European Review for Medical and Pharmacological Sciences 2019; 23: 3838-3846

COX-2 potentiates cisplatin resistance of non-small cell lung cancer cells by promoting EMT in an AKT signaling pathway-dependent manner

G.-B. JIANG, H.-Y. FANG, D.-Y. TAO, X.-P. CHEN, F.-L. CAO

Department of Oncological Surgery, Taizhou Enze Medical Center (Group) Enze Hospital, Zhejiang, China

Abstract. – OBJECTIVE: To elucidate the effect of cyclooxygenase 2 (COX-2) on cisplatin resistance of NSCLC and its molecular mechanisms, with special attention to its pro-EMT (epithelial-mesenchymal transition) properties.

MATERIALS AND METHODS: COX-2 levels were compared in two NSCLC cell lines, A549 and H460, by qPCR (quantitative Polymerase Chain Reaction) and Western blot. Cytotoxicity of cisplatin was also determined in the two cell lines using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay. The expression of EMT-related proteins and activation of AKT (protein kinase B) signaling were detected in H460 cells with ectopic COX-2 expression.

RESULTS: Cisplatin-induced apoptosis was assessed in COX-2 overexpressing H460 cells by FACS. NS398, a COX-2 inhibitor, was also applied to determine EMT status and effect on cisplatin sensitivity in H460 cells. COX-2 levels were positively correlated with cisplatin resistance in both NSCLC cell lines tested. In response to COX-2 overexpression, EMT-related proteins, such as E-cadherin, were inhibited, while vimentin and N-cadherin were upregulated. The AKT signaling pathway was also activated in H460 cells. Ectopic expression of COX-2 potentiated cisplatin resistance of H460 cells, which was accompanied by decreased levels of apoptosis. Notably, NS398 effectively increased the cytotoxicity of cisplatin in A549 cells by inhibiting EMT and the AKT pathway.

CONCLUSIONS: COX-2 might promote cisplatin resistance in NSCLC by promoting EMT through the AKT signaling pathway activation.

Key Words

COX-2, Lung cancer, Cisplatin, Drug resistance, EMT, AKT.

Introduction

Lung cancer is one of the most common causes of cancer-related deaths worldwide. Approximately 85% of all lung cancer deaths were the result of non-small-cell lung cancer (NSCLC)[1]. Among numerous factors, resistance to apoptosis is the most important in the mechanism of NSCLC pathophysiology[2], leading to resistance of NSCLC to cisplatin treatment[3] and making NSCLC the most widespread cause of cancer-associated mortality[4]. Therefore, is urgent to investigate the underlying mechanisms of NSCLC resistance to chemotherapy.

Cyclooxygenases (COX) are critical enzymes in the conversion of arachidonic acid to prostaglandins and other eicosanoids[5]. There are two isoforms of the COX enzyme. COX-1 is constitutively expressed in all tissues, while COX-2 is induced by various cytokines, growth factors and tumor promoters[6]. COX-2 is up-regulated in many malignant cancers, including gastric, colon, breast, esophagus, pancreas, hepatocellular carcinoma and NSCLC, while COX-2 is nearly undetectable in most normal tissues[7-12]. Previous studies have found that tumor growth requires COX-2 expression and that enhanced COX-2 expression is sufficient to induce tumorigenesis[13]. These findings indicate that COX-2 plays an indispensable role in carcinogenesis.

Chemotherapy, including cisplatin treatment, is commonly used to treat advanced NSCLC, unfortunately with limited success. The resistance mechanisms by which tumors escape from drug-induced cell death have been attributed to variations in the apoptosis pathway[14]. Based on recent evidence, epithelial-mesenchymal transition (EMT) of cancer cells is believed to be a critical process for the development of drug resistance in cancer. EMT is a process by which epithelial cells lose their cell polarity and cell-cell adhesions to become mesenchymal stem cells. A number of transcription factors, including Twist, Snail, Slug and ZEB1 can induce EMT. Furthermore, EMT is essential for numerous developmental process-

Corresponding Author: Feilin Cao, MD; e-mail: caofl@enzemed.com

es, not only including mesoderm formation and neural tube formation but also involving wound healing, organ fibrosis and initiation of cancer cell metastasis[15]. Moreover, the EMT trans-differentiation programme can confer cells with stem-like properties[15]. Therefore, EMT is not only the initial step for cell migration but also a critical regulator of the treatment resistance, including drug resistance of cancer cells[16]. However, little is known about whether COX-2 could impact the development of the resistance of NSCLC to anticancer drugs, including cisplatin, by inducing EMT in cancer cells.

In the present work, we demonstrated that the overexpression of COX-2 effectively potentiates the cisplatin resistance of NSCLC cells by promoting EMT. NS398, a COX-2 inhibitor, induced apoptosis and additionally potentiated chemosensitivity to cisplatin-mediated apoptosis in human non-small cell lung cancer by targeting the AKT (protein kinase B) pathway. Furthermore, we provided novel insight into the function of COX-2 with respect to regulation of the sensitivity of human NSCLC to cisplatin, as well as the mechanisms involved.

Materials and Methods

Cell Line and Culture Conditions

The human NSCLC cell lines A549 and H460 were cultured in Dulbecco's modification of Eagle's Medium supplemented with 10% FBS (fetal bovine serum) (Gibco, Grand Island, NY, USA), 100 U/mL penicillin and 100 μg/mL streptomycin. All cell lines were cultured in a humidified incubator in an atmosphere of 5% (v/v) CO_2 at 37 °C.

