LncRNA SBF2-AS1 inhibits apoptosis and promotes proliferation in lung cancer cell *via* regulating FOXM1

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Summary

Purpose: *To discover pivotal long non-coding ribonucleic acids (lncRNAs) in lung cancer, and to explore the related molecular mechanism by which the lncRNAs affect lung cancer cells.*

Methods: *The differentially expressed lncRNAs and messenger RNAs (mRNAs) in cancer tissues and paracancer tissues in lung cancer patients were first excavated via gene chips. Then, the differentially expressed lncRNAs were verified using reverse transcription-quantitative polymerase chain reaction (RT-qPCR). The key genes were screened from the differentially expressed genes using bioinformatics, and their correlations with the expression levels of lncRNAs were explored using Pearson's correlation coefficient. Finally, overexpression and knockdown techniques were employed to verify the influences of changes in the content of lncRNAs and relevant genes on the proliferation and apoptosis of lung cancer A546 cells.*

Results: *The expression profiles of lncRNAs and mRNAs showed notable differences between cancer and paracancer tissues in lung cancer patients, and 28 differentially expressed lncRNAs and 914 differentially expressed mRNAs were screened. Through the survival analysis, it was found that the expression levels of SBF2-antisense RNA 1 (AS1) and LINC00968 had significant influence on the survival of patients. The Pearson's correlation coefficient analysis results revealed that the expression level of SBF2-AS1 was positively correlated with the core genes forkhead box protein M1 (FOXM1), the cyclin-dependent kinase 1(CDK1), centromere protein F (CENPF), cyclin B2 (CCNB2), and cell division cycle 20 (CDC20). The CCK-8 and EdU assay results showed that the proliferation ability of lung cancer A549 cells was enhanced in lncRNA SBF2-AS1 overexpression group, and that their proliferation rate was substantially lowered, when FOXM1 was knocked down.*

Conclusions: *Both lncRNA SBF2-AS1 and FOXM1 play crucial roles in the pathological process of lung cancer, and lncRNA SBF2-AS1 up-regulates FOXM1 to inhibit the apoptosis of lung cancer cells, and promote their proliferation.*

Key words: *lncRNA SBF2-AS1, FOXM1, lung cancer, proliferation, apoptosis*

Introduction

Lung cancer remains one of the most lethal diseases in the world. There are an estimated 1.8 million new cases [1], and as many as 1.6 million deaths of this disease annually [2]. Although significant advances have been made in the treatment means, including surgery and medications in the past decade, the total mortality rate is still stubbornly high mainly due to the lack of effective methods for early diagnosis. Since early lung cancer has insidious symptoms, nearly 60% of patients have not been definitely diagnosed until the progressive stages of lung cancer [3]. According to the results of epidemiological research, after initial treatment, the 5-year survival rate of the patients with progressive lung cancer is less than 10%, while that of patients with early lung cancer

is 70% or so [4]. Therefore, it is essential to explore biomarkers for the early diagnosis of lung cancer. Further understanding the cause and mechanisms of lung cancer is one key step to prevent the disease and improve the survival rate.

A study manifested that long non-coding ribonucleic acids (lncRNAs) play important roles in multiple biological processes such as chromosome remodeling, post-transcriptional regulation and intracellular signaling [5]. Research has suggested that the dysregulated lncRNAs are vital players in the pathological process of various cancers. It has been reported that some lncRNAs are closely associated with the development and progression of lung cancer [6], which can be taken as the potential biomarkers for cancer diagnosis and prognosis judgment. LncRNAs affect tumors mainly by the following mechanisms: First, lncRNAs serve as "molecular sponges" and bind to micro RNAs (miRNAs) or messenger RNAs (mRNAs) to block their biological functions. Second, lncRNAs act as transcription factors to regulate gene expression. Third, lncRNAs bind to signaling proteins to interfere in the activity of the signaling pathways [7-9]. At present, there is a consensus that the lncRNAs serve as the biomarkers for cancer diagnosis with the major advantages as follows: LncRNAs can synergistically regulate the synthesis of RNAs, deoxyribonucleic acids (DNAs) and proteins as well as their biological functions, possessing high biological value. Besides, it is relatively easy to detect lncRNAs in body fluids using reverse transcription-polymerase chain reaction (RT-PCR). Compared with miRNAs and mRNAs, lncRNAs have stronger tissue specificity, so they can become a kind of strikingly attractive biomarkers for tumor diagnosis and prognosis prediction [10]. However, the roles of most lncRNAs in the pathological process of tumors have not been well understood currently.

The onset of lung cancer is a complicated process driven by genetic and epigenetic alterations. In recent years, high-throughput gene microarray techniques have developed rapidly and been widely applied to reveal the general genetic alterations in the progression of diseases, which can be used to screen target genes for tumor diagnosis, treatment and prognosis. In this study, differentially expressed lncRNAs and mRNAs in cancer and paracancer tissues in lung cancer patients were first assessed *via* lncRNA gene microarray technique. Then, RT-qPCR was performed to verify the differentially expressed lncRNAs. Besides, the key lncRNAs were assessed *via* bioinformatics, and their correlations with relevant mRNAs were analyzed. Subsequently, the influence of changes in the con-

tent of lncRNAs on the proliferation and apoptosis of lung cancer A546 cells were verified using overexpression and knockdown techniques. The present study aimed to discover lncRNAs that play crucial roles in lung cancer, explore the related molecular mechanism of lncRNAs affecting lung cancer cells and illustrate the molecular biological evidence for the progression of lung cancer, in the hope of searching novel targets for the diagnosis and treatment of lung cancer.

