

Regulatory mechanism of interaction between Y-box-binding protein 1 and heterogenous nuclear ribonucleoprotein K in cell division cycle 25a signal pathway and lung cancer metastasis

Lvping Fu¹, Mengmeng Yang², Yuquan Ma^{2*}¹Department of Oncology, Handan Central Hospital, Handan, 056000, Hebei Province, China²Department of Thoracic Surgery, HanDan Central Hospital, Handan, 056000, Hebei Province, China

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ABSTRACT

This research aimed to explore the influences of the interaction between Y-box-binding protein 1 (YBX1) and heterogeneous nuclear ribonucleoprotein K (HNRNPK) on the cell division cycle protein 25 phosphatase A (CDC25a) signal pathway and the regulatory mechanism of lung cancer (LC) metastasis. A total of 34 patients diagnosed with LC pathologically were selected, and the expression levels of YBX1, HNRNPK, and CDC25a in LC non-metastasis and metastasis tissues were detected using immunohistochemistry and Western blot (WB). High-expression stable cell lines YBX1/A549 and HNRNPK/A549 were established in the LC A549 cell strain. The number of migratory cells in YBX1/A549 and HNRNPK/A549 was measured, and the influences of the interaction between YBX1 and HNRNPK on CDC25a were analyzed using co-immunoprecipitation (co-IP). The findings indicated significantly elevated levels of YBX1, HNRNPK, and CDC25a proteins in LC metastasis tissues versus LC non-metastasis tissues (YBX1: $P < 0.001$; HNRNPK: $P < 0.01$; CDC25a: $P < 0.05$). Moreover, the mRNA and relative protein expressions of YBX1 and HNRNPK were notably heightened in the YBX1/A549 and HNRNPK/A549 cell lines versus the Control Group ($P < 0.001$). In the co-IP, the YBX1 antibody, when coated with magnetic beads, captured a substantial amount of HNRNPK protein in the YBX1/HNRNPK complex. Additionally, YBX1 and HNRNPK mRNA and protein levels were notably higher in both YBX1/A549 and HNRNPK/A549 cell lines than in the Control Group ($P < 0.001$). Induction by either YBX1 or HNRNPK led to an increased count of migratory CDC25/A549 cells compared to the Control Group ($P < 0.05$). This significant rise in CDC25a expression levels in the YBX1/HNRNPK/A549 cell line compared to both YBX1/A549 and HNRNPK/A549 underscored the regulatory impact of the YBX1 and HNRNP interaction on CDC25a expression, further implicating their involvement in LC metastasis.

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Introduction

Lung cancer (LC) is one of the malignant tumors with the highest incidence and mortality worldwide. In the last 20 years, LC has been the most common tumor among both males and females in European and American countries (1). With the development of domestic industry and the aggravation of environmental pollution, the incidence of LC is dramatically growing. In some coastal cities in China, the incidence of LC is the highest among males (2), while it is ranked in the second or third place among females (3). According to related statistics, the number of new domestic LC patients every year occupies about 22% of total number of LC patients (4). LC grows fast, and distal metastasis occurs frequently. At the initial phase of the disease, no obvious symptom can be detected among most patients. However, most patients are diagnosed with advanced LC when they are diagnosed for the first time, and advanced LC results in very high mortality. Distal metastasis is one of the major causes of the death of LC patients (5). With the development of medical technology in recent years, the therapeutic methods of LC are improved constantly. Due to LC metastasis, therapeutic effects

are not satisfactory. Although LC patients are treated by the combination of surgery and postoperative auxiliary chemotherapy, the 5-year survival rate of these patients ranges between 60% and 80% (6). Therefore, LC metastasis has become the current hot topic in the research into LC, and the research on LC metastasis-related molecule mechanism and pathology has significant meaning for LC treatment.

LC metastasis is one of the main causes of failure in treatment and mortality of patients. Heterogenous nuclear ribonucleoprotein K (HNRNPK) is a kind of ribonucleic acid (RNA)-binding protein, whose expression levels in breast cancer, myeloma, and cervical cancer enhance significantly (7). Besides, it is related to the proliferation, migration, and invasion of liver cancer cells (8). Y-box-binding-protein 1 (YBX1) is an essential regulatory factor in the cell cycle and a repair factor for deoxyribonucleic acid (DNA) damage. For patients with breast cancer, colon cancer, and gastric cancer, YBX1 can accelerate the progress of the tumor cell cycle directly or indirectly, and inhibit the activity of inhibitory proteins during the cell cycle (9). The interaction between HNRNPK and YBX1 was involved in the proliferation of breast cancer cells and

* Corresponding author. Email: fulvping@163.com

the regulation of the cell cycle (10). Cell division cycle protein 25 phosphatase A (CDC25a) plays a significant role in the transformation of cell cycle G1/S, and multiple nuclear transcription factors can regulate the activity of CDC25a promoter transcription (11). The analysis of related research into the CDC25a promoter region demonstrated that three specificity sequences might be combined by the YBX1 protein in the CDC25a nucleic acid sequence (12). Therefore, it is inferred that the interaction between HNRNPK and YBX1 induced the change in the expression level of CDC25, and further got involved in LC cell metastasis.