Cell Viability Assay

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay (Sigma-Aldrich, St. Louis, MO, USA) was used to assess cell viability. Briefly, cells were seeded into 96-well plates at a density of 2,500 cells/well and allowed to attach for 24 h. After attachment, variable concentrations of NS398 and/or cisplatin were added to cells in full growth medium. Cells were exposed to NS398 and/or cisplatin for 24 h followed by MTT assay to assess cell survival rates. We used GraphPad Prism software (GraphPad Software, Inc., La Jolla, CA, USA) to calculate IC50 values. The absorbance of each sample was read at 570 nm using a microplate reader.

Measurement of Apoptosis by Flow Cytometry

FITC-labeled annexin-V and propidium iodide (PI) were used to assess externalization by flow cytometry (FACSCalibur) (BD Biosciences, Franklin Lakes, NJ, USA). Briefly, after the treatments indicated, human NSCLC cells were collected and washed with ice-cold PBS (phosphate-buffered saline). Next, cells were stained with Annexin V-FITC for 15 min in the dark at room temperature, followed by PI staining for another 5 min. Soon afterward, cells containing the fluorescent signal were assessed by flow cytometry (FACSCalibur) (BD Biosciences, Franklin Lakes, NJ, USA). WinMDI 2.9 software was used to analyze the data.

Real Time-Polymerase Chain Reaction (RT-PCR)

Total RNA was isolated using TRIzol (Invitrogen, Carlsbad, MA, USA) according to the manufacturer's protocols. Complementary DNA synthesis was performed using the PrimeScript RT Reagent Kit (TaKaRa, Otsu, Shiga, Japan). Total RNA was dissolved in 30 μl diethylpyrocarbonate (DEPC)-treated water, and concentrations were determined by spectrophotometric analysis at 260 nm. Next, 472 ng RNA was reverse-transcribed using PrimeScript TM Reverse Transcriptase (RT) Master Mix (TaKaRa, Otsu, Shiga, Japan) under conditions of 37°C for 10 min. One microliter of cDNA generated in the RT reaction was added to each SYBR® Premix Ex TapTM mix (TaKaRa, Otsu, Shiga, Japan) using the LightCycler® manual (Roche Diagnostics, Basel, Switzerland). PCR cycling conditions were 40 cycles after initial denaturation (95°C, 30 s) with the following parameters: 95°C for 5 s, 58 °C for 20 s, and 72°C for 40 s. Fluorescence acquisition and melting curve analyses were performed as per the product manual instructions. The expression levels of COX-2 mRNA (forward: 5'-GAATGGGGTGATGAGCAGTT-3'; reverse 5'-CAGAAGGGCAGGATACAGC -3') were evaluated using SYBR green PCR master mix (Applied Biosystems, Foster City, CA, USA) and normalized to β-actin (forward: 5'-AGCACAGAGCCTCGCCTTTGC-3'; reverse 5'-CTGTAGCCGCGCTCGGTGAG-3'). All reactions were performed in triplicate. The threshold cycle (Ct) value was measured and the comparative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method.

Western Blotting

Cells were lysed in Laemmli lysis buffer (Sigma-Aldrich, St. Louis, MO, USA). Proteins

were loaded in denaturing 12% or 15% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) gels. After running, gels were transferred to nitrocellulose membranes, which were blocked with 5% nonfat dry milk for 2 h at room temperature. Membranes were then washed with PBS containing 0.1% Tween-20, followed by incubation with primary antibodies (antibodies against PARP, caspases, XIAP, COX-2, p-AKT, and AKT were from Cell Signaling Technology (Danvers, MA, USA) at 4 °C overnight. After washing with Phosphate-buffered Saline-Tween (PBS-T), membranes were incubated with secondary antibodies conjugated with horseradish peroxidase for another 2 h. Finally, signals were visualized with chemiluminescence (Amersham Life Sciences, Inc., Buckinghamshire, UK). β-actin was used as a loading control.

Measurement of Changes in Mitochondrial Membrane Potential

Changes in mitochondrial membrane potential change were detected by JC-1 staining. Human NSCLC cells were seeded in cell culture dishes overnight followed by incubation with various concentrations of NS398 and/or cisplatin for another 24 h. Then, cells were harvested, washed with PBS, and resuspended in JC-1 staining solution (10 μM) for 10 min at room temperature. Cells were then assessed by a FACSCanto flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA).

Plasmid Construction and Transfection

Coding sequences of human COX-2 mRNA were synthesized and subcloned into pcDNA3.1. DNA sequencing was used to confirm construct integrity of the respective plasmid. The plasmid and Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) complex were allowed to form for 20 min at room temperature, and the transfection was performed at 37°C for 24 h.

Statistical Analysis

All data are expressed as the means ± S.D. of at least three separate experiments. All statistical analyses were performed using GraphPad Prism 5.0 (GraphPad Software, Inc., La Jolla, CA, USA). Statistical significance was determined using the two-sided Student's *t*-test, and $p<0.05$ was considered significant.

Results

COX-2 Expression is Correlated With the Cisplatin Sensitivity of NSCLC Cells

To preliminarily elucidate the relationship between COX-2 expression levels and cisplatin resistance of NSCLC cells, COX-2 levels in A549 and H460 cells were assessed by Western blot and Real-time PCR assays. The results revealed that both protein and mRNA levels of COX-2 were much higher in NSCLC cell line A549 compared to H460 (Figure 1A and 1B). The MTT assay also indicated that A549 cells were more resistant to cisplatin than were H460 cells (Figure 1C). Our finding suggests that COX-2 expression may be positively associated with cisplatin resistance, at least in these two cell lines.

