

# LncRNA BC200 regulates the cell proliferation and cisplatin resistance in non-small cell lung cancer via PI3K/AKT pathway

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**Abstract. – OBJECTIVE:** Long non-coding RNA (lncRNA) exerts tissue specificity and regulates the occurrence and progression of tumors. Previous bioinformatics showed that lncRNA BC200 is served as an oncogene. However, the specific role of BC200 in lung cancer (LC) is rarely reported. The aim of this study is to elucidate the regulatory effects of BC200 on tumor development and cisplatin resistance in non-small cell lung cancer (NSCLC).

**PATIENTS AND METHODS:** The expression level of BC200 in 76 pairs of NSCLC tissues and adjacent normal tissues was detected by quantitative Real Time-Polymerase Chain Reaction (qRT-PCR). Correlation analyses were conducted to investigate the relation between BC200 expression and prognosis of NSCLC patients. Subsequently, BC200 expression in LC cell lines was detected. After construction of si-BC200 and si-NC, the cellular functions of LC cells were detected through colony formation, flow cytometry and transwell assay, respectively. Western blot was performed to detect the protein expressions of key genes in the PI3K/AKT pathway in LC cells. Finally, cell counting kit-8 (CCK-8) assay was carried out to explore the effect of BC200 on cisplatin resistance of LC cells via calculating IC50.

**RESULTS:** Higher expression of BC200 was found in NSCLC tissues than that of adjacent normal tissues. BC200 expression was positively correlated with tumor stage, lymph node metastasis and distant metastasis of NSCLC patients, whereas not correlated to age and sex. Knockdown of BC200 inhibited proliferation, invasion and migration of LC cells. Western blot results showed that protein expressions of PI3K, AKT and STAT3 were downregulated after BC200 knockdown in LC cells. Additionally, the IC50 in H1299/DDP cells transfected with si-BC200 was lower than in those transfected with si-NC. The apoptotic rate in H1299 cells transfected with si-BC200 was remarkably lower than those transfected with si-NC.

**CONCLUSIONS:** BC200 is highly expressed in NSCLC, which is positively correlated with tu-

mor stage and metastasis of NSCLC patients. BC200 promotes the malignant progression of NSCLC via regulating cisplatin-induced apoptosis of H1299/DDP cells.

*Key Words:*

BC200, NSCLC, Cisplatin resistance, Malignant progression.

## Introduction

Lung cancer (LC) is a common primary tumor of the respiratory system, which is the leading malignancy throughout the world<sup>1,2</sup>. Non-small cell lung cancer (NSCLC) is the most common type of LC, accounting for about 80% of LC cases. According to the pathological types, NSCLC is divided into squamous cell carcinoma, adenocarcinoma and large cell carcinoma<sup>3,4</sup>. About 75% of NSCLC patients are already in an advanced stage of diagnosis, leading to the low 5-year survival rate<sup>3,4</sup>. Genetics factors, diet, unhealthy lifestyles and precancerous lesions are all closely related to NSCLC occurrence. It is reported that over 50% of NSCLC patients experience micrometastasis before radical surgery, which is the direct cause of postoperative metastasis and recurrence<sup>3-6</sup>. The comprehensive understanding of the molecular mechanism of NSCLC contributes to develop effective therapeutic approaches and to improve the clinical outcomes of affected people. Currently, surgical resection and chemotherapy are the preferred options for treating NSCLC, which can greatly prevent recurrence and metastasis of NSCLC. However, chemotherapy resistance is the main cause of chemotherapy failure, remarkably limiting its clinical application<sup>5,6</sup>. With the in-depth researches on NSCLC, it is believed that NSCLC is the result of genetic and environmental factors. Alterations in certain oncogenes and

tumor-suppressor genes finally lead to disordered cell proliferation, apoptosis and differentiation<sup>7,8</sup>.

