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LncRNA HOXA-AS2 regulates microRNA-216a-5p to promote malignant progression of non-small cell lung cancer

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Abstract. – OBJECTIVE: Previous studies have shown that long non-coding RNA (lncRNA) HOXA-AS2 is a cancer-promoting gene. However, the role of HOXA-AS2 in non-small cell lung cancer (NSCLC) has not been reported. This study aims to investigate the expression characteristics of HOXA-AS2 in NSCLC and whether HOXA-AS2 can promote the malignant progression of NSCLC by regulating microRNA-216a-5p.

PATIENTS AND METHODS: Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) was used to examine the HOXA-AS2 level in 40 pairs of NSCLC tumor tissue samples and adjacent ones. Then, the relationship between HOXA-AS2 expression and clinical indicators and prognosis of NSCLC was analyzed. Meanwhile, qRT-PCR further verified the expression level of HOXA-AS2 in NSCLC cell lines. Also, HOXA-AS2 knockdown and overexpression models were constructed using lentivirus in NSCLC cell lines, and the effects of HOXA-AS2 on the biological function of NSCLC cells were analyzed using the Cell Counting Kit-8 (CCK-8), transwell migration, and cell wound healing assays. Finally, Western blotting assay and cell recovery experiment were used to explore the regulatory mechanism of HOXA-AS2 and microRNA-216a-5p in NSCLC.

RESULTS: In this experiment, qRT-PCR results revealed that HOXA-AS2 level in NSCLC tumor tissue specimens was remarkably higher than that in adjacent tissues. Compared with those with low expression of HOXA-AS2, the patients with high expression had a higher incidence of distant metastases and a lower overall survival rate. The proliferative and metastasis abilities of the cells in the HOXA-AS2 overexpression group were remarkably increased when compared with the control group, while the opposite results were observed in HOXA-AS2 silence group. Subsequently, qRT-PCR verified that microRNA-216a-5p level was remarkably decreased in NSCLC tissues and negatively correlated with HOXA-AS2 expression. In addition, the result of the cell recovery experiment and Western blotting revealed that there might be a mutual regulation between HOXA-AS2 and microRNA-216a-5p, the two of which could jointly regulate the malignant progression of NSCLC.

CONCLUSIONS: The results indicate that lncRNA HOXA-AS2 is upregulated in NSCLC and is remarkably associated with distant metastasis and poor prognosis of NSCLC patients. In addition, lncRNA HOXA-AS2 is found to be able to promote the malignant progression of NSCLC via regulating microRNA-216a-5p.

Key Words:
LncRNA HOXA-AS2, MicroRNA-216a-5p, Lung cancer, Malignant progression.

Introduction

Lung cancer has become the most common malignant tumor with an extremely high incidence in the world. According to statistics[1,2], the mortality rate of lung cancer is high among all kinds of cancers. In recent years, with the rapid economic development and the environmental degradation caused by urbanization, the harm of lung cancer has gradually increased, and the urgency of lung cancer treatment has been listed as the first among all cancer treatments in China[3,4]. According to the specific location and histopathological manifestations of lung cancer, lung cancer can be further divided into many types, among which non-small cell lung cancer (NSCLC), second only to malignant small cell lung cancer, is also a type of cancer with an extremely high incidence[5,6]. The current treatment of NSCLC mainly relies on surgery and chemotherapy. However, although great progress has been made in the treatment of NSCLC in recent years, due to the untimely detection and diagnosis of cancer, the overall 5-year survival rate of NSCLC patients is still very low,

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which remains only about 15%[7,8]. Therefore, the in-depth study on the molecular mechanism of the development and metastasis of NSCLC and the discovery of new molecular biomarkers for the diagnosis of lung cancer have become particularly important for the early diagnosis and early treatment of NSCLC[9,10].

According to the central principle, genetic information is transmitted from DNA to RNA and then forms proteins with different functions through translation and other processes[11,12]. However, with the completion of human genome sequencing, it has been found that only 2% of the total RNA can be translated, and most of the RNA has not been transcribed, and this type of RNA is collectively referred to as non-coding RNA[13,14]. According to previous studies[15,16], the occurrence and development of lung cancer are always accompanied by the changes in the expression of non-coding RNA, and some ncRNAs also play an important regulatory role and become important targets for NSCLC research. Long non-coding RNA (lncRNA) is a new member of newly discovered ncRNAs, and its expression is specific in time, space, and tissue[17]. So far, a large amount of reports have shown that lncRNAs are involved in many biological processes in many organisms, including X chromosome inactivation, reactivation of pluripotent stem cells, and regulation of muscle cell differentiation, apoptosis, and invasion[13,18]. Wang et al[19] have suggested that lncRNA HOXA-AS2 is an oncogenic gene, but its role in NSCLC has not been reported.