To conclude, the interaction between HNRNPK and YBX1 engaged in the proliferation of cancer cells. CDC25a contained the specificity sequence that could be combined with the YBX1 protein. Hence, it was speculated that the interaction between HNRNPK and YBX1 engaged in LC metastasis through the CDC25a signal pathway. LC metastasis patients were selected as the research objects in the research, and the regulatory mechanism of CDC25a signal pathway by the interaction between HNRNPK and YBX1 and its function in LC metastasis were discussed to offer new ideas for the diagnosis and treatment of LC metastasis patients.

Materials and Methods

Experimental specimen collection

The specimens of LC tissues from 34 cases were collected from patients who received surgical incisions at the thoracic surgery department and were diagnosed with LC pathologically in Handan Central Hospital between December 2019 and March 2021. Among these selected patients, there were 22 male cases and 12 female cases aged between 25 and 74. Their average age was (54.18±6.72). 23 out of these cases suffered from squamous carcinoma, and 11 cases suffered from adenocarcinoma. Besides, 17 cases got LC metastasis, and the other 17 were not diagnosed with LC metastasis. The inclusion standard of this research included: a. patients who received pneumonectomy. b. patients without undergoing any anti-tumor treatment. c. patients whose clinical data and imaging data were both complete. d. patients without suffering from primary tumors in other body positions. Patients not eligible for surgical treatment were excluded. The experimental procedures of this research were approved by the Handan Central Hospital Ethics Committee, and all objects included in the research signed the informed consent form.

Examination of protein expression level in lung tissue by immunohistochemistry

All lung tissue specimen was collected directly during surgery. The LC tissues with obvious metastasis and the LC tissues without metastasis of patients were both extracted from primary lesions without inflammation and necrosis. Extracted lung tissues were embedded in paraffin wax, and lung tissue slices of 20µm were obtained by section. After baking, dewaxing, hydration, antigen retrieval, and inactivation of endogenous peroxidase, slices were washed with phosphate buffer solution (PBS) and added with 5% fetal calf serum to seal slices for 1 hour. After that, 50 µL of the first antibody was added to incubate slices at low temperature overnight. After incubation, slices were washed again by PBS, and then a second antibody marked with

horseradish peroxidase (HRP) followed by incubation at room temperature for 2 hours. Then, diaminobenzidine was added for dyeing for 5 minutes, and hematoxylin was also applied to dye slices for 2 minutes. After hydration and transparency, slices were sealed and photo-taken by a laser scanning confocal microscope (LSCM).

Construction of YBX1 and HNRNPK high-expression stable cell lines

1.5mL of Eppendorf (EP) tube was taken and added with 500uL of opti-minimal essential medium(Opti-MEM) and 20µg of plasmid. After that, 60µL of Lipofectamine2000 was added and placed still at room temperature for 15 minutes. 293T cells that would enter growth to logarithmic phases were transfected for 5 hours, and virus liquid was collected. Cancer A549 cell strain was added to Dulbecco's modification of Eagle's medium (DMEM) for cultivation. The culture medium was changed every other day. When cells developed into the logarithmic phase, the cell culture medium was changed into a DMEM culture medium containing 10% fetal serum, and 800 uL of collected virus liquid was added into it. After cultivation at 37°C for 24 hours, cells were selected by puromycin and then blank plasmid control cell line, YBX1 over-expression stable cell line (YBX1/A549), and HNRNPK over-expression stable cell line (HNRNPK/A549) were obtained, respectively.