Methods

Collection of tissue samples

Cancer and paracancer tissues were collected from 50 cases of tumor samples surgically resected in our hospital from January to April 2019. After resection, the cancer and paracancer tissues were immediately preserved in RNA-fixer reagent (BioTeke Corporation, Beijing, China) and then stored in a refrigerator at -80°C for later use. To exclude the factors that affect circular RNA (circRNA) expression profiles, the subjects who received chemotherapy, radiotherapy or targeted drug therapy were excluded from this study. This study was approved by the Ethics Committee of Linyi Cancer Hospital. Signed informed consents were obtained from all participants before the study entry.

Gene chip assay

Total RNAs were extracted from the lung cancer and paracancer tissues and quantified using NanoDrop kit (Thermo Fisher Scientific, Waltham, MA, USA), and their integrity was assessed with 2100 Bioanalyzer (Agilent, Santa Clara, CA, USA). Then, 100 ng of total RNAs were prepared into complementary RNAs (cRNAs) using Affymetrix 3' IVT Express kit, and the cRNAs were hybridized on Affymetrix Primeview Human Gene Expression Array for 16 h according to the user manual of Affymetrix GeneChip 3' Array (Affymetrix, Santa Clara, CA, USA). Additionally, the arrays were washed and stained on Affymetrix Fluidics Station 450 and scanned using Affymetrix GeneChip scanner according to the manufacturer's protocol (Affymetrix, Santa Clara, CA, USA). The raw data in CEL files were imported into the Partek Genomics Suite Software 6.6, and the probe set was standardized by the robust multiarray average. Finally, the significance of differentially expressed genes was determined using one-way analysis of variance (ANOVA), and p value was corrected with false discovery rate (FDR) [10].

Gene functional and pathway enrichment analyses

In this study, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were performed in the differentially expressed genes using the online annotation tool the Database for Annotation, Visualization and Integrated Discovery (DAVID) (Error! Hyperlink reference not valid. Error! Hyperlink reference not valid.

Protein-protein interaction (PPI) network establishment and key module selection

The PPI networks of differentially expressed genes were built using STRING (http://string.embl.de/), and confidence score >0.9 was set as the confidence value in order to ensure the reliability of the PPI networks. Then, the PPI networks were visualized with Cytoscape software, and the core modules were dug using the Cytoscape plugin cytoHubba. Finally, the functions and key pathways of the core modules were annotated with the DAVID.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

The expressions of RNAs were detected *via* RT and qPCR as follows: The tissue samples were first taken out from cryopreservation tubes, drained, ground in liquid nitrogen, moved to a 5 mL tube, and homogenized, and the resulting homogenate was added into a clean 1.5 mL Eppendorf (EP) tube, let stand at room temperature for 5-10 min for complete lysis, and centrifuged at 1,200 rpm for 5 min. Then, with the deposits removed, the tissues were added with chloroform at 200 µL (chloroform/1 mL) and TRIzol (Invitrogen, Carlsbad, CA, USA), shaken and mixed evenly, placed at room temperature for 15 min, and centrifuged at 4°C and 1,200 rpm for 15 min. The supernatant was transferred into another centrifugal tube, added with isopropanol, let stand at room temperature for 10-30 min and centrifuged at 12,000 rpm for 10 min. After the supernatant was discarded, RNAs were deposited at the bottom of the centrifugal tube, added with 75% ethanol at 1 mL of 75% ethanol/1 mL of TRIzol, moderately shaken to suspend the precipitates, and centrifuged again at 4°C and 12,000 rpm for 5 min. Subsequently, the supernatant was removed, and the precipitates were blown dry on an ultra-clean bench for 10-20 min and dissolved with 10-50 µL of diethyl pyrocarbonate (DEPC)-treated H_2O. Finally, the concentration of RNAs was determined using OneDrop microspectrophotometer. The RT reaction was performed using 2 µL of RNAs, 0.5 µL of reverse transcriptase, 4.5 µL of RNase-free dH_2O, 0.5 µL of random primers, 0.5 µL of Oligo dT and 2 µL of 5× RT reaction buffer. Then, the complementary (cDNA) samples were divided into three portions and each portion was diluted by 20 folds. Subsequently, 3 µL of cDNAs were taken for PCR amplification. The amplification levels of target genes were verified using 5% agarose gel electrophoresis, and quantification and data processing were conducted using LabWorks 4.0 image acquisition and analysis software. To ensure the accuracy of data, the above operation was repeated 3 times in each group of samples. In the present study, the relative expression levels of target genes were analyzed using $2^{-\Delta\Delta Ct}$. The primers used in this study were synthesized by Shanghai Generay Biotech Co., Ltd. (Shanghai, China) (Table 1).

Cell culture

Human non-small cell lung cancer cell line A549 (the Institute of Biology, Chinese Academy of Sciences, Shanghai, China), phosphate buffered saline (PBS), trypsin, fetal bovine serum (FBS) and 1640 medium (Gibco, Rockville, MD, USA) and small interfering RNAs (siRNAs) (Wuhan Servicebio Technology Co., Ltd., Wuhan, China) were used in cell culture. The A549 cells were cultured in an incubator with 5% CO_2 at 37°C, and when growing to cover the whole culture dish, the cells were digested by 0.25% trypsin-EDTA (ethylenediaminetetraacetic acid) and sub-cultured.