Previous studies[20,21] have found that microRNA-216a-5p gene can play a role in the inhibition of the occurrence and development of tumors, especially in tumor metastasis and proliferation. According to the hypothesis of ceRNA (competitive endogenous RNA), through bioinformatics retrieval, we have discovered that the genes with sequence correlation of microRNA-216a-5p include HOXA-AS2 gene, which has not been included in the studies of its correlation with lung cancer[22]. Therefore, in this study, the expressions of HOXA-AS2 and microRNA-216a-5p in 40 NSCLC tumor tissue samples and adjacent ones were analyzed, respectively, and the effects of HOXA-AS2 and microRNA-216a-5p on the biological functions of NSCLC cells were explored. Meanwhile, the relevant mechanism of the effects of lncRNA HOXA-AS2 on clinical parameters, prognosis, and malignant progression of lung cancer through sponge adsorption of microRNA-216a-5p was further explored.

Patients and Methods

Patients and NSCLC Samples

The tumor tissue samples and adjacent ones of 40 patients with lung cancer were collected. All specimens were obtained from the surgical specimens of the Department of Oncology, Thoracic Surgery, and Respiratory Medicine, as well as biopsy or bronchoscopy. In addition, the paracancerous tissues of all specimens were more than 5 cm away from cancerous tissues, and no anti-tumor treatment (e.g., radiotherapy or chemotherapy) was performed before surgery. The study was approved by the Ethics Committee of Fujian Provincial Hospital, and all patients signed informed consent. All patients were followed-up through telephone and outpatient follow-up, including general conditions, clinical symptoms, and imaging examination. This study was conducted in accordance with the Declaration of Helsinki.

Cell Lines and Reagents

Five kinds of non-small cell lung cancer cell lines including NL9980, H1299, H460, SPC-A1, A549, and a normal human bronchial epithelial cell Beas-2B were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA). Dulbecco's Modified Eagle's Medium (DMEM) medium and fetal bovine serum (FBS) were obtained from Life Technologies (Gaithersburg, MD, USA). The cells were cultured at 37°C under 5% CO_2 in DMEM containing 10% FBS.

Transfection

The control group and the lentivirus containing the HOXA-AS2 overexpression and knockdown sequences were purchased from Shanghai Jima Company (Shanghai, China). The cells were plated in 6-well plates and grown to a cell density of 40%, transfected according to the manufacturer's instructions, and were harvested 48 h later for quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) analysis and cell function experiments.

Cell Proliferation Assays

The cells after 48 h of transfection were harvested and plated into 96-well plates at 2000 cells per well. Then, the cells were cultured for 6 h, 24 h, 48 h, and 72 h respectively, and then added with the Cell Counting Kit-8 (CCK-8; Dojindo Laboratories, Kumamoto, Japan) reagent. After incubation for 2 h, the optical density (OD) value of each well was measured in the microplate reader at an absorption wavelength of 490 nm.

Transwell Cell Migration Assay

After transfection for 48 h, the cells were trypsinized and resuspended in the serum-free medium. After cell counting, the diluted cell density was adjusted to 2.0×10^5/mL, and the transwell chamber containing Matrigel and no Matrigel was placed in a 24-well plate. 200 μL of the cell suspension was added to the upper chamber, while 500 μL of the medium containing 10% FBS was added to the lower chamber. After 48 h, the chamber was removed and fixed with 4% paraformaldehyde for 30 minutes. After staining with the crystal violet for 15 minutes, the cells were washed with, and the inner surface of the basement membrane of the chamber was carefully cleaned to remove the inner layer cells. The perforated cells stained in the outer layer of the basement membrane of the chamber were observed in 5 randomly selected fields of view under the microscope.

Wound Healing Assay

After transfection for 48 h, the cells were digested, centrifuged, and resuspended in the serum-free medium to adjust the density to 5 x 10^5 cells/mL. The density of the plated cells was determined according to the size of the cells (the majority of the number of cells plated was set to 50000 cells/well). After the stroke, the cells were gently rinsed with PBS for 2-3 times, added with low-concentration serum medium (such as 1% FBS), and incubated for other 24 h. According to the pre-experiment of scratches, it was judged whether the cells had healing ability according to the migration area. For the scratch test, the difference in cell healing ability was judged according to the migration area.

ORT-PCR

The total RNA was extracted from the tissue samples using the TRIzol (Invitrogen, Grand Island, NY) method, and reversely transcribed into cDNA. Then, the complementary deoxyribose nucleic acid (cDNA) was applied for Real Time-PCR using 2xSYBR Green PCR Master Mix (TaKaRa, Otsu, Shiga, Japan) with β-actin as an internal reference. The PCR was carried out on a quantitative PCR reactor. The data obtained after three independent experiments were analyzed by the formula $RQ=2^{-\Delta\Delta Ct}$.

The following primers were used for qRT-PCR reactions: lncRNA HOXA-AS2: forward: 5'-CCCGTAGGAAGAACCGATGA-3', reverse: 5'-TTTAGGCCTTCGCAGACAGC-3'; β-actin: forward: 5'-CCTGGCACCCAGCACAAT-3', reverse: 5'-TGCCGTAGGTGTCCCTTTG-3'; microRNA-216a-5p: forward: 5'-GGGCTGGACCGAGAGAGTTT-3', reverse: 5'-CCTTGTACGTGGTGGGATTGA-3'; U6: forward: 5'-CTCGCTTCGGCAGCACA-3', reverse: 5'-AACGCTTCACGAATTTGCGT-3'.