Ectopic Expression of COX-2 Promoted the Cisplatin Resistance of NSCLC Cells

To further show the role of COX-2 in cisplatin resistance in NSCLC cells, a COX-2 overexpression plasmid was constructed and transfected into H460 cells, which express relatively low levels of endogenous COX-2. Western blotting was performed to determine the efficiency of the overexpression plasmid and to detect the expression of EMT-related biomarkers, including E-cadherin, vimentin and N-cadherin. As shown in Figure 2A, E-cadherin was significantly downregulated, while vimentin and N-cadherin were upregulated in response to COX-2 overexpression. These findings indicated that the ectopic expression of COX-2 promoted the EMT process in H460 cells. Furthermore, increased p-AKT expression was observed when COX-2 was overexpressed in H460 cells, with no change in total AKT levels (Figure 2B). Meanwhile, the MTT assay results indicated that H460 cells were more resistant to cisplatin after COX-2 overexpression (Figure 2C). The data from the FACS assay also revealed that endogenous COX-2 significantly attenuated cisplatin-induced apoptosis in H460 cells (Figure 3A and 3B). Taken together, our data demonstrate that COX-2 may potentiate cisplatin resistance by promoting EMT through the AKT signaling pathway activation.

NS398 Potentiates Chemosensitivity to Cisplatin in NSCLC Cells via Induction of Apoptosis

Subsequently, whether NS398 could sensitize NSCLC cells to cisplatin was also investigated.

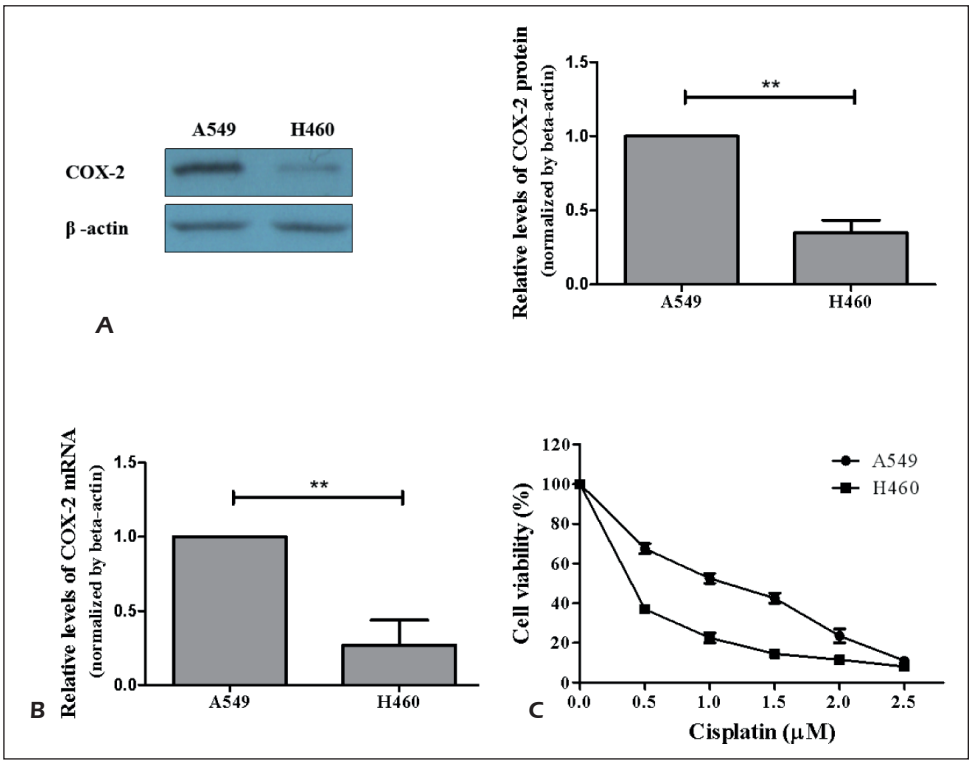

Figure 1. COX-2 expression is positively associated with cisplatin resistance in human non-small cell lung cancer. **A**, Western blotting analysis of COX-2 protein levels in the indicated cell lines. **B**, Real-Time PCR analysis of COX-2 mRNA levels in the indicated cell lines. **C**, H460 and A549 cells were treated with the indicated concentrations of cisplatin for 24 h. The MTT assay was performed to determine cell viability. Data are presented as means ± SD of three separate experiments. ***p*<0.01 *vs.* control.