Non-coding RNAs have been well recognized in recent years. Among them, long non-coding RNA (lncRNA) is a kind of non-coding RNA with over 200 bases in length<sup>9-11</sup>. lncRNAs may be differentially expressed in malignancies, which are closely related to the progression of tumors. Certain lncRNAs could be served as diagnostic and prognostic markers<sup>12,13</sup>. Functionally, lncRNA degrades or inhibits the target mRNA, thereafter regulating target gene expressions<sup>14,15</sup>. Misexpression of lncRNA may result in multiple misexpressed proteins<sup>14,15</sup>. Accumulating evidence has shown that lncRNAs are closely related to the occurrence and progression of malignancies, which are served as tumor hallmarks<sup>16</sup>. For example, lncRNA BC200 is differentially expressed in tumors and is utilized as a biomarker<sup>17-19</sup>. Our previous studies<sup>20,21</sup> have already found that BC200 is highly expressed in NSCLC as an oncogene, which promotes proliferation and induces apoptosis of LC cells. However, the potential mechanism of BC200 in regulating NSCLC development has not been fully elucidated. Through literature review<sup>22,23</sup>, we concluded that BC200 is involved in regulating proliferation, apoptosis, differentiation and metastasis of tumor cells. The specific role of BC200 in NSCLC still needs to be further investigated.

In the present work, we first detected the expression level of BC200 in NSCLC tissues and adjacent normal tissues. The regulatory effect of BC200 on cisplatin resistance of LC cells and NSCLC tissues were further explored.

## Patients and Methods

### Sample Collection

76 pairs of NSCLC tissues and adjacent normal tissues were surgically resected. Enrolled patients were all pathologically diagnosed as NSCLC and received DDP (cisplatin) chemotherapy (Table I). Based on the therapeutic outcomes of DDP chemotherapy, patients were divided into DDP-sensitive group (n=31) and DDP-insensitive group (n=45). This study was approved by the Shandong Provincial Hospital Affiliated to Shandong University Ethics Committee and all patients signed the informed consent.

### Cell Culture

Human LC cell lines (SKMES1, A549, PC-9, H358, H1299 and SPCA1) and human bronchial epithelial cell line (16HBE) were obtained from Cell Bank, Chinese Academy of Sciences. DDP-resistance H1299 cells (H1299/DDP) were obtained from Runcheng (Shanghai, China). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM; Gibco, Rockville, MD, USA) containing 10% fetal bovine serum (FBS; Gibco, Rockville, MD, USA) and maintained in a 5% CO<sub>2</sub> incubator at 37°C.

### Transfection

H1299 and H1299/DDP cells in logarithmic growth phase were seeded in the 6-well plates at a density of 5×10<sup>4</sup> cells per well. Cells were transfected with si-BC200 or si-NC according to the instructions of Lipofectamine 2000 (Invitrogen,

**Table I.** Association of lncRNA BC200 expression with clinicopathologic characteristics of non-small cell lung cancer.

| Parameters            | Number of cases | lncRNA BC200 expression |      | p-value |
|-----------------------|-----------------|-------------------------|------|---------|
|                       |                 | Low                     | High |         |
| Age (years)           |                 |                         |      | 0.488   |
| <60                   | 32              | 20                      | 12   |         |
| ≥60                   | 44              | 24                      | 20   |         |
| Gender                |                 |                         |      | 0.096   |
| Male                  | 37              | 25                      | 12   |         |
| Female                | 39              | 19                      | 20   |         |
| T stage               |                 |                         |      | 0.017   |
| T1-T2                 | 43              | 30                      | 13   |         |
| T3-T4                 | 33              | 14                      | 19   |         |
| Lymph node metastasis |                 |                         |      | 0.019   |
| No                    | 45              | 31                      | 14   |         |
| Yes                   | 31              | 13                      | 18   |         |
| Distance metastasis   |                 |                         |      | 0.010   |
| No                    | 60              | 40                      | 20   |         |
| Yes                   | 16              | 4                       | 12   |         |

Carlsbad, CA, USA). Culture medium was replaced 5-6 hours later.

### **Cell Proliferation Assay**

Transfected cells were digested for the preparation of cell suspension ( $5 \times 10^4$ /mL). 100  $\mu$ L of the suspension was added in each well of the 96-well plates. 24 hours after cell adherence, 100  $\mu$ L of medium containing 0, 1, 2, 4, 8, 16, 32, and 64  $\mu$ g/mL DDP was added. Each concentration had 5 replicates. After cell culture for 48 h, 10  $\mu$ L of cell counting kit-8 (CCK-8) solution (Dojindo, Kumamoto, Japan) was added and the optical density was determined at the wavelength of 450 nm 1 hour later. The  $IC_{50}$  was finally calculated.