The data analysis was performed using the ABI Step One software (Applied Biosystems, Foster City, CA, USA) and the relative expression levels of mRNA were calculated using the $2^{-\Delta\Delta Ct}$ method.

Western Blotting

The transfected cells were lysed using cell lysis buffer, shaken on ice for 30 minutes, and centrifuged at 14,000 g for 15 minutes at 4°C. The total protein concentration was calculated by bicinchoninic acid (BCA) Protein Assay Kit (Pierce, Rockford, IL, USA). The extracted proteins were separated using a 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) gel and subsequently transferred to a polyvinylidene difluoride membrane. The primary antibodies N-cadherin, Vimentin, β-catenin, MMP-9, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH), and the secondary antibodies were all purchased from Cell Signaling Technology (Danvers, MA, USA).

Statistical Analysis

The Statistical Product and Service Solutions (SPSS) 19.0 statistical software (IBM Corp., Armonk, NY, USA) was used for data analysis. The comparison between multiple groups was done using the One-way ANOVA test followed by the post-hoc test (Least Significant Difference). The expression of lncRNA HOXA-AS2 or microRNA-216a-5p in tumor and paracancerous tissues of NSCLC and its relationship with clinical parameters were analyzed by the Chi-square test. The Kaplan-Meier method was used to analyze the relationship between the expressions of lncRNA HOXA-AS2 or microRNA-216a-5p and the survival time and prognosis, and the Cox proportional risk model was used to analyze the factors affecting the prognosis of NSCLC. The mean standard deviation was used for the data, and $p<0.05$ was considered statistically significant.

Results

HOXA-AS2 Was Highly Expressed in NSCLC Tissues and Cell Lines

The expression of HOXA-AS2 in 40 pairs of NSCLC tumor tissues and their corresponding

adjacent tissues and NSCLC cell lines were detected by qRT-PCR. The results showed that HOXA-AS2 expression was remarkably higher in NSCLC tumor tissues compared with adjacent tissues (Figure 1A), meanwhile, HOXA-AS2 expression was also remarkably increased in the NSCLC cell line than in BEAS-2B, with a statistically significant difference (Figure 1B). Among them, the expression of the expression was higher in H460 and A549 cell lines, so we chose these two cells for subsequent experiments.

HOXA-AS2 Expression Was Correlated With Distance Metastasis and Overall Survival in NSCLC Patients

NSCLC tumor tissues were divided into high and low expression group according to HOXA-AS2 level in the tissue samples. As shown in Table I, a high level of HOXA-AS2 had no significant correlation with age, gender, pathological stage, and lymph node metastasis of NSCLC patients, but it was remarkably associated with distant metastasis. Meanwhile, the Kaplan-Meier survival curves indicated that the high level of HOXA-AS2 was remarkably associated with poor prognosis of NSCLC (p<0.05; Figure 1C). The above results suggested that HOXA-AS2 may be a new biological indicator for predicting the prognosis of NSCLC.

HOXA-AS2 Promotes NSCLC Cell Proliferation, Invasion, and Migration

To explore the effect of lncRNA HOXA-AS2 on NSCLC cell proliferation and invasion and migration, the HOXA-AS2 overexpression and knockdown models were successfully constructed (Figure 2A). Subsequently, CCK-8, transwell migration, and cell scratch assay showed that the proliferation, invasion, and metastasis ability of NSCLC cells in HOXA-AS2 overexpressing group HOXA-AS2 was remarkably increased compared with the NC group. In the HOXA-AS2 silencing group, the ability of NSCLC cells to proliferate, invade, and metastasize was remarkably reduced in Anti-HOXA-AS2 (Figures 2B-2D). These results demonstrated that HOXA-AS2 can promote NSCLC cell proliferation, invasion, and migration.

HOXA-AS2 Regulates EMT Signaling Pathway in NSCLC

In order to analyze the potential mechanism of HOXA-AS2 promoting NSCLC cell proliferation and invasion, and migration, the key proteins N-cadherin, Vimentin, β-catenin, and MMP-9 in EMT signaling pathway after knockdown and overexpression of HOXA-AS2 were detected by Western blotting. The results showed that the expression level of the above protein was remarkably downregulated after knocking down of