LncRNA SBF2-AS1 knockdown and overexpression assays

LncRNA SBF2-AS1 was first knocked down using siRNAs (shRNA, Qiagen, Cambridge, MA, USA), and 30 nM siRNAs targeting lncRNA SBF2-AS1 were transfected into A549 cells using Lipofectamine 2000™ transfection reagent (Invitrogen, Carlsbad, CA, USA). After 6 h, the medium containing the transfection reagent was discarded, and the transfected A549 cells were cultured in the fresh medium for another 48 h.

Detection of influence of lncRNA SBF2-AS1 expression on lung cancer cell proliferation via cell counting kit (CCK)-8 assay

First, the A549 cells in the logarithmic growth phase were evenly seeded into a 96-well plate at $1×10^4$

Table 1. Primer sequences

Name	Primer sequence
Forkhead box protein M1 (FOXM1)	5'-TCTGCCAATGGCAAGGTCTCCT-3'
	5'-CTGGATTCGGTCGTTTCTGCTG-3'
Actin filament associated protein 1- antisense RNA 1 AFAP1-AS1)	5' TCG CTC AAT GGA GTG ACG GCA 3'
	5'-CGGCTGAGACCGCTGAGAACTT-3'
SBF2-AS1	5'-CCACGACCCAGAAGGAGTCT-3'
	5'-GCATTGATGGAGCATTGCGA-3'
Long intergenic non-protein coding RNA 968 (LINC00968)	5'-CCACTCCTTTAC TTCCTTTCTTCTC-3'
	5'-GGTCCCTCATTCCTATCCC-3'
Tstis-specific transcript, Y-linked 14 (TTTY14)	5'-CTTTCATCACTCTGCCTGCAACT-3'
	5'-GCATGGCAAAGATGCACACAATG-3'
U6	5'-CTCGCTTCGGCAGCACA-3'
	5'-AACGCTTCACGAATTTGCGT-3'

cells/well and cultured in the incubator for 72 h. After the original medium was discarded, the cells were incubated with 20 µL of CCK-8 reaction solution (Dojindo, Kumamoto, Japan) and 170 µL of cell culture medium at 37°C in the dark for 2 h. The resulting cells were shaken on a micro-vibrator for 3 min. Finally, the absorbance was measured using a microplate reader at the wavelength of 450 nm.

Detection of influence of lncRNA SBF2-AS1 expression level on lung cancer cell apoptosis via Annexin V/propidium iodide (PI) double staining

The apoptosis of cells treated with lncRNA SBF2-AS1 was detected using Annexin V/PI double staining kit. A total of 5×10^5 cells were trypsinized, and rinsed with PBS at 4°C twice. Then, the centrifuged cells were re-suspended in 500 µL of staining buffer, and stained with 5 µL each of Annexin V-FITC and PI staining solution at 37°C in the dark for 15 min. Finally, the resulting cells were detected using Guava flow cytometer.

Statistics

SPSS 20.0 (IBM, Armonk, NY, USA) was used for statistical analyses, and all data were presented as mean ± standard deviation. Differences between two groups were analyzed by using the Student's t-test. Comparison between multiple groups was done using one-way ANOVA test followed by *post hoc* test (Least Significant Difference). The survival curves were plotted using the Kaplan-Meier method and log-rank test was used to compare the differences between groups. P<0.05 denoted statistically significant differences.
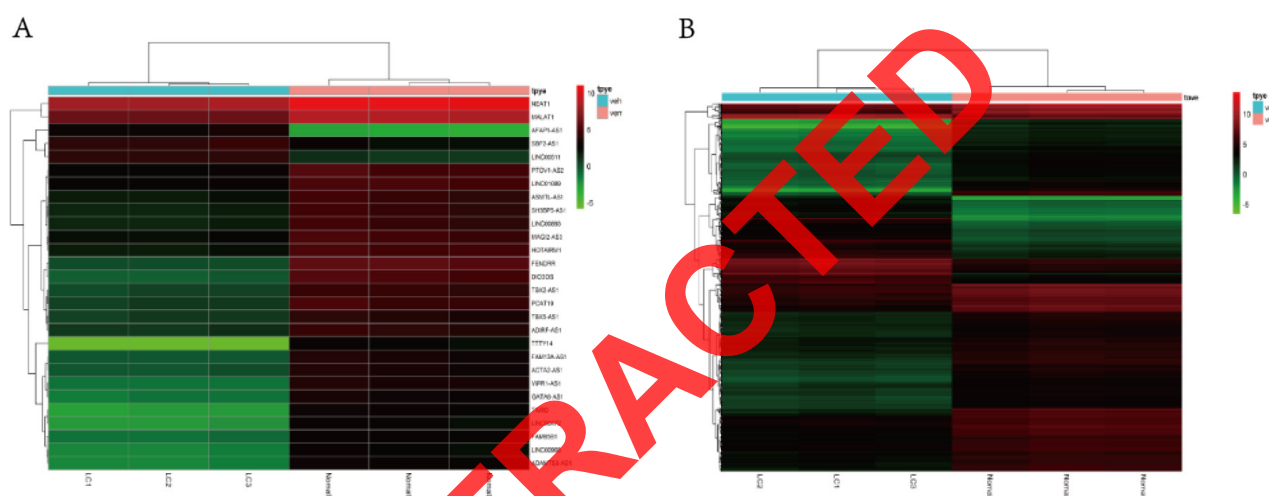

Figure 1. Differentially expressed lncRNAs and mRNAs screened using gene chip assay. **A and B:** Heatmaps of differentially expressed lncRNAs and mRNAs. LC: Lung cancer tissues and Con: normal tissues. In the maps, each row represents a circRNA, and red and blue denote a high expression level and a low expression level, respectively.