Real-time fluorescence quantification polymerase chain reaction (PCR) detection of messenger ribonucleic acid (mRNA) level of YBX1 and HNRNPK in over-expression cell line

The total RNA of cells was extracted by the TRIzol method. After the examination of extracted total RNA by agarose gel electrophoresis, Hifair® III 1st Strand cDNA Synthesis SuperMix for qPCR reverse transcription kit (Takara Biomedical Technology Co., Ltd) was adopted in reverse transcription. The procedure of reverse transcription was 37°C, 15min; 85°C, 5s. Real-time fluorescence quantitative PCR primers of synthesized YBX1 gene were YBX1-F: 5'-GGGTGCAGGAGAA-CAAGGTA-3' and YBX1-R: 5'-TCTTCATTGCCGTCCTCTCT-3'. Real-time fluorescence quantitative PCR primers of synthesized HNRNPK gene included HNRNP-F: 5'-CAATGGTGAATTTGGTAAACGCC-3' and HNRNP-R: 5'-GTAGTCTGTACGGAGAG CCTTA-3'. Phosphoglyceraldehyde dehydrogenase (GAPDH) was used as an internal reference, which included two primers, namely Fm: AGTTCAACGGCACAGTCAAGG and Rm: CAGCCTTCTCCATGGTGGTG. Each reaction was repeated three times and the relative expression level was calculated by $2^{-\Delta\Delta ct}$ method.

Examination of protein expression level by Western blot (WB)

Processed lung tissue or cells were added with 1mL of prepared protein lysate for grinding, which split tissue to extract total protein. The protein content of extracted total protein was measured by the Bisynic acid (BCA) method. Protein was divided by Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene fluoride (PVDF) membrane by wet transfer method. After that, the protein was sealed with 5% skimmed milk, and then the first antibody was added

followed by overnight incubation at 4°C. Next, the protein was washed by tris buffered saline tween (TBST), and a second antibody coupled with HRP was added before incubation at room temperature for 2 hours. Finally, the protein was automatically exposed on the visualizer after the color liquid was added, and then it was photographed for the gray scan.

Cell invasion and migration experiments

A549 cells that grew well were taken to be cultivated in DMEM culture medium without serum for 5 hours. After that, centrifugation at 1000rpm was performed for 3 minutes to collect these cells, and then a single cell suspension was formed by resuspension. Next, cells were diluted to 5×10^5 /mL. 600 μ L DMEM culture medium was added into each hole of 24 orifice and then a transwell chamber was inserted. The upper chamber was added with 200 μ L of diluted cell suspension and cultivated in an incubator at 37°C. Next, the inverted microscope was used to observe if cells penetrated through the culture medium at the lower chamber. When cells penetrated through the lower chamber, the transwell chamber was taken out, fixed with 4% paraformaldehyde, and dyed for 10 minutes. After the transwell chamber became dry, it was observed under an inverted microscope and then photographed from 5 different visions randomly selected.

A549 cells at the logarithmic phase were extracted, and trypsin digestion cells were adopted to prepare a single-cell suspension. Based on certain cell density, cells were inoculated into 6-well plates, and the final cell density was adjusted to 5×10^5 cells/mL. When the cell fusion rate reached 50%, cells were transfected. Based on the observation with a microscope, cell density was close to 90% when the bottom of culture wells was filled with cells. The spear tip with a volume of 10 μ L was utilized to make a character one-shaped scratch with equal width along the bottom of the culture plate. After that, cells were phototaken with a microscope. At that time, inter-cellular width was selected as the base width at the time of 0 hours. Next, the culture medium was washed gently with PBS twice, and then 500 μ L of DMEM complete culture medium was added into each well for continuous cultivation. After 4 hours, cell growth in each group was observed by microscope and cell migration rate was calculated.

Examination of interaction between YBX1 and HNRNP by co-immunoprecipitation

20 μ L of protein G magnetic beads were put into 1.5mL of EP tube, and 300 μ L of Tris-triton was added into the tube to absorb beads. After magnetic beads were washed with Tris-triton three times, they were added with 4 μ g of YBX1 and immunoglobulin G (IgG) antibody. After that, magnetic beads were vibrated for 6 hours at 4°C. After washing the cells with PBS, they were added 300 μ L of Tris-triton protein lysate and vibrated for 30 minutes at 4°C. After that, centrifugation at 12000rpm was carried out to collect supernatant, which was then mixed with BCA to examine the concentration. Next, 20 μ g of protein was taken as input and incubated at 90°C for 10 minutes. After that, the protein was added into beads that absorbed the antibody before being incubated at 4°C overnight. Reactants were placed on the magnetic rack, and the supernatant was removed. Then, Tris-triton was added to wash magnetic beads three times. At the end, 2 \times Loding buffer was added

and then incubated at 95°C for 10 minutes before WB examination.

Statistical methods

Experimental data was processed by Statistical Product and Service Solutions (SPSS) 19.0. Measurement data was denoted by mean \pm standard deviation ($\bar{x} \pm s$) and tested by t. Enumeration data was expressed by percentage rate (%) and tested by χ^2 . Besides, $P < 0.05$ indicated that the differences had statistical meaning.