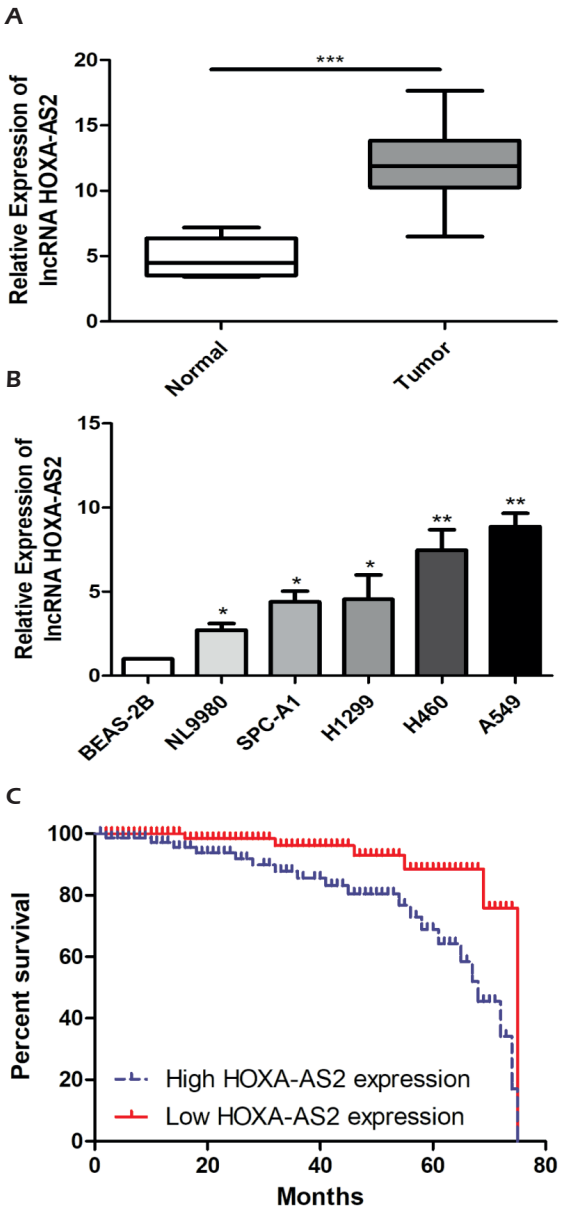

Figure 1. HOXA-AS2 is highly expressed in NSCLC tissues and cell lines. ***A,*** qRT-PCR was used to detect the difference in expression of HOXA-AS2 in NSCLC tumor tissues and adjacent tissues. ***B,*** qRT-PCR was used to detect the expression level of HOXA-AS2 in NSCLC cell lines. ***C,*** The Kaplan-Meier survival curve of lung cancer patients based on HOXA-AS2 expression; the prognosis of patients with high expression was significantly worse than that of the low expression group. Data are mean ± SD, *p<0.05, **p<0.01, ***p<0.001.

Table I. Association of lncRNA HOXA-AS2 and miR-216a-5p expression with clinicopathologic characteristics of NSCLC

Parameters	Number of cases	HOXA-AS2 expression		*p*-value	miR-216a-5p expression		*p*-value
		Low (%)	High (%)		High (%)	Low (%)	
Age (years)				0.327			0.505
<60	15	9	6		5	10	
≥60	25	11	14		10	15	
Gender				0.527			0.519
Male	20	11	9		6	14	
Female	20	9	11		9	11	
T stage				0.102			0.109
T1-T2	25	15	10		7	18	
T3-T4	15	5	10		8	7	
Lymph node metastasis				0.168			0.075
No	28	16	12		8	20	
Yes	12	4	8		7	5	
Distance metastasis				0.022			0.023
No	25	16	9		6	19	
Yes	15	4	11		9	6	