### **Colony Formation Assay**

200 transfected cells were seeded in each well of the 6-well plates for 2-week cell culture. The medium was replaced once a week. Cells were then washed with phosphate-buffered saline (PBS), fixed with methanol for 20 min and stained with 0.1% crystal violet for 20 min. Colonies were captured using a microscope.

### **Flow Cytometry Analysis of Cell Apoptosis**

H1299 and SPCA1 cells in logarithmic growth phase were seeded in the 6-well plates. After transfection for 24 h, cells were centrifuged and resuspended in 500  $\mu$ L of Binding Buffer. Subsequently, cells were incubated with 5  $\mu$ L of Annexin V-APC and 5  $\mu$ L of 7-AAD in the dark for 15 min. Flow cytometry analyses were performed for accessing the cell apoptotic rate.

### **Transwell Assay**

Transfected cells were centrifuged and resuspended in serum-free DMEM at a density of  $2.0 \times 10^5$ /mL. Transwell chambers pre-coated with Matrigel were placed in 24-well plates. 200  $\mu$ L of cell suspension and 600  $\mu$ L of medium containing 10% FBS were added in the upper and lower chamber, respectively. After cell culture for 48 h, cells were fixed with 4% paraformaldehyde for 15 min and stained with crystal violet for 15 min. Inner cells were carefully cleaned. Penetrating cells were captured in 5 randomly selected fields of each sample for cell counting.

### **Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)**

TRIzol kit (Invitrogen, Carlsbad, CA, USA) was used to extract the total RNA, which was then reversely transcribed into complementary

Deoxyribose Nucleic Acid (cDNA). The sequence of specific BC200 RT primers was 5'-AGACCTGCCTGGGCAATATAGC-3', glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was 5'-CATGAGAAGTATGACAACAGCCT-3'. The sequences of specific BC200 PCR primers: forward, 5'-AGACCTGCCTGGGCAATATAGC-3' and reverse, 5'-GTTGTTGCTTTGAGGGAAGTTACG-3'. The expression levels of BC200 were normalized to GAPDH (forward, 5'-CATGAGAAGTATGACAACAGCCT-3' and reverse, 5'-AGTCCTTCCACGATACCAAAGT-3'). After the cDNA was amplified, qRT-PCR was performed to detect the expressions of related genes.

### **Western Blot**

Cells were lysed for protein extraction. The concentration of each protein sample was determined by a BCA (bicinchoninic acid) kit (Abcam, Cambridge, MA, USA). The protein sample was separated by gel electrophoresis and transferred to PVDF (polyvinylidene difluoride) membranes (Millipore, Billerica, MA, USA). After incubation with the primary and secondary antibody (Cell Signaling Technology, Danvers, MA, USA), immunoreactive bands were exposed by enhanced chemiluminescence method (ECL).