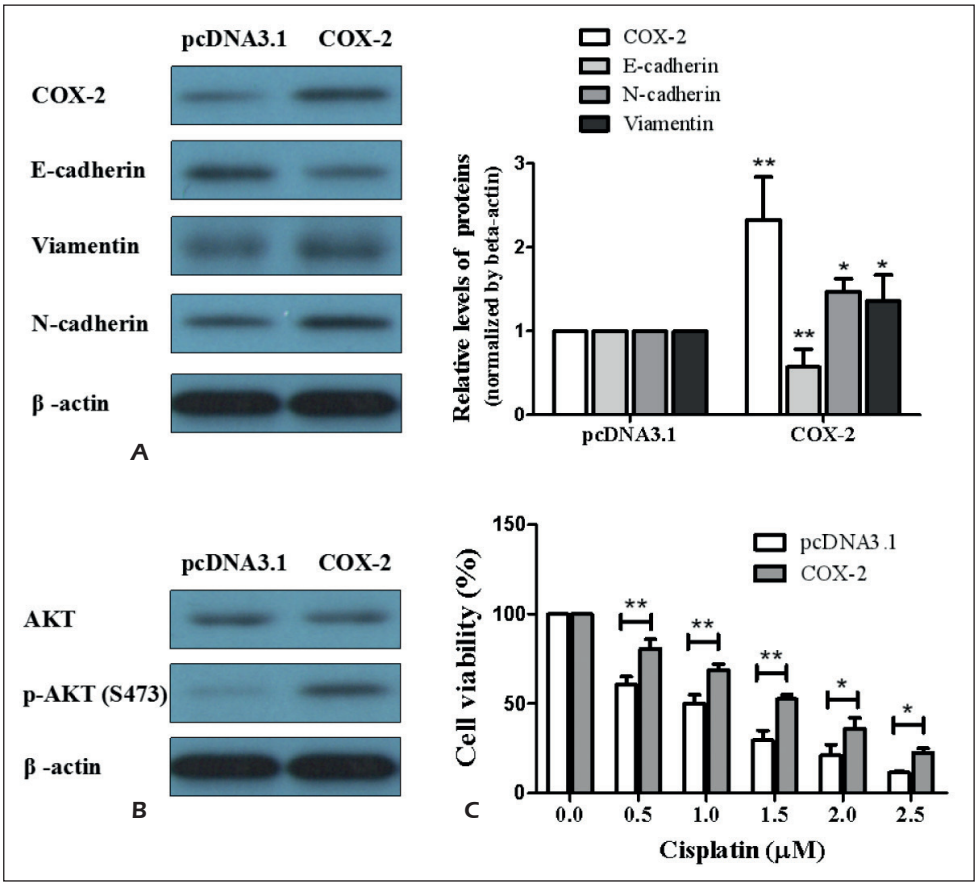

Figure 2. COX-2 potentiates the chemoresistance of non-small cell lung cancer (NSCLC) cells to cisplatin. **A**, and **B**, After H460 cells were transfected with COX-2 overexpression plasmid or empty vector pcDNA3.1 for 24 h, Western blotting was used to detect the indicated proteins. **C**, After H460 cells were transfected with COX-2 overexpression plasmid or empty vector pcDNA3.1 following cisplatin treatment with the indicated concentrations, cell viability was determined by MTT assays. Data are presented as means ± SD of three separate experiments. **p*<0.05 *vs.* control; ***p*<0.01 *vs.* control.

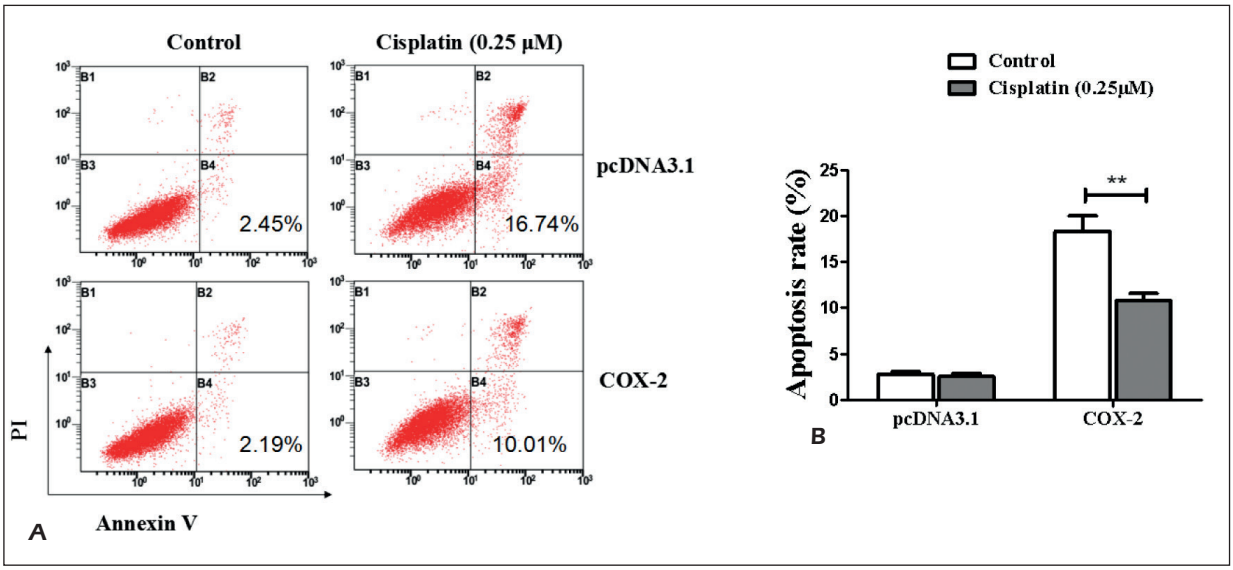

Figure 3. COX-2 overexpression attenuates cisplatin-induced apoptosis in H460 cells. After H460 cells were transfected with COX-2 overexpression plasmid or empty vector pcDNA3.1 for 24 h, cells were treated with 0.25 μM cisplatin for another 24 h. FACS was performed to assess apoptosis following cell staining with Annexin V-FITC and PI. Data are presented as means ± SD of three separate experiments. $^{**}p<0.01$ *vs.* control.