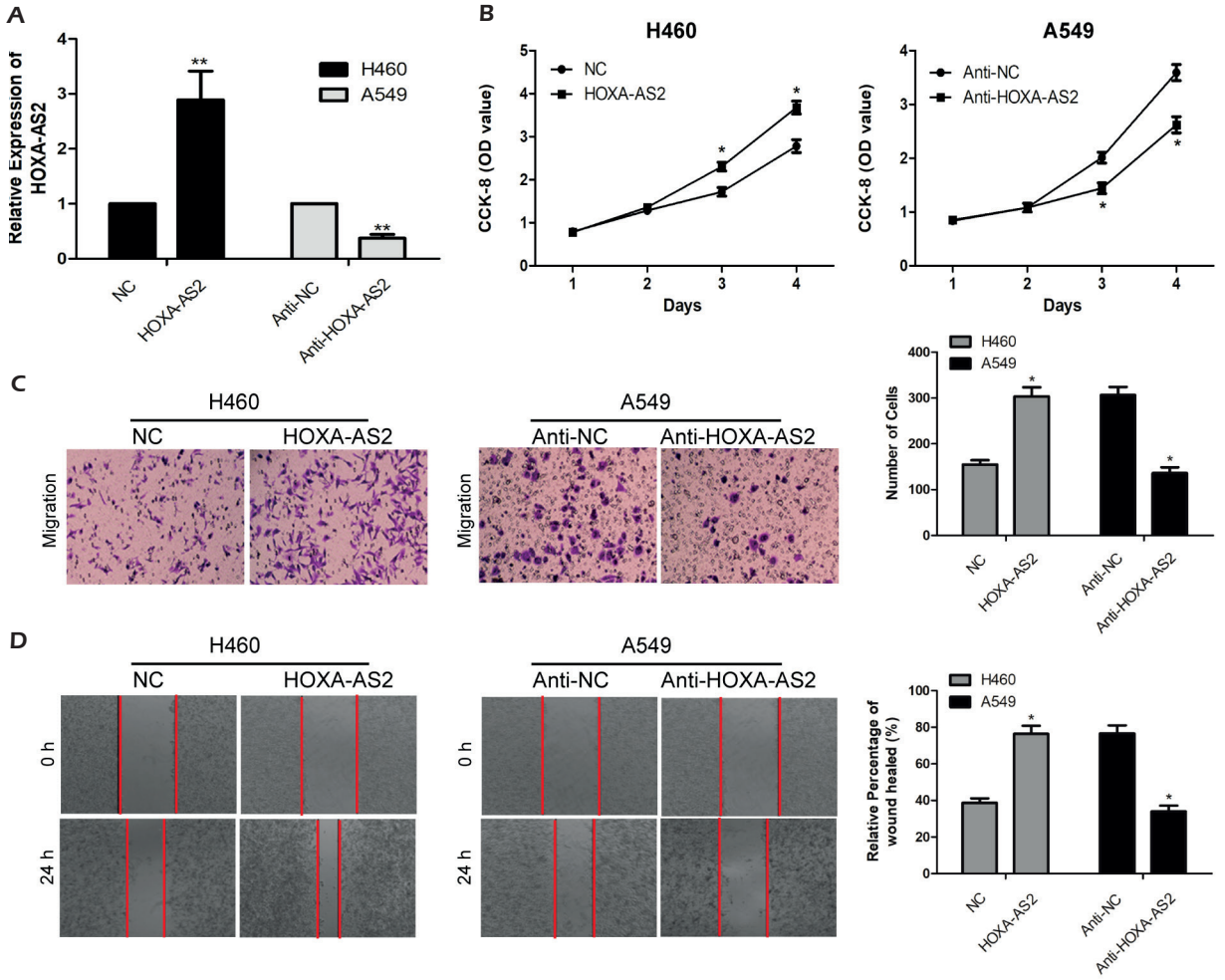

Figure 2. HOXA-AS2 regulates proliferation and invasion and migration of NSCLC cells. ***A,*** qRT-PCR verified the interference efficiency of HOXA-AS2 overexpression vector in H460 cell line and HOXA-AS2 after transfection of HOXA-AS2 knockout vector in A549 cell line. ***B,*** The CCK-8 assay detects the effect of HOXA-AS2 on proliferation of NSCLC cells in H460 and A549 cell lines. ***C,*** The transwell migration invasion assay detects the ability of H460 and A549 cell lines to interfere with the migration of HOXA-AS2 to NSCLC cells (× 40). ***D,*** The cell scratch assays tested the ability of H460 and A549 cell lines to interfere with HOXA-AS2 crawling on NSCLC cells (× 20). Data are mean ± SD, *p<0.05, **p<0.01.

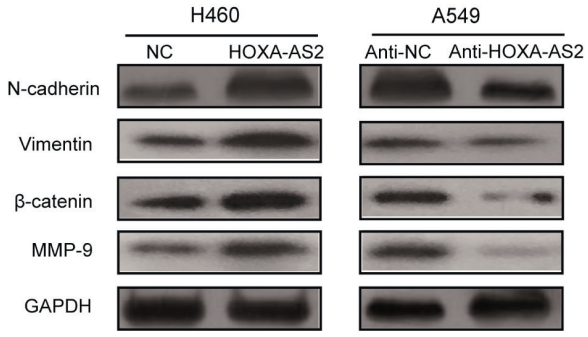

Figure 3. Overexpression/knockdown HOXA-AS2 upregulates/downregulates the expression of key proteins N-cadherin, Vimentin, β-catenin, and MMP-9 in the EMT signaling pathway.

HOXA-AS2; whereas the expression level of the above protein was remarkably upregulated after the overexpression of HOXA-AS2 (Figure 3), suggesting that HOXA-AS2 may regulate NSCLC through the EMT signaling pathway.

MicroRNA-216a-5p Was Lowly Expressed in NSCLC Tissues and Cell Lines

In order to explore the malignant progression of microRNA-216a-5p in NSCLC, the qRT-PCR experiment was performed to detect microRNA-216a-5p expression in 40 pairs of NSCLC tumor tissues and their corresponding paracancerous tissues, as well as in NSCLC cell lines. The expression level of microRNA-216a-5p was remarkably decreased in NSCLC tumor tissues, and the difference was statistically significant (Figure 4A). Compared with normal human bronchial epithelial cells BEAS-2B, microRNA-216a-5p was also remarkably lower in