Table 2. GO enrichment results of differentially expressed genes

GO ID	GO term	Ontology	Gene number	GO term
Up-regulated genes				
GO:0140014	Mitotic nuclear division	Biological process (BP)	24	7.19E-15
GO:1903047	Mitotic cell cycle process	BP	35	2.03E-12
GO:0000086	G2/M transition of mitotic cell cycle	BP	11	0.000418315
GO:0000075	Cell cycle checkpoint	BP	11	0.000152674
GO:0016477	Cell migration	BP	21	0.0010936638
Down-regulated genes				
GO:0045765	Regulation of angiogenesis	BP	39	4.57E-12 level
GO:0031589	Cell-substrate adhesion	BP	37	6.51E-12 level
GO:0050727	Regulation of inflammatory response	BP	36	3.81E-06 level
GO:0007266	Rho protein signal transduction	BP	19	8.70E-06 level
GO:0070372	Regulation of ERK1 and ERK2 cascade	BP	28	1.83E-07
GO:0010942	Positive regulation of cell death	BP	37	0.000842704
GO:0045926	Negative regulation of growth	BP	30	1.33E-10

Results

Excavation of differentially expressed genes

According to the results of screening using gene expression profile chips, the expression profiles of lncRNAs were notably different between cancer and paracancer tissues in lung cancer patients (Figure 1A), and 28 lncRNAs showed notable differences (Log$_2$ (fold change) >2, p<0.01), of which 3 circRNAs were remarkably up-regulated, while 25 lncRNAs were considerably down-regulated.

Additionally, based on the gene expression profile chip assay results, the mRNA expression profiles differed substantially between cancer and paracancer tissues in lung cancer patients (Figure 1B), and 914 RNAs showed considerable differences [Log$_2$ (fold change) >2, p<0.01), which consisted of 239 up-regulated mRNAs and 675 down-regulated mRNAs.

Differentially expressed lncRNAs verified via RT-qPCR

Then, 4 lncRNAs (AFAP1-AS1, SBF2-AS1, LINC00968 and TTTY14) showing remarkable differences were further verified using RT-qPCR. The RT-qPCR results revealed that their expression levels in 50 cases of lung cancer tissues were considerably different from those in the paracancer tissues, and the differences were statistically significant (p<0.01) (Figure 2).