First, MTT assay was performed to determine the toxicity of NS398 to NSCLC cell lines A549 and H460 (Figure 4A). Next, we examined whether combined treatment of NS398 and cisplatin exerted enhanced lethality in human NSCLC cells. After treatment with the indicated concentrations of NS398, cisplatin alone, or NS398 combined with cisplatin for 24 h, samples were stained with Annexin V-FITC and PI and then subjected to FACS assays. Interestingly, the combination of NS398 and cisplatin dramatically induced apoptosis in A549 cells (the percentage of Annexin-V-positive cells increased from 9.42% to 64.64%) (Figure 4B). Significant cleavage of caspase 3 and PARP was observed in A549 cells treated with combined NS398 and cisplatin (Figure 4C). These results suggest that NS398 potentiates the chemosensitivity of NSCLC cells to cisplatin *via* inducing apoptosis.

The Intrinsic Apoptotic Pathway is Initiated in Response to Cisplatin Sensitization by NS398 in Human NSCLC, with EMT and AKT Pathway Suppression

There are the two primary pathways of apoptosis in tumor cells, the extrinsic and intrinsic apoptotic pathways[17,18]. Cleavage of caspases 3, 8, and 9 is a hallmark of activation of the extrinsic and intrinsic apoptosis pathways[19,20]. Our observation that both caspase 8 and caspase 9 are cleaved in NS398- and cisplatin-treated A549 cells suggests that both intrinsic and extrinsic apoptosis pathways are likely involved in NS398- and cisplatin-induced apoptosis (Figure 4C). JC-1 staining was used to determine the effect of NS398 and cisplatin on mitochondrial membrane potential (MMP). As shown in Figure 5A, the disruption of MMP was significantly induced by NS398 and cisplatin. After co-treatment with 40 μM NS398 and 0.25 μM cisplatin, the percentage of A549 cells with depolarized MMP increased to 50.4%. These findings indicate that NS398 potentiates cisplatin-induced apoptosis *via* the activation of both the intrinsic and extrinsic apoptotic pathways.

Furthermore, the functions of NS398 in EMT and AKT pathway activation in A549 cells were determined. As shown in Figure 5B, phosphorylation of AKT was effectively inhibited, while E-cadherin was upregulated and N-cadherin was downregulated in A549 cells treated with 40 μM NS398. Our data suggest that NS398 may potentiate the effects cisplatin on NSCLC cells by suppressing both the EMT process and the AKT signaling pathway activation.