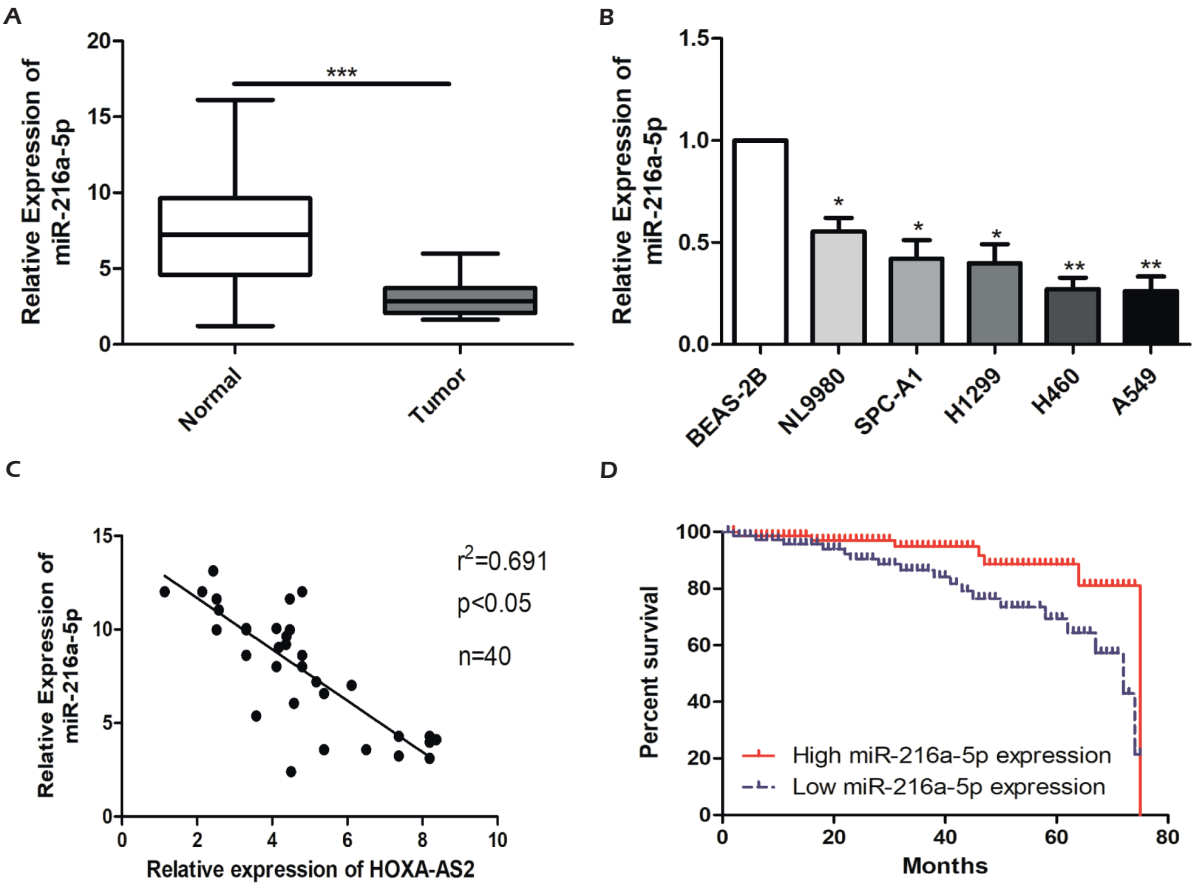

Figure 4. High expression of miR-216a-5p in NSCLC tissues and cell lines. ***A,*** qRT-PCR was used to detect the difference in expression of miR-216a-5p in NSCLC tumor tissues and adjacent tissues. ***B,*** qRT-PCR was used to detect the expression level of miR-216a-5p in NSCLC cell lines. ***C,*** qRT-PCR showed a significant negative correlation between HOXA-AS2 and miR-216a-5p expression in breast cancer tissues. ***D,*** The Kaplan-Meier survival curve of lung cancer patients based on miR-216a-5p expression; the prognosis of patients with low expression was significantly worse than that of the high expression group. Data are mean ± SD, *p<0.05, **p<0.01, ***p<0.001.

NSCLC cell lines, and the difference was statistically significant (Figure 4B). In addition, the expression of HOXA-AS2 and microRNA-216a-5p were detected by qRT-PCR, and the results showed that HOXA-AS2 and microRNA-216a-5p showed a negative correlation between mRNA expression levels in NSCLC tissue samples (Figure 4C). To explore the relationship between the expression of microRNA-216a-5p and the prognosis of patients with NSCLC, relevant follow-up data were collected. The Kaplan-Meier survival curves showed that low expression of microRNA-216a-5p was remarkably associated with poor prognosis of NSCLC ($p<0.05$; Figure 4D).

MicroRNA-216a-5p Modulated HOXA-AS2 Expression in NSCLC

In order to further explore the ways in which HOXA-AS2 regulated the malignant progression of NSCLC, a possible relationship between microRNA-216a-5p and HOXA-AS2 was found by the related bioinformatics analysis. Furthermore, in order to demonstrate the interaction between HOXA-AS2 and microRNA-216a-5p in NSCLC cells, the H460 cell line overexpressing HOXA-AS2 was transfected with microRNA-216a-5p mimics, while the A549 cell line with silenced HOXA-AS2 was further transfected with microRNA-216a-5p inhibitor, so as to investigate the role of microRNA-216a-5p and HOXA-AS2 in NSCLC, and the transfection efficiency of microRNA-216a-5p was examined by qRT-PCR (Figure 5A). Subsequently, it was found by CCK-8, transwell migration, and cell scratching experiments that microRNA-216a-5p mimics can reverse the effects of overexpressing HOXA-AS2 on the proliferation and invasion and metastasis of NSCLC cells, while microRNA-216a-5p inhibitor can reverse the effects of HOXA-AS2 silencing on proliferation, invasion, and metastasis of NSCLC cells (Figure 5B-5D).

In addition, Western blot was used to detect the expression changes of the key proteins N-cadherin, Vimentin, β-catenin, and MMP-9 in EMT signaling pathway after co-transfection of HOXA-AS2 and microRNA-216a-5p. The results showed that microRNA-216a-5p mimics reversed the overexpression of HOXA-AS2 and upregulated the EMT signaling pathway-related proteins, whereas microRNA-216a-5p inhibitor reversed the trend of the downregulating EMT signaling pathway protein after HOXA-AS2 silencing (Figure 6). These results revealed that microRNA-216a-5p could modulate HOXA-AS2 expression in NSCLC.