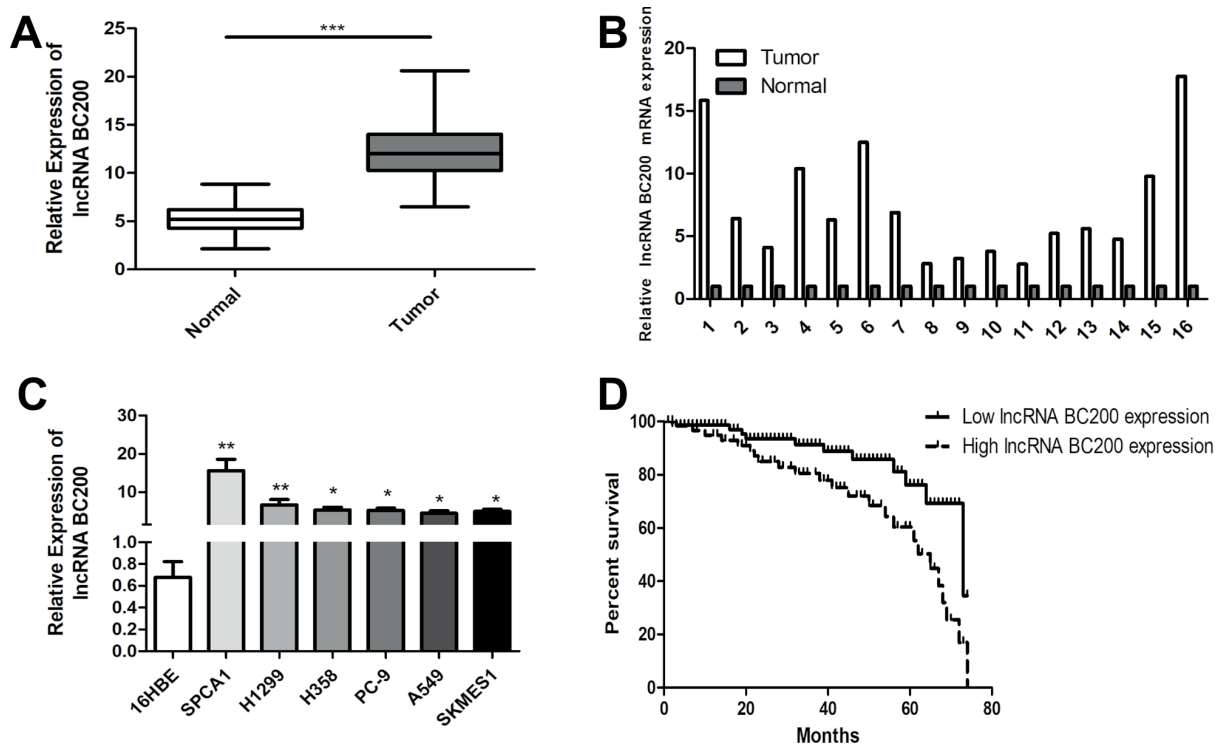
### **Statistical Analysis**

We used Statistical Product and Service Solutions (SPSS) 22.0 software (IBM, Armonk, NY, USA) for statistical analysis. The quantitative data were represented as mean  $\pm$  standard deviation ( $\bar{x} \pm s$ ). The *t*-test was used for comparing differences between the two groups. Differences among groups were compared with the one-way ANOVA, followed by LSD analyses (Least Significant Difference). The prognosis of NSCLC patients was analyzed by Kaplan-Meier and differences between curves were compared by the Log-rank test.  $p < 0.05$  was considered statistically significant.

## **Results**

### **BC200 Was Highly Expressed in NSCLC Tissues and Cell Lines**

We detected the expression level of BC200 in NSCLC tissues and adjacent normal tissues by qRT-PCR. The data showed higher expression of BC200 in NSCLC tissues than that of adjacent normal tissues (Figure 1A and 1B). Similarly, BC200 was highly expressed in LC cell lines



**Figure 1.** A-B, BC200 expression in 76 pairs of NSCLC tissues and adjacent normal tissues. C, Expression levels of BC200 in LC cell lines (H1299, SPCA1, MLC76H, SMMC-7221, Huh7, Hep3B) and normal hepatic cell (LO2). D, Kaplan-Meier survival curves of NSCLC patients based on BC200 expression. Patients in the high-level BC200 group had a significantly more unfavorable prognosis than those in the low-level BC200 group.

compared with that of controls (Figure 1C). In particular, H1299 and SPCA1 cells exerted a relatively higher expression of BC200, which were selected for the following experiments.

#### **BC200 Expression Was Correlated with Clinical Stage, Lymph Node Metastasis, Distance Metastasis, and Overall Survival in LC Patients**

Based on the expression level of BC200, NSCLC patients were assigned into high-level BC200 and low-level BC200 group, respectively. The correlation between BC200 expression with age, sex, tumor stage, lymph node metastasis and distant metastasis of NSCLC patients was analyzed. The data showed that BC200 expression was positively correlated with tumor stage, lymph node metastasis and distant metastasis of NSCLC patients, whereas not correlated to age and sex. Follow-up data of each patient was collected for analyzing the relationship between BC200 expression and prognosis of NSCLC. Kaplan-Meier results indicated that higher expression of BC200 is remarkably associated with worse prognosis of NSCLC patients ( $p < 0.05$ , Figure 1D). It is suggested that