KEGG and GO enrichment analysis results of differentially expressed mRNAs

The GO enrichment analysis results suggested that the up-regulated differentially expressed genes in lung cancer tissues were remarkably enriched in mitotic nuclear division, mitotic cell cycle process, G2/M transition of mitotic cell cycle, cell
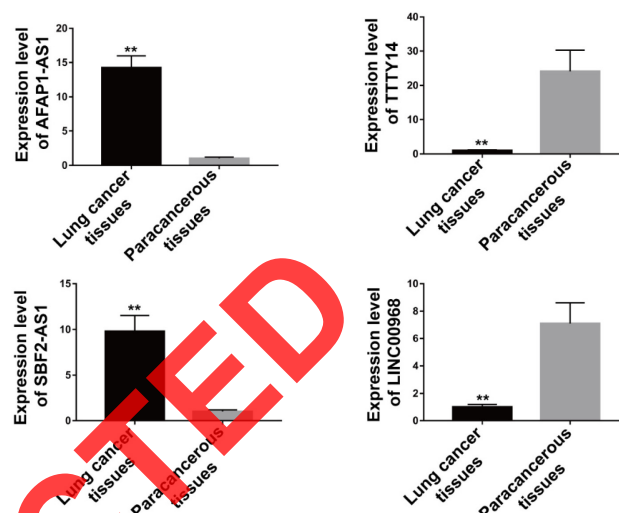
Figure 2. Differences in the expression levels of AFAP1-AS1, SBF2-AS1, LINC00968 and TTTY14 between cancer tissues and paracancerous tissues detected *via* RT-qPCR. **p<0.01.
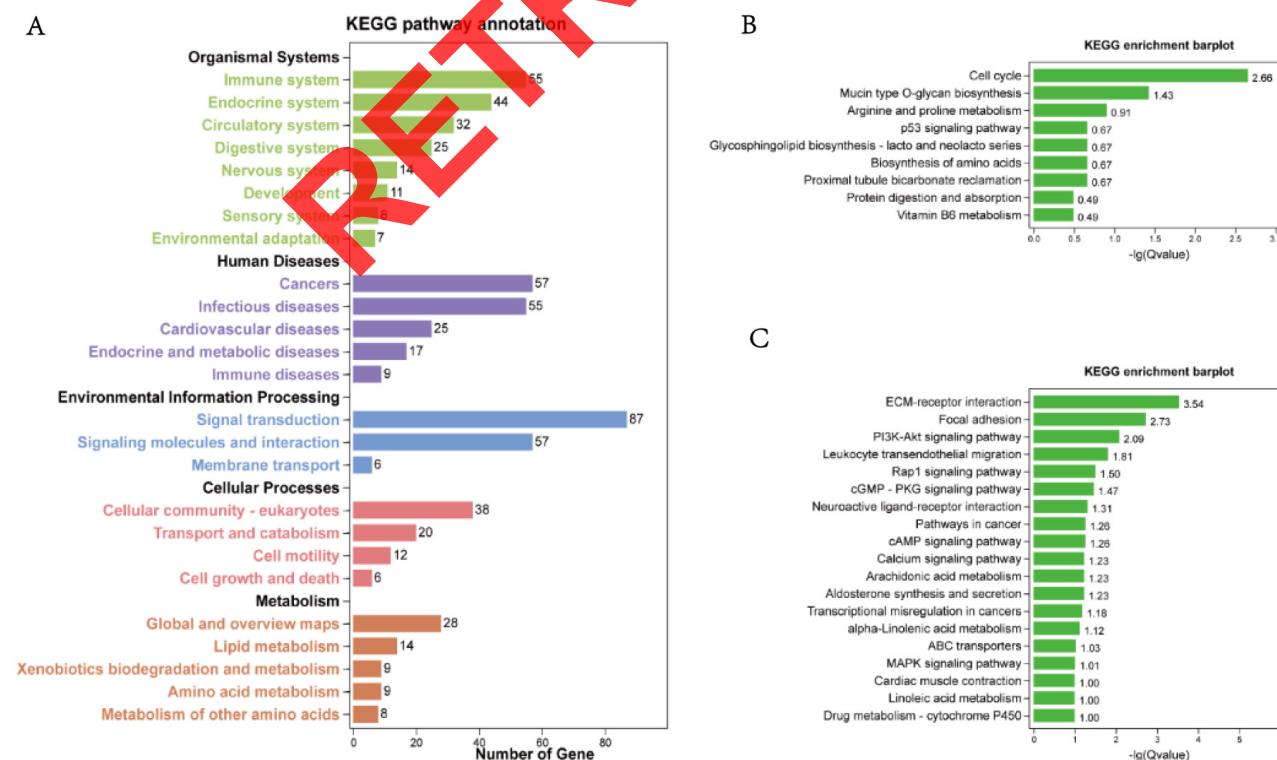
Figure 3. KEGG enrichment results of differentially expressed genes. **A:** Annotation of pathways enriched in differentially expressed genes. **B:** Signaling pathways enriched in the up-regulated genes. **C:** Signaling pathways enriched in the down-regulated genes.
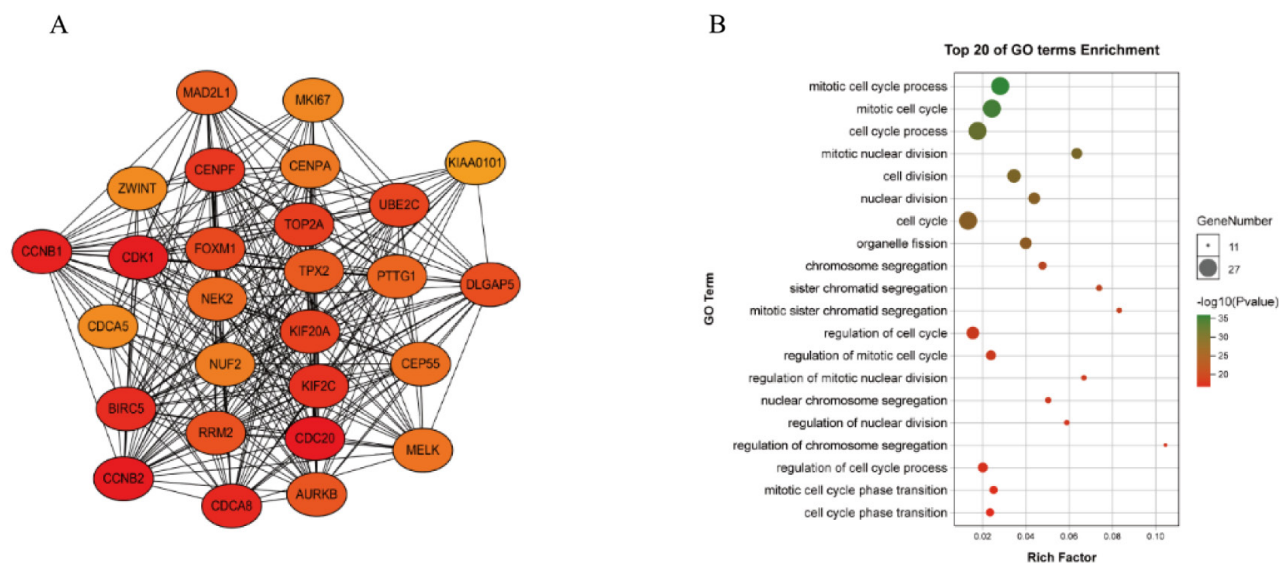
A

B

Figure 4. PPI network analysis results of differentially expressed genes. **A:** Dug core modules in PPI network. **B:** BPs in which the core modules were involved.
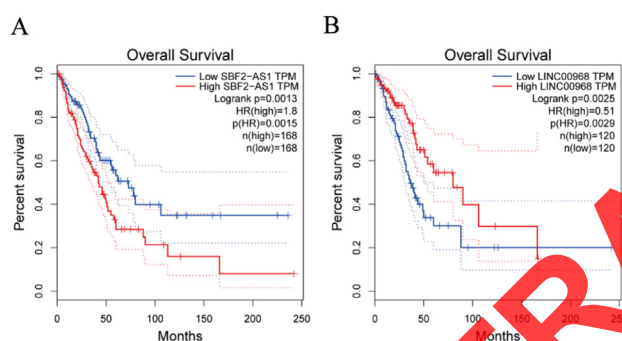
A