Discussion

As we all know, lung cancer is recognized as one of the malignant tumor diseases with the highest mortality[1-3]. Lung cancer can be divided into non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC) according to different degrees of differentiation and pathological morphology[5,6]. Despite the improvement of diagnosis and treatment conditions of NSCLC, it is still diagnosed at advanced stages in most cases, which leads to the final high mortality rate of lung cancer and poor prognosis of patients with a high degree of malignancy. Therefore, it is of great urgency to study the prognostic indicators of lung cancer[7-10].

According to the results of the genome sequencing, only 2% of the genes in the human body have the function of the encoding proteins, and the remaining 90% or more of the genes, although transcribed into RNA, are not in the process of being translated into proteins[11-13]. These RNAs that do not have the function of coding into proteins are called non-coding RNAs (ncRNAs), which can be divided into housekeeping ncRNA (housekeeping ncRNA) and regulatory ncRNA (regulatory ncRNA)[14-16]. LncRNAs were initially recognized to have no function, however, as the discovery of studies deepens, these RNAs are also found to be regulated by multiple genes, including the transcription process regulation, epigenetic regulation, and translation process regulation[17]. LncRNA also has certain tissue specificity, and it is expressed in different species, and the expression level of lncRNA detected varies from tissues to tissues[18]. The spatial and temporal specificity of lncRNA is manifested in the same tissue or organ, or unified tissue or organ, and the expression level of lncRNA detected also varies in different growth stages[18,19].

Many studies have revealed the relationship between lncRNA and different tumors, and lncRNA plays different roles in the occurrence and development of cancer[14-16]. The occurrence and development of NSCLC are precisely regulated by a variety of molecules and processes, including the activation of NSCLC oncogenes and the silencing of cancer suppression-related genes[9,10]. Therefore, to explore the abnormal expression of lncRNA in NSCLC and analyze its function will