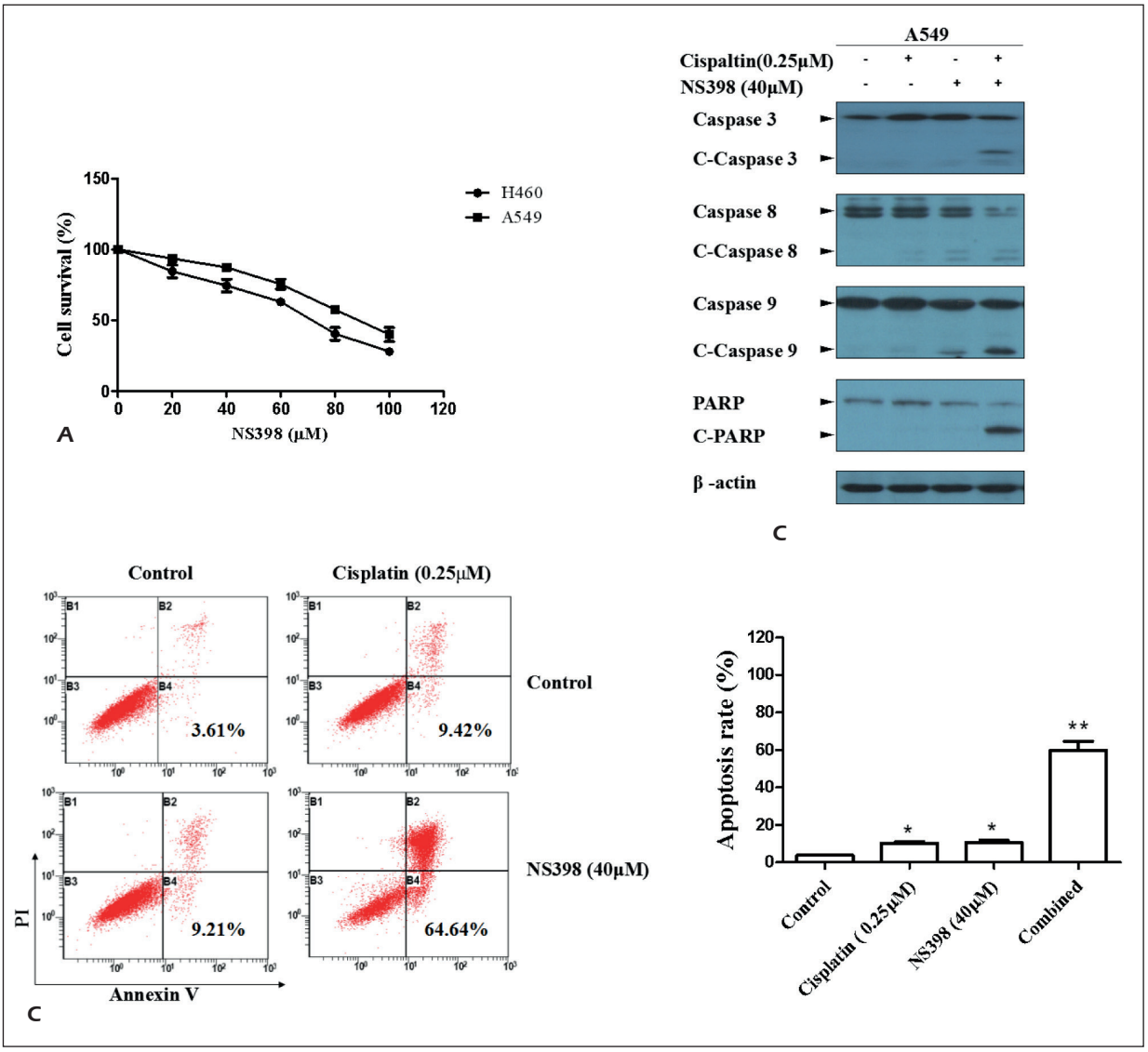

Figure 4. NS398 sensitizes human NSCLC cell line A549 to cisplatin-induced apoptosis. **A**, H460 cells were treated with the indicated concentrations of NS398 for 24 h. The MTT assay was performed to determine cell viability. Data represent three separate experiments. **B**, After treatment as above, FACS was performed to assess apoptosis in A549 cells by co-staining with Annexin V-FITC and PI. Data are presented as means ± SD of three separate experiments. *p<0.05 *vs.* control; **p<0.01 *vs.* control. **C**, After A549 cells were treated with 0.25 μM cisplatin, 20 μM NS398, or both in combination for 24 h, Western blotting was used to detect the proteins indicated. Representative data from three independent experiments are shown.

Discussion

Over the decades, the optimization of chemotherapy has observably improved the survival of patients with advanced NSCLC[21]. However, nearly all patients yield to relapse due to the development of drug resistance, and no valid therapy is available at present[22]. Therefore, it is urgent to understand the mechanism whereby drug resistance occurs in human NSCLCs. Many studies[23,24] have shown that some human NSCLC cell lines are resistant to cisplatin, and co-treatment with chemotherapeutic drugs and radiation can promote cisplatin-resistant cells to become sensitive to cisplatin.

It has been reported that COX-2-derived prostaglandin E2 contributes to lung tumorigenesis[25]. Increasing studies indicate that COX-2 also plays an important role in the drug resistance of cancer cells. Upregulated tumor expression of COX-2 is associated with an increased risk for angiogenesis, as well as resistance to apoptosis[26]. Furthermore,