B

Figure 5. Influences of lncRNA expression levels on patient survival. **A:** Influence of SBF2-AS1 expression level on patient survival. **B:** Influence of LINC00968 expression level on patient survival.

cycle checkpoint and cell migration, whereas the down-regulated ones in the regulation of angiogenesis, cell-substrate adhesion, regulation of inflammatory response, Rho protein signal transduction, regulation of extracellular signal regulated kinase (ERK)1 and ERK2 cascade, positive regulation of cell death and negative regulation of growth (Table 2). Besides, it was found through the KEGG enrichment analysis that in the lung cancer tissues, the up-regulated genes were substantially enriched in cell cycle and the p53 signaling pathway (Figure 3A-B), while the down-regulated genes were substantially enriched in the ECM-receptor, PI3K/Akt, cancer and MAPK signaling pathways (Figure 3C).

PPI network analysis results of differentially expressed genes

After isolated nodes were eliminated, the PPI network of differentially expressed genes was com-

posed of 319 nodes and 1,112 sides, and the most notable PPI modules in the network were dug using the cytoHubba (Figure 4A). It was found that CDK1, FOXM1, CENPF, CCNB2, CDC20 and CCNB1 proteins in the core modules possessed the highest degree of connectivity and closeness centrality, indicating that they play important roles in the network. According to the GO enrichment analysis results, the core genes were considerably enriched in cell cycle-related BPs (Figure 4B).

Influence of critical lncRNAs on patient survival

Subsequently, GEPIA database was used to analyze the influence of differentially expressed lncRNAs on the survival of patients, and it was found that only the expression levels of SBF2-AS1 and LINC00968 in all verified lncRNAs had marked influence on the survival of patients, and that the survival was substantially prolonged in the patients with lowly expressed SBF2-AS1 (Figure 5A-B).

Correlations of lncRNA SBF2-AS1 with core genes

Through the Pearson's correlation coefficient analysis, it was discovered that the expression level of SBF2-AS1 was positively correlated with the core genes FOXM1 (r=0.46), CDK1 (r=0.35), CENPF (r=0.21), CCNB2 (r=0.43), CDC20 (r=0.22) and CCNB1 (r=0.41) (Figure 6A). Moreover, the positive correlations of lncRNA SBF2-AS1 with FOXM1, CCNB1 and CCNB2 were moderate, while those with other molecules were weak. The RT-qPCR results were analyzed using Pearson's correlation coefficient, and it was found that lncRNA SBF2-AS1 was strongly associated with FOXM1 (r=0.79) (Figure 6B).

Effect of lncRNA SBF2-AS1 expression level on A549 cell proliferation

According to CCK-8 assay results, the proliferation ability of lung cancer A549 cells in lncRNA SBF2-AS1 overexpression group was notably enhanced at 36, 48, 60 and 72 h, suggesting that lncRNA SBF2-AS1 can accelerate the proliferation of lung cancer cells (p<0.05), while knocking down FOXM1 notably decreased the proliferation rate of A549 cells (p<0.05). Besides, the 5-Ethynyl-2'-deoxyuridine (EdU) staining results showed that at 36 h after lncRNA SBF2-AS1 overexpression, the amount of red fluorescence was obviously larger than that in A549 + empty vector group, implying that lncRNA SBF2-AS1 overexpression is able to promote lung cancer cell proliferation, whereas lowering the expression level of FOXM1 caused marked decrease in the proliferation rate of A549 cells (Figure 7A-B).

Impact of lncRNA SBF2-AS1 expression level on A549 cell apoptosis

The influence of lncRNA SBF2-AS1 expression level on A549 cell apoptosis was explored using a flow cytometer and Annexin V/PI staining, which showed that the apoptosis rate in lncRNA SBF2-AS1 low-expression group rose obviously and declined notably in lncRNA SBF2-AS1 low-expression + FOXM1 overexpression group (Figure 8).