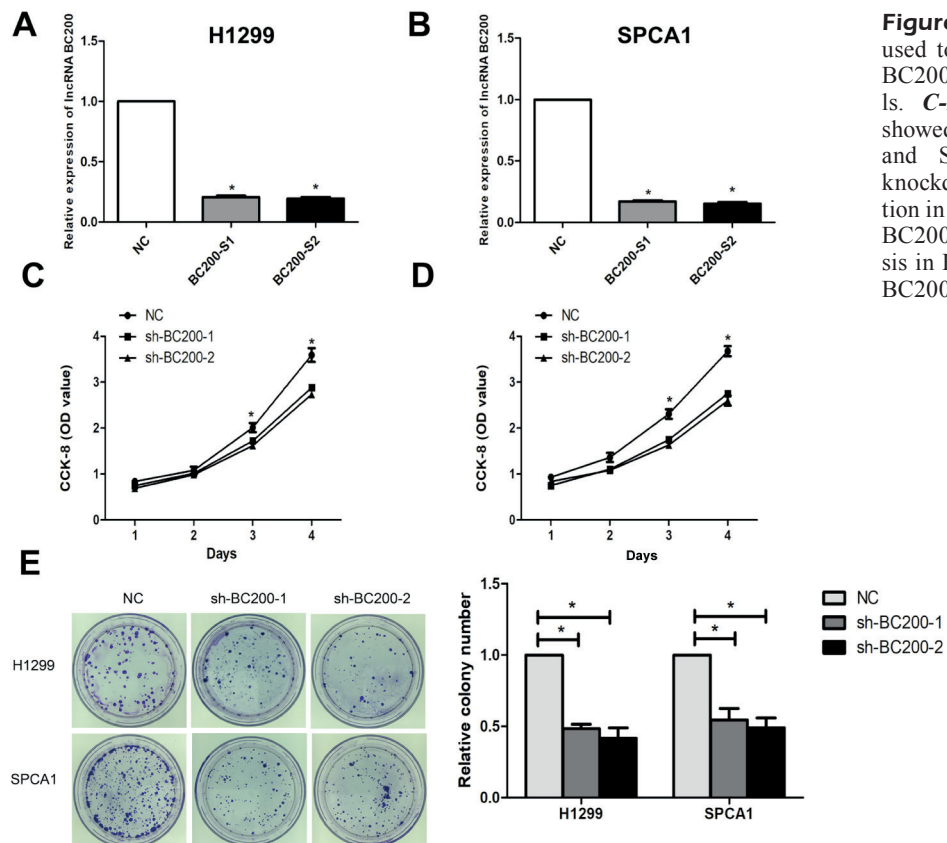
BC200 may serve as a new biological hallmark for predicting the prognosis of NSCLC.

#### **Knockdown of BC200 Inhibited Proliferation of LC Cells**

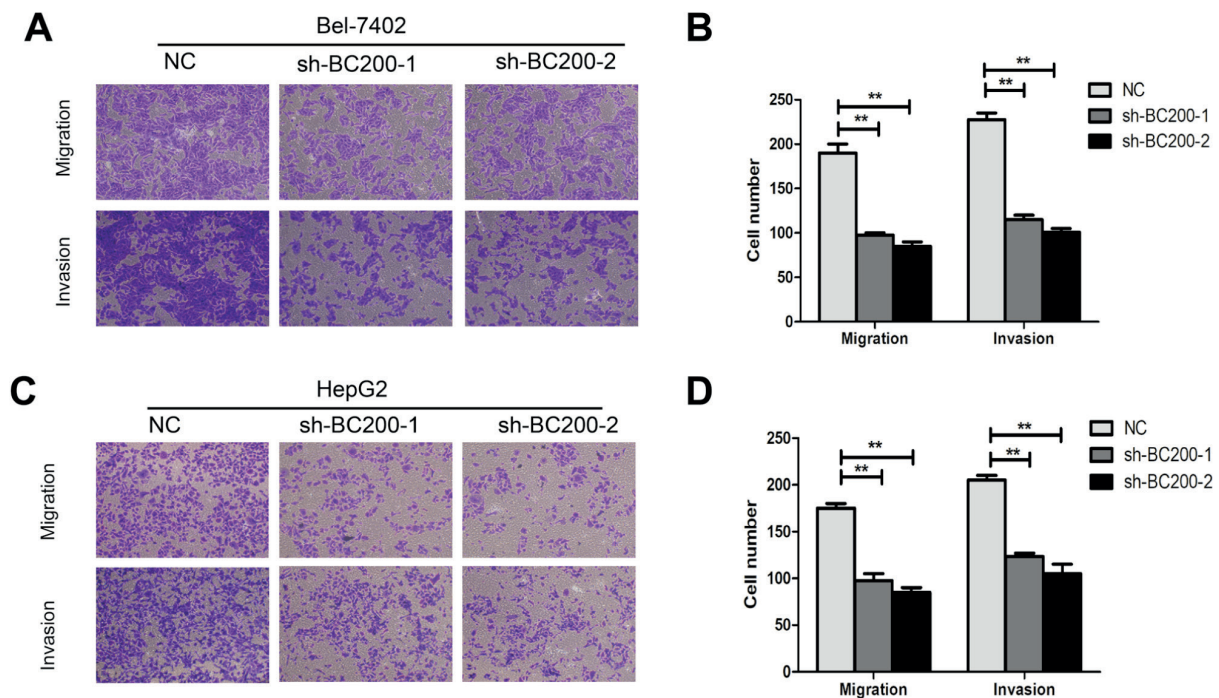
To explore the regulatory effect of BC200 on the proliferation of LC cells, we first constructed si-BC200 and si-NC. Transfection efficacies of them in LC cells were verified (Figure 2A and 2B). CCK-8 results demonstrated that BC200 knockdown remarkably decreased the proliferation of LC cells (Figure 2C and 2D). Colony formation assay obtained the similar results (Figure 2E).

