Wang H, Murage EN, Dennison JB, Watkins DN, Levine H, Ostrin EJ, Taguchi A and Hanash SM. MCAM Mediates chemoresistance in small-cell lung cancer via the PI3K/AKT/SOX2 signaling pathway. Cancer Res 2017; 77: 4414-4425.

[15] Zhang C, Zhu K and Ma X. Antisense lncRNA FOXC2-AS1 promotes doxorubicin resistance in osteosarcoma by increasing the expression of FOXC2. Cancer Lett 2017; 396: 66-75.

[16] Xiong H, Ni Z, He J, Jiang S, Li X, He J, Gong W, Zheng L, Chen S, Li B, Zhang N, Lyu X, Huang G, Chen B, Zhang Y and He F. LncRNA HULC triggers autophagy via stabilizing Sirt1 and attenuates the chemosensitivity of HCC cells. Oncogene 2017; 36: 3528-3540.

[17] Zhang S, Yang Y, Weng W, Guo B, Cai G, Ma Y and Cai S. Fusobacterium nucleatum promotes chemoresistance to 5-fluorouracil by upregulation of BIRC3 expression in colorectal cancer. J Exp Clin Canc Res 2019; 38: 14.

[18] Nakagawa MM and Rathinam CV. Constitutive activation of the canonical NF-κB pathway leads to bone marrow failure and induction of erythroid signature in hematopoietic stem cells. Cell Rep 2018; 25: 2094-2109.

[19] Jiang Y, Zhou J, Luo P, Gao H, Ma Y, Chen Y, Li L, Zou D, Zhang Y and Jing Z. Prosaposin promotes the proliferation and tumorigenesis of glioma through toll-like receptor 4 (TLR4)-mediated NF-κB signaling pathway. Ebiomedicine 2018; 37: 78-90.

[20] Jacamo R, Chen Y, Wang Z, Ma W, Zhang M, Spaeth EL, Wang Y, Battula VL, Mak PY, Schallmoser K, Ruvolo P, Schober WD, Shpall EJ, Nguyen MH, Strunk D, Bueso-Ramos CE, Konoplev S, Davis RE, Konopleva M and Andreeff M. Reciprocal leukemia-stroma VCAM-1/VLA-4-dependent activation of NF-kappaB mediates chemoresistance. Blood 2014; 123: 2691-2702.

[21] Zhang Y, Wei Y, Zhu Z, Gong W, Liu X, Hou Q, Sun Y, Chai J, Zou L and Zhou T. Icariin enhances radiosensitivity of colorectal cancer cells by suppressing NF-κB activity. Cell Biochem Biophys 2014; 69: 303-310.