Discussion

Lung cancer results from accumulative gene mutations, epigenetic abnormalities, and somatic
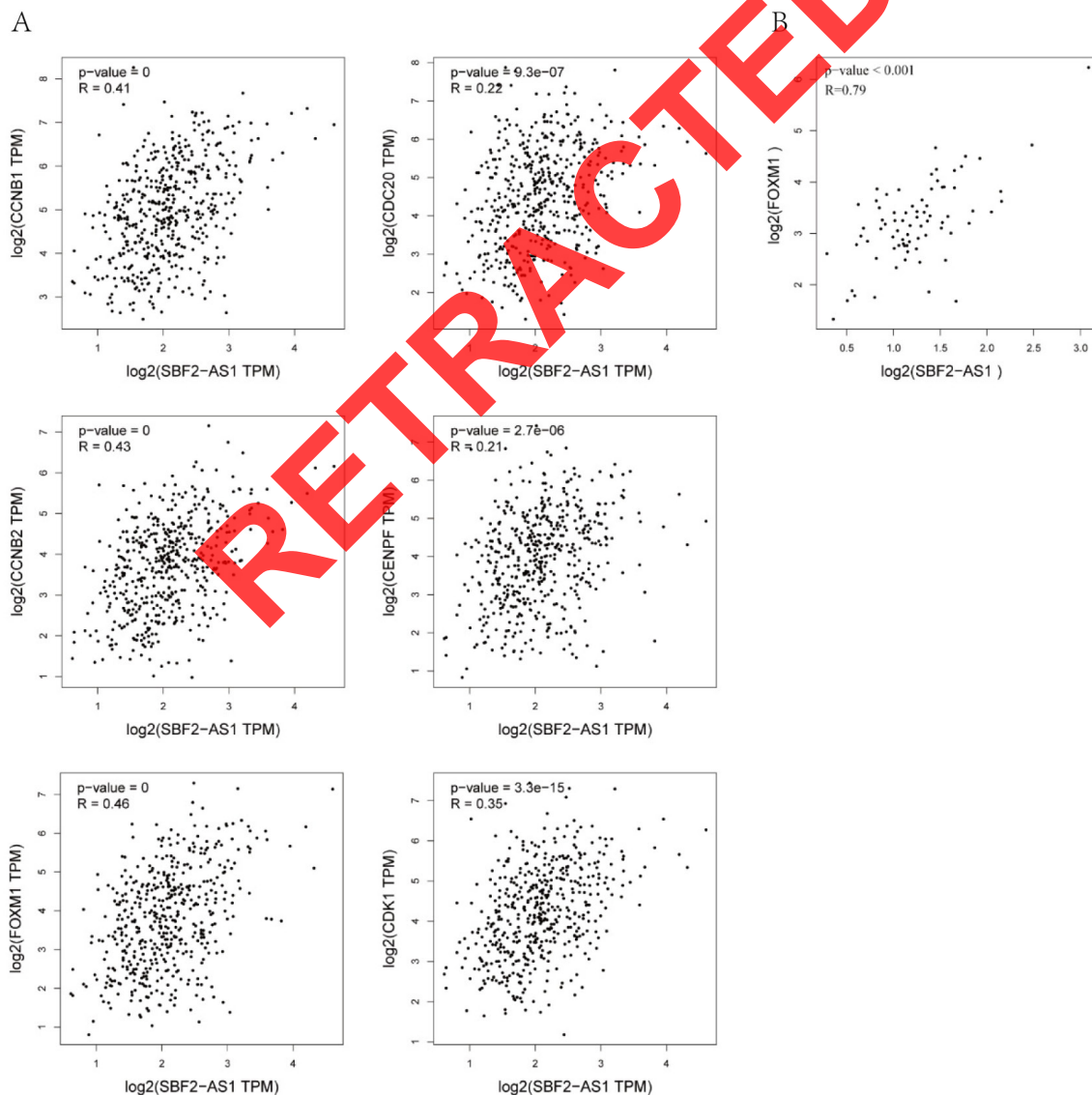

Figure 6. Correlations of lncRNA-SBF2-AS1 with core genes. **A:** Correlations of lncRNA-SBF2-AS1 expression level with core genes. **B:** Correlation between SBF2-AS1 expression level and FOXM1 expression level determined *via* RT-qPCR based on the Pearson's correlation coefficient analysis.
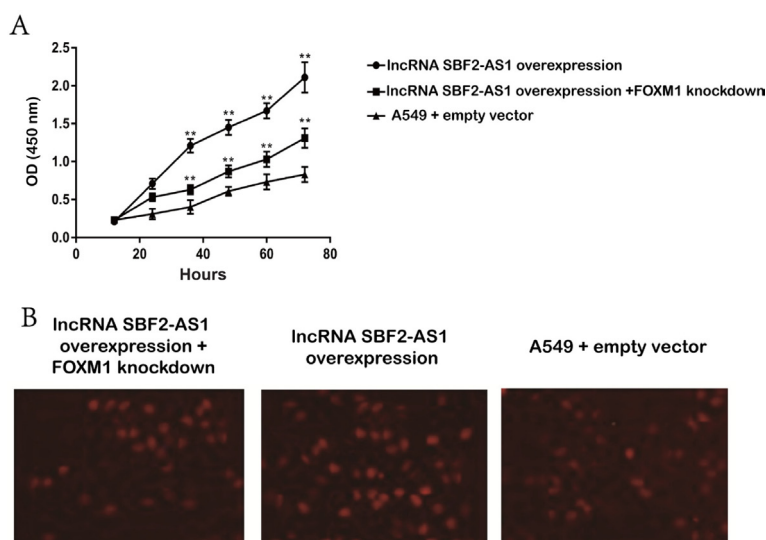
Figure 7. Influence of lncRNA SBF2-AS1 expression level on A549 cell proliferation. **A:** Influence of lncRNA SBF2-AS1 expression level on A549 cell proliferation detected *via* CCK-8 assay. **B:** Effect of lncRNA SBF2-AS1 expression level on A549 cell proliferation detected using EdU staining. **p<0.01 *vs.* A549 + empty vector group.
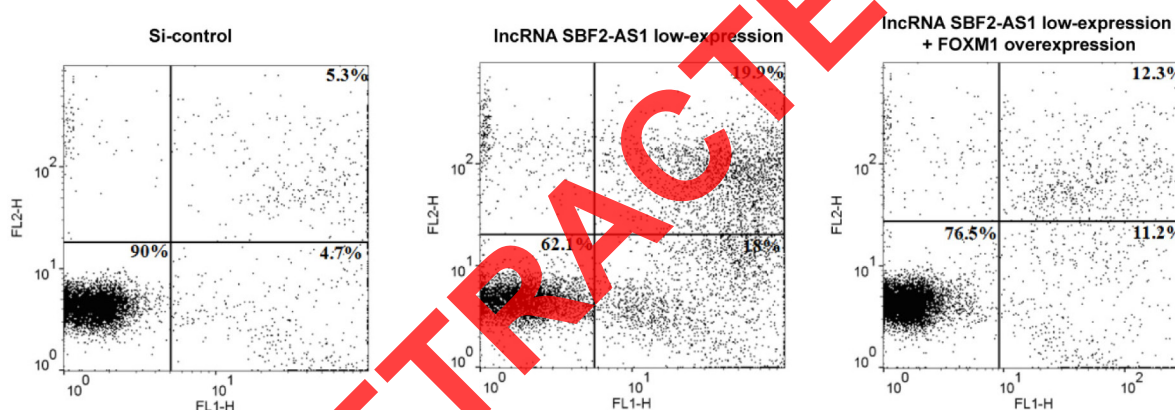