#### **Knockdown of BC200 Inhibited Migration and Invasion of LC Cells**

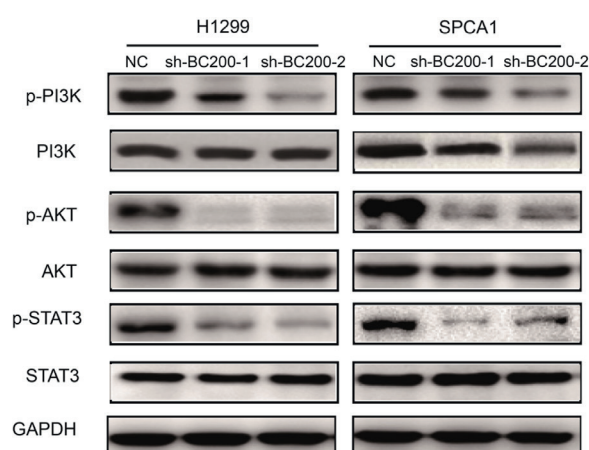
Subsequently, transwell assay was conducted to explore the migratory and invasive abilities after BC200 knockdown in LC cells. We found that the amount of penetrating cells in LC cells transfected with si-BC200 was lower than those of controls, suggesting the inhibited migratory capacity (Figure 3A and 3B). Similarly, cell invasion was inhibited by BC200 knockdown in LC cells as well (Figure 3C and 3D).



**Figure 2.** A-B, QRT-PCR were used to verify the efficiency of si-BC200 in H1299 and SPCA1 cells. C-D, Growth curve analysis showed the cell growth of H1299 and SPCA1 cells after BC200 knockdown. E, Cell colony formation in H1299 and SPCA1 cells after BC200 knockdown. F, Cell apoptosis in H1299 and SPCA1 cells after BC200 knockdown.



**Figure 3.** A-B, H1299 and SPCA1 cells transfected with si-BC200 displayed significantly lower migratory capacity. C-D, H1299 and SPCA1 cells transfected with si-BC200 displayed significantly lower invasive capacity.



**Figure 4.** Knockdown of BC200 significantly decreased protein expressions of key genes in PI3K/AKT signal pathway, including PI3K, AKT and STAT3.

#### **Knockdown of BC200 Inhibited PI3K/AKT Pathway**

PI3K/AKT pathway has been confirmed to regulate cellular functions, including cell migration and invasion. Hence, we speculated whether BC200 regulated NSCLC development *via* PI3K/AKT pathway. Western blot results showed that protein expressions of PI3K, AKT and STAT3 were downregulated after BC200 knockdown in LC cells (Figure 4).

#### **Cisplatin Modulated BC200 Expression in LC Cells**

To further investigate the role of BC200 in cisplatin resistance of LC cells, cell viability was detected after cisplatin treatment. The  $IC_{50}$  in H1299 cells transfected with si-BC200 after cisplatin treatment was  $15.22 \pm 0.91$   $\mu\text{g/mL}$ , which was  $3.42 \pm 0.90$   $\mu\text{g/mL}$  in H1299 cells transfected with si-NC (Figure 5A). On the contrary, the  $IC_{50}$  in H1299/DDP cells transfected with si-BC200 was lower than those transfected with si-NC (Figure 5B). The above data demonstrated that BC200 knockdown elevated the cisplatin resistance in LC cells.