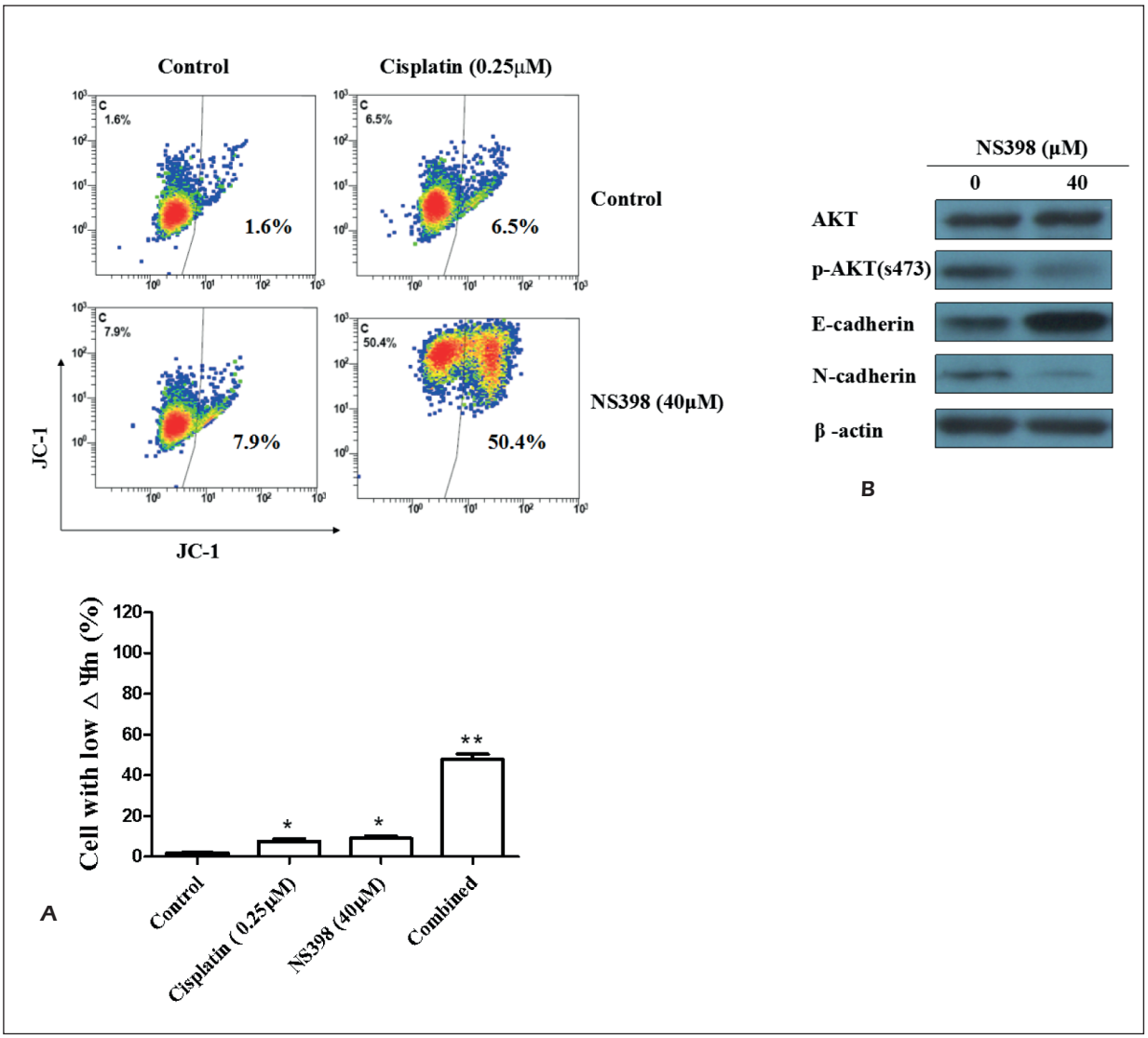

Figure 5. The intrinsic apoptotic pathway and AKT pathway are involved in induction of apoptosis in cisplatin-sensitized A549 cells. **A**, After A549 cells were treated with 0.25 μM cisplatin, 20 μM NS398, or both in combination for 24 h, cells were stained with JC-1 and subjected to flow cytometry to assess mitochondrial membrane permeabilization. Data are presented as means ±SD of three separate experiments. *p<0.05 vs. control; **p<0.01 *vs.* control. **B**, After A549 cells were treated with 20 μM NS398 for 24 h, Western blotting was performed to detect the proteins indicated. Representative data from three independent experiments are shown.

YAP can increase COX-2 expression to promote the development of drug resistance in human colon cancer[27]. In addition, COX-2 expression is a predictive factor for early relapse and aromatase inhibitor resistance in patients with ductal carcinoma in situ of the breast and is a target for treatment[28].

In the current work, our results indicated that COX-2 expression may be positively associated with cisplatin resistance in two NSCLC cell lines, A549 and H460 (Figure 1). The overexpression of COX-2 significantly enhanced cisplatin resistance in H460 cells through the activation of EMT and AKT signaling (Figure 2). As a result, the ectopic expression of COX-2 attenuated cisplatin-induced apoptosis in H460 cells (Figure 3). These findings all indicate that COX-2 expression levels are a predictor of cisplatin resistance and that EMT may play a critical role in this process. In a previous study[29], some COX-2 inhibitors, such as celecoxib, unfortunately failed to benefit the survival of NSCLC patients through EMT in human lung cancer *via* MEK-ERK signaling. Therefore, different COX-2 inhibitors may function in multiple ways and likely introduce other unexpected side effects.

Several investigations have shown that NS398 suppresses cell growth and induces apoptosis in various cancer cell lines, and a variety of anti-tumor effects of COX-2 inhibitors have been proposed as well[30]. In the present work, NS398 was chosen to test its function in the cisplatin resistance of NSCLC cells and to explore the underlying mechanism. Our data revealed that NS398 significantly sensitized the human NSCLC cell line A549 to cisplatin-induced apoptosis through both extrinsic and intrinsic apoptotic pathway activation (Figure 4). More importantly, NS398 also inhibited the EMT process with concomitant suppression of the AKT signaling (Figure 5).

Conclusions

We demonstrated that COX-2 promotes the cisplatin resistance of NSCLC cells and that the COX-2 inhibitor NS398 augments cisplatin chemosensitivity *via* the inhibition of the EMT and AKT pathway signaling. These results suggest that therapies combining the COX-2 inhibitor NS398 with cisplatin may prove to be an effective new strategy for the treatment of cisplatin-resistant human NSCLC.

Acknowledgements

This work was supported by the Technology Division of Taizhou (No. 14SF07).

Conflict of Interests

The authors declare that they have no conflict of interest.

References

1) Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015; 65: 87-108.
2) Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011; 144: 646-674.
3) Zhao N, Wilkerson MD, Shah U, Yin X, Wang A, Hayward MC, Roberts P, Lee CB, Parsons AM, Thorne LB, Haithcock BE, Grilley-Olson JE, Stinchcombe TE, Funkhouser WK, Wong KK, Sharpless NE, Hayes DN. Alterations of LKB1 and KRAS and risk of brain metastasis: comprehensive characterization by mutation analysis, copy number, and gene expression in non-small-cell lung carcinoma. Lung Cancer 2014; 86: 255-261.
4) Blanco R, Maestu I, de la Torre MG, Cassinello A, Nunez I. A review of the management of elderly patients with non-small-cell lung cancer. Ann Oncol 2015; 26: 451-463.
5) Williams CS, Smalley W, DuBois RN. Aspirin use and potential mechanisms for colorectal cancer prevention. J Clin Invest 1997; 100: 1325-1329.
6) Dubois RN, Abramson SB, Crofford L, Gupta RA, Simon LS, Van De Putte LB, Lipsky PE. Cyclooxygenase in biology and disease. FASEB J 1998; 12: 1063-1073.
7) Leung WK, To KF, Ng YP, Lee TL, Lau JY, Chan FK, Ng EK, Chung SC, Sung JJ. Association between cyclo-oxygenase-2 overexpression and missense p53 mutations in gastric cancer. Br J Cancer 2001; 84: 335-339.
8) Gong WH, Zhao N, Zhang ZM, Zhang YX, Yan L, Li JB. The inhibitory effect of resveratrol on COX-2 expression in human colorectal cancer: a promising therapeutic strategy. Eur Rev Med Pharmacol Sci 2017; 21: 1136-1143.
9) Simonsson M, Bjorner S, Markkula A, Nodin B, Jirstrom K, Rose C, Borgquist S, Ingvar C, Jernstrom H. The prognostic impact of COX-2 expression in breast cancer depends on oral contraceptive history, preoperative NSAID use, and tumor size. Int J Cancer 2017; 140: 163-175.
10) Tucker ON, Dannenberg AJ, Yang EK, Zhang F, Teng L, Daly JM, Soslow RA, Masferrer JL, Woerner BM, Koki AT, Fahey TR. Cyclooxygenase-2 expression is up-regulated in human pancreatic cancer. Cancer Res 1999; 59: 987-990.
11) Xiong H, Li B, He J, Zeng Y, Zhang Y, He F. lncRNA HULC promotes the growth of hepatocellular carcinoma cells via stabilizing COX-2 protein. Biochem Biophys Res Commun 2017; 490: 693-699.
12) Pastuszak-Lewandoska D, Domanska D, Czarnecka KH, Kordiak J, Migdalska-Sek M, Nawrot E, Kiszalkiewicz J, Antczak A, Gorski P, Brzezianska E. Expression of STAT5, COX-2 and PIAS3 in correlation with NSCLC histhopathological features. PLoS One 2014; 9: e104265.
13) Guerrant W, Kota S, Troutman S, Mandati V, Fallahi M, Stemmer-Rachamimov A, Kissil JL. YAP mediates tumorigenesis in neurofibromatosis type 2 by promoting cell survival and proliferation through a COX-2-EGFR signaling axis. Cancer Res 2016; 76: 3507-3519.
14) Morin PJ. Drug resistance and the microenvironment: nature and nurture. Drug Resist Updat 2003; 6: 169-172.
15) Thiery JP, Acloque H, Huang RY, Nieto MA. Epithelial-mesenchymal transitions in development and disease. Cell 2009; 139: 871-890.
16) Mani SA, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhou AY, Brooks M, Reinhard F, Zhang CC, Shipitsin M, Campbell LL, Polyak K, Brisken C, Yang J, Weinberg RA. The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell 2008; 133: 704-715.
17) Green DR. Apoptotic pathways: paper wraps stone blunts scissors. Cell 2000; 102: 1-4.
18) Wang X. The expanding role of mitochondria in apoptosis. Genes Dev 2001; 15: 2922-2933.
19) Nunez G, Benedict MA, Hu Y, Inohara N. Caspases: the proteases of the apoptotic pathway. Oncogene 1998; 17: 3237-3245.
20) Martinou JC, Youle RJ. Mitochondria in apoptosis: Bcl-2 family members and mitochondrial dynamics. Dev Cell 2011; 21: 92-101.