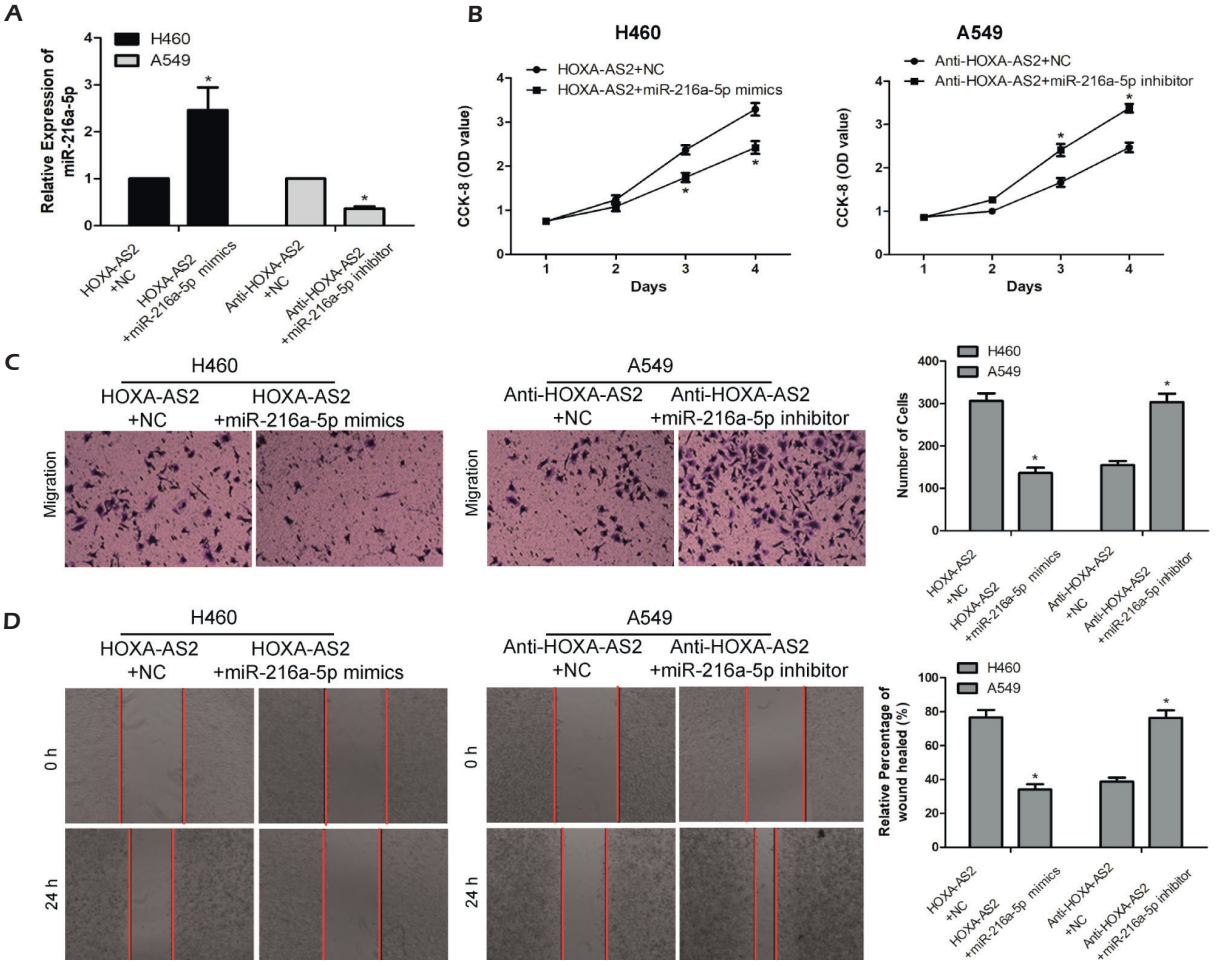

Figure 5. HOXA-AS2 regulates the role of miR-216a-5p in NSCLC cell lines. ***A,*** The expression level of miR-216a-5p in the NSHLC cell line co-transfected with HOXA-AS2 and miR-216a-5p was detected by qRT-PCR. ***B,*** CCK-8 detects the role of HOXA-AS2 and miR-216a-5p in co-transfection to regulate the proliferation of NSCLC cells. ***C,*** The transwell migration assay detects the role of HOXA-AS2 and miR-216a-5p in co-transfection to regulate NSCLC cell migration (× 40). ***D,*** The cell scratch assay was used to detect the role of HOXA-AS2 and miR-216a-5p in co-transfection of NSCLC cells (× 20). Data are mean ± SD, *$p<0.05$.

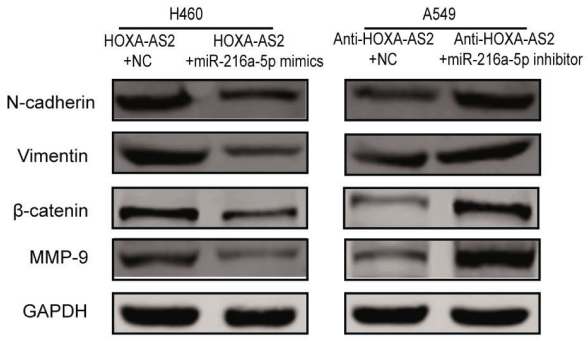

Figure 6. Western blotting detects the expression of the key proteins N-cadherin, Vimentin, β-catenin, and MMP-9 in EMT signaling pathway after co-transfection of HOXA-AS2 and miR-216a-5p.

help improve the diagnosis and treatment level and improve the prognosis.

In this study, the expressions of HOXA-AS2 and microRNA-216a-5p were verified in 40 pairs of NSCLC tumor tissue samples and adjacent ones. The results showed that the expression of lncRNA HOXA-AS2 was remarkably upregulated and that of microRNA-216a-5p was remarkably downregulated, which were positively correlated with distant metastasis and poor prognosis of NSCLC. Therefore, it is believed that HOXA-AS2 plays a role in promoting cancer in NSCLC while microRNA-216a-5p plays an inhibitory role in NSCLC. In order to further explore the influence of HOXA-AS2 and microRNA-216a-5p on the bi-

ological function of NSCLC, the overexpression/knockdown model of HOXA-AS2 was constructed by LPV. The results of CCK-8, transwell migration, and cell scratch experiments showed that HOXA-AS2 could promote the occurrence and development of NSCLC, but its specific molecular mechanism remains unclear.

To explore the regulatory effect and mechanism of lncRNA on NSCLC through the regulation of miRNA, it will be helpful to find new therapies for NSCLC[22]. MicroRNAs (miRNAs) are small non-coding RNA molecules discovered in recent years, containing about 22 nucleotides and widely distributed in eukaryotes[23]. MicroRNA-216a-5p is a key molecule in the miRNA family. The results of this experiment showed that microRNA-216a-5p was lower expressed in the tumor tissues of NSCLC than in the adjacent tissues, and microRNA-216a-5p could inhibit proliferation, invasion, and migration in NSCLC cells. By using bioinformatics software, it was found that HOXA-AS2 could interact with microRNA-216a-5p, and our experimental results also confirmed the possible interaction between the two. In addition, recent studies[22,23] on the function of lncRNA have proposed a new ceRNA mechanism, which has been confirmed in some cancers to explain the functional relationship between lncRNA and miRNA. On this basis, we assume that HOXA-AS2 gene also acts as a ceRNA in NSCLC, and our results also showed that the knockdown of HOXA-AS2 can upregulate the expression of microRNA-216a-5p. On the contrary, the expression of HOXA-AS2 gene was also inhibited when microRNA-216a-5p was overexpressed, suggesting that HOXA-AS2 and microRNA-216a-5p may form a mutually inhibitory feedback regulation.

Conclusions

We demonstrated that HOXA-AS2 expression was remarkably increased in NSCLC and was correlated with distant metastasis and poor prognosis of NSCLC. Additionally, HOXA-AS2 had the ability to promote malignant progression of NSCLC by regulating microRNA-216a-5p.

Conflict of Interests

The Authors declare that they have no conflict of interests.

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