Furthermore, cell apoptosis was detected after BC200 knockdown by flow cytometry. The apoptotic rate in H1299 cells transfected with si-BC200 was remarkably lower than those transfected with si-NC. However, H1299/DDP cells transfected with si-BC200 presented a higher apoptotic rate than in those transfected with si-NC (Figure 5C). Therefore BC200 knockdown could alleviate the cisplatin-induced apoptosis of LC cells.

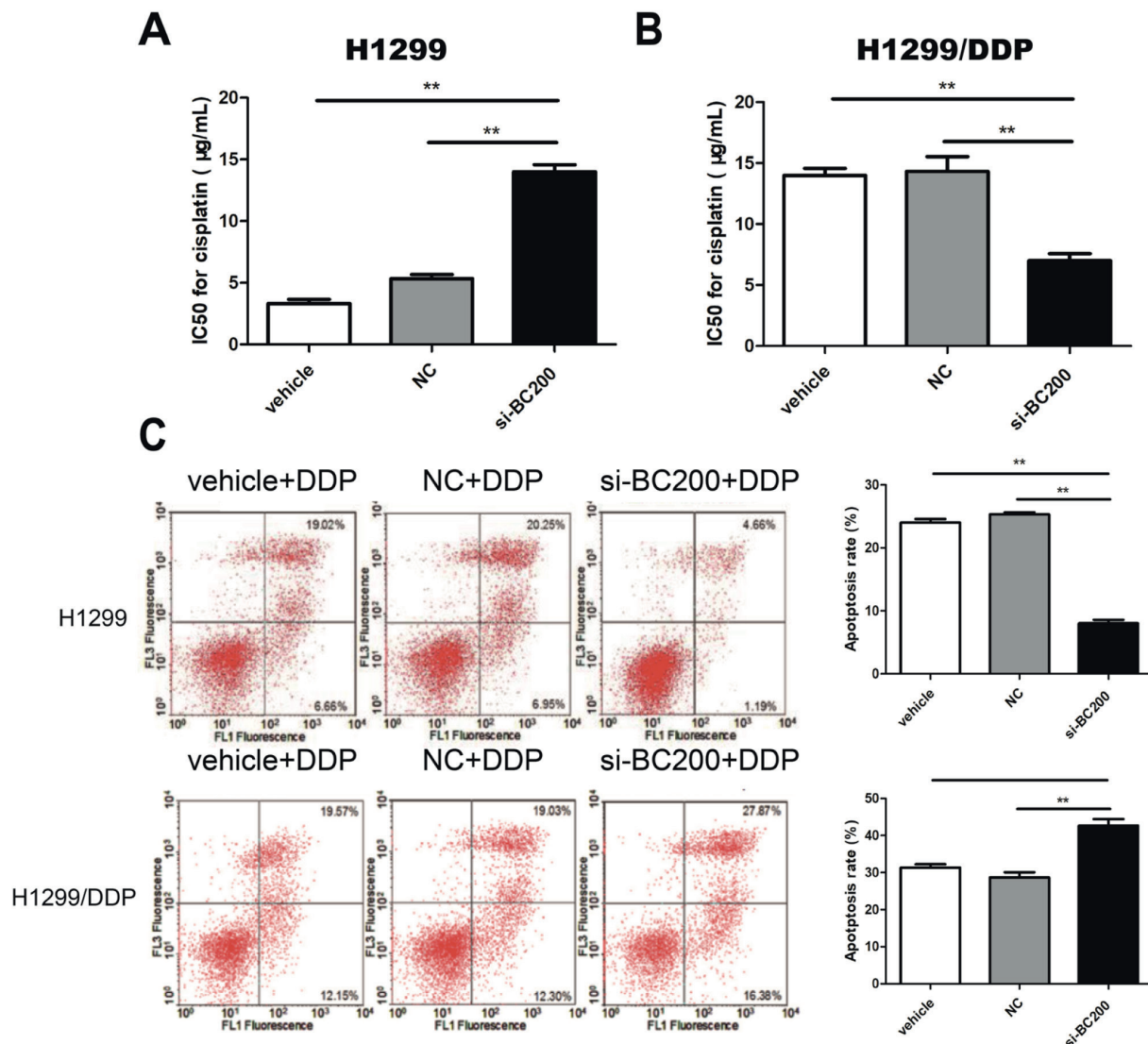
## **Discussion**

LC is a common malignancy with a high morbidity<sup>1,3</sup>. Currently, the combined treatment of platinum-based drugs with rh-endostatin exerts an optimal therapeutic efficacy in treating NSCLC<sup>3,4</sup>. However, cisplatin resistance poses a huge challenge in chemotherapy of affected people, which requires for effective approaches to overcome the chemotherapy limitations<sup>3-5</sup>. At present, chemotherapy is the major approach in treating NSCLC besides surgical resection, and it markedly decreases the incidence of postoperative recurrence and metastasis<sup>5,6</sup>. It is noteworthy that chemotherapy resistance during the postoperative treatment remarkably restricts its clinical application.

In recent years, lncRNAs have shown their capacities in regulating drug resistance of tumor treatment<sup>9-11</sup>. For example, lncRNA MEG3 knockdown promotes DDP resistance in LC cells *via* Wnt pathway<sup>24</sup>. lncRNA GAS5 regulates DDP resistance in NSCLC cells *via* autophagy pathway<sup>25</sup>. Additionally, lncRNA NEAT1 serves as a microRNA sponge that enhances the DDP-sensitivity of LC cells<sup>26</sup>. Differentially expressed lncRNAs may exert vital roles in diagnosing and treating LC<sup>10-22</sup>.

Previous studies<sup>9,10</sup> have proved the regulatory effects of lncRNAs on the occurrence and progression of tumors. Based on the specific function in tumor development, lncRNAs are divided into carcinogenic lncRNAs and carcinostatic lncRNAs<sup>11</sup>. Under normal condition, the dynamic balance of carcinogenic and carcinostatic lncRNAs could repair DNA damage and promote apoptosis of abnormal cells. However, oncogenes break out this balance, thereafter leading to carcinogenesis<sup>12-15</sup>. LC development involves multiple gene changes. For