21) Liu X, Ma M, Duan X, Zhang H, Yang M. Knockdown of OCT4 may sensitize NSCLC cells to cisplatin. Clin Transl Oncol 2017; 19: 587-592.
22) Boyer MJ, Gu L, Wang X, Kelsey CR, Yoo DS, Onaitis MW, Dunphy FR, Crawford J, Ready NE, Salama JK. Toxicity of definitive and post-operative radiation following ipilimumab in non-small cell lung cancer. Lung Cancer 2016; 98: 76-78.
23) Ziemann F, Arenz A, Preising S, Wittekindt C, Klussmann JP, Engenhart-Cabillic R, Wittig A. Increased sensitivity of HPV-positive head and neck cancer cell lines to x-irradiation +/- Cisplatin due to decreased expression of E6 and E7 oncoproteins and enhanced apoptosis. Am J Cancer Res 2015; 5: 1017-1031.
24) Comella P, Scoppa G, Daponte A, Musetta G, Anania C, Maiorino A, Curcio C, Casaretti R, Comella G. Alternated approach with local irradiation and combination chemotherapy including cisplatin or carboplatin plus epirubicin and etoposide in intermediate stage non-small cell lung cancer. Cancer 1994; 74: 1874-1881.
25) Chang J, Xue M, Yang S, Yao B, Zhang B, Chen X, Pozzi A, Zhang MZ. Inhibition of 11beta-hydroxysteroid dehydrogenase type II suppresses lung carcinogenesis by blocking tumor COX-2 expression as well as the ERK and mTOR signaling pathways. PLoS One 2015; 10: e127030.
26) Murono S, Inoue H, Tanabe T, Joab I, Yoshizaki T, Furukawa M, Pagano JS. Induction of cyclooxygenase-2 by Epstein-Barr virus latent membrane protein 1 is involved in vascular endothelial growth factor production in nasopharyngeal carcinoma cells. Proc Natl Acad Sci U S A 2001; 98: 6905-6910.
27) Li W, Cao Y, Xu J, Wang Y, Li W, Wang Q, Hu Z, Hao Y, Hu L, Sun Y, Xu G, Ao G. YAP transcriptionally regulates COX-2 expression and GCCSysm-4 (G-4), a dual YAP/COX-2 inhibitor, overcomes drug resistance in colorectal cancer. J Exp Clin Cancer Res 2017; 36: 144.
28) Generali D, Buffa FM, Deb S, Cummings M, Reid LE, Taylor M, Andreis D, Allevi G, Ferrero G, Byrne D, Martinotti M, Bottini A, Harris AL, Lakhani SR, Fox SB. COX-2 expression is predictive for early relapse and aromatase inhibitor resistance in patients with ductal carcinoma in situ of the breast, and is a target for treatment. Br J Cancer 2014; 111: 46-54.
29) Wang ZL, Fan ZQ, Jiang HD, Qu JM. Selective Cox-2 inhibitor celecoxib induces epithelial-mesenchymal transition in human lung cancer cells via activating MEK-ERK signaling. Carcinogenesis 2013; 34: 638-646.
30) Sun Y, Tang XM, Half E, Kuo MT, Sinicrope FA. Cyclooxygenase-2 overexpression reduces apoptotic susceptibility by inhibiting the cytochrome c-dependent apoptotic pathway in human colon cancer cells. Cancer Res 2002; 62: 6323-6328.