Figure 8. Impact of lncRNA SBF2-AS1 expression level on A549 cell apoptosis. Influence of lncRNA SBF2-AS1 expression level on A549 cell apoptosis detected using flow cytometer and Annexin V/PI staining.

mutations. Massive studies have proposed that it is vital to understand the molecular mechanism of lung cancer for its early diagnosis and treatment. High-throughput gene chip and transcriptome sequencing techniques have been widely applied to discover and assess tumor molecular markers, explore the potential pathogenesis of tumors, and search for therapeutic targets for tumors.

The present study mainly sought to discover lncRNAs that serve as crucial players in lung cancer, and to explore the related molecular mechanism by which the lncRNAs affect the phenotypic change of lung cancer cells. First, the differentially expressed lncRNAs and mRNAs between cancer and paracancer tissues in lung cancer patients were searched using gene expression profile chips, and a total of 28 differentially expressed lncRNAs were screened, of which 3 circRNAs were remarkably up-regulated, while 25 lncRNAs were considerably

down-regulated. Several genes (MALAT1 AFAP1-AS1 and NEAT1) in the differentially expressed genes dug in this study have already been confirmed to play pivotal roles in the progression of lung cancer [11-14], corroborating the reliability of screening in this study.

Subsequently, 4 remarkably different and less researched lncRNAs were selected and further verified using RT-qPCR. The results showed that there were notable differences in all the differentially expressed lncRNAs screened using gene chips. Additionally, the influence of differentially expressed lncRNAs on the survival of patients was analyzed using GEPIA database, and it was found that only the expression levels of SBF2-AS1 and LINC00968 in all verified lncRNAs had marked influence on the survival of patients, and that the survival was substantially prolonged in the patients with lowly expressed SBF2-AS1, indicating that SBF2-AS1

serves as a crucial player in the progression of lung cancer. Therefore, SBF2-AS1 was taken as the object of this study. Although some reports have pointed out that SBF2-AS1 can promote cancer cell proliferation, apoptosis, metastasis and invasion by various mechanisms, the mechanism of its influence on lung cancer cells remains to be fully elucidated [15,16].

In this study, the expression profile screening results showed that a total of 914 RNAs were notably different between tumor and paracancer tissues in lung cancer patients [Log_2 (fold change) >2, p<0.01]. Then, the PPI network of the differentially expressed genes was established, and the core modules in the network were explored using cytoHubba. It was discovered that the core modules CDK1, FOXM1, CENPF, CCNB2, CDC20 and CCNB1 had the highest degree and closeness centrality, suggesting that these core proteins are crucial in the whole pathological mechanism of lung cancer. Besides, the GO enrichment analysis results revealed that the function of the core gene modules was associated with such BPs as cell proliferation and cell cycle.

One interesting finding in this study is that the expression level of lncRNA SBF2-AS1 was positively correlated with the cell cycle-associated genes FOXM1 (r=0.46), CDK1 (r=0.35), CENPF (r=0.21), CCNB2 (r=0.43), CDC20 (r=0.22) and CCNB1 (r=0.41) in the core modules to different degrees according to the Pearson's correlation coefficient analysis results of transcriptome data in 232 cases of lung cancer from the GEPIA database. This may further explain the molecular mechanism by which SBF2-AS1 facilitates lung cancer cell proliferation. Moreover, SBF1-AS1 was positively associated

with FOXM1 to the highest degree. In the present study, the RT-qPCR results of lncRNA SBF2-AS1 and FOXM1 in lung cancer tissues were analyzed using Pearson's correlation coefficient, and it was found that lncRNA SBF2-AS1 was strongly positively correlated with FOXM1 (r=0.79). According to the results of the study conducted by Gao et al [17], lncRNA SBF2-AS1 modulates the miR-361-5p/FOXM1 axis to accelerate the progression of cervical cancer, similar to those of this study. However, the detailed regulatory mechanism between lncRNA SBF2-AS1 and FOXM1 is still being explored.

In vivo experiment results manifested that overexpressing lncRNA SBF2-AS1 increased the proliferation rate of lung cancer cells, while knocking down FOXM1 remarkably lowered the proliferation rate, implying that lncRNA SBF2-AS1 increases the expression level of FOXM1 to promote the proliferation of A549 cells. In addition, knocking down lncRNA SBF2-AS1 considerably raised the apoptosis rate of lung cancer cells, but the apoptosis rate was remarkably decreased when FOXM1 was overexpressed.

Conclusions

In summary, the results of this study demonstrated that both lncRNA SBF2-AS1 and FOXM1 play pivotal roles in the pathological process of lung cancer, and lncRNA SBF2-AS1 up-regulates FOXM1 to inhibit the apoptosis of lung cancer cells, and promote their proliferation.

Conflict of interests

The authors declare no conflict of interests.

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