instance, p52 inhibits tumor development by directly inducing the transcription of lncRNA p21, thereby forming a regulatory network to further mediate the downstream genes<sup>16-19</sup>. In this investigation, we first detected BC200 expression in NSCLC tissues and adjacent normal tissues. BC200 expression was found to be positively correlated to tumor stage, lymph node metastasis and distant metastasis of NSCLC patients. Further *in vitro* experiments revealed the role of BC200 in regulating migration and invasion of LC cells.

PI3K/AKT pathway is an important signaling pathway involved in tumorigenesis<sup>27,28</sup>. PI3K/AKT exerts its anti-apoptotic effect mainly by affecting multiple effector molecules<sup>29,30</sup>. At present, apoptosis promotion has become the focus



**Figure 5.** A-B, IC<sub>50</sub> in DDP-induced H1299 and SPCA1 cells. C, Apoptotic rate of LC cells after transfection was detected by FCM assay. A representative data set was displayed as mean ± SD values (\**p*<0.05, \*\**p*<0.01).

of tumor treatment<sup>31,32</sup>. Therefore, the PI3K/AKT pathway is believed to be significant for developing new therapeutic targets for tumor cell metastasis<sup>33,34</sup>. We found that the expression levels of PI3K, AKT and STAT3 were remarkably down-regulated after BC200 knockdown in LC cells, indicating that BC200 promotes invasion and metastasis in NSCLC through the PI3K/AKT pathway.

Furthermore, we speculated the underlying role of BC200 in chemotherapy resistance of NSCLC. BC200 expression was downregulated in H1299/DDP cells than that of H1299 cells. DDP-sensitivity of H1299/DDP cells was markedly elevated after BC200 overexpression, indicating that

BC200 regulates drug resistance in NSCLC. However, the specific mechanism of BC200 in regulating chemotherapy resistance of NSCLC still needs to be further investigated.

## Conclusions

We found that BC200 was highly expressed in NSCLC, which was positively correlated with tumor stage and metastasis of NSCLC patients. BC200 promoted malignant progression of NSCLC *via* regulating cisplatin-induced apoptosis of H1299/DDP cells.

## Conflict of Interest

The Authors declare that they have no conflict of interest.

## Acknowledgements

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