Results

Analysis of expression levels of YBX1, HNRNP, and CDC25a in lung tissues

The expression levels of YBX1, HNRNP, and CDC25a in different groups of lung tissues were examined by immunohistochemistry and WB methods (Figures 1 and 2). According to Figures 1 and 2, the YBX1 protein expression level in LC metastasis tissues was obviously higher than that in LC non-metastasis tissues ($P < 0.001$), HNRNP protein expression level in LC metastasis tissues was significantly higher than that in LC non-metastasis tissues ($P < 0.01$), and CDC25a protein expression level in LC metastasis tissues was higher than that in LC non-metastasis tissues ($P < 0.05$).

Identification of YBX1 and HNRNP over-expression cell lines

Reverse transcription-polymerase chain reaction (RT-

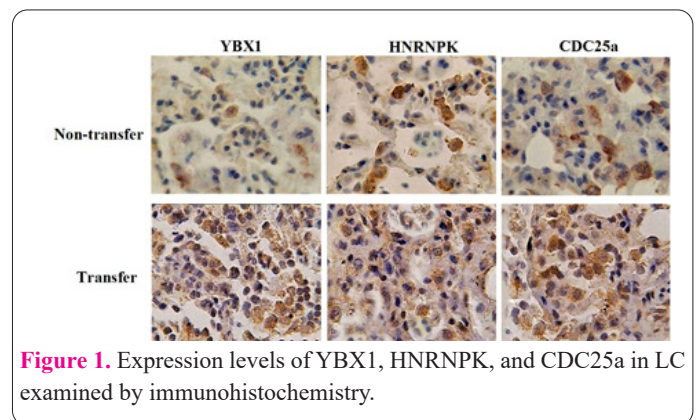


Figure 1. Expression levels of YBX1, HNRNP, and CDC25a in LC examined by immunohistochemistry.

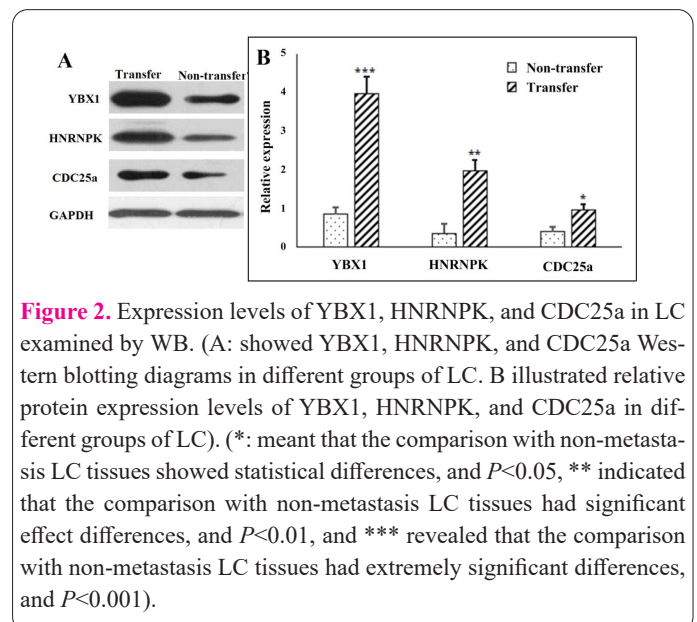


Figure 2. Expression levels of YBX1, HNRNP, and CDC25a in LC examined by WB. (A: showed YBX1, HNRNP, and CDC25a Western blotting diagrams in different groups of LC. B illustrated relative protein expression levels of YBX1, HNRNP, and CDC25a in different groups of LC). (*: meant that the comparison with non-metastasis LC tissues showed statistical differences, and $P < 0.05$, ** indicated that the comparison with non-metastasis LC tissues had significant effect differences, and $P < 0.01$, and *** revealed that the comparison with non-metastasis LC tissues had extremely significant differences, and $P < 0.001$).

PCR) and WB methods were adopted to compare and analyze YBX1 expression level in YBX1 over-expression stable cell line YBX1/A549 (Figure 3). Compared with the Control Group of A549 cell strain and transfected blank plasmid, the mRNA level and relative protein expression level of YBX1 in the YBX1/A549 cell line were both significantly enhanced ($P<0.001$).

RT-PCR and WB methods were adopted to compare and analyze HNRNPK expression levels in HNRNPK over-expression stable cell line HNRNPK/A549 (Figure 4). The mRNA level and protein expression level of HNRNPK in the HNRNPK/A549 cell line were 1.95 ± 0.23 and 1.58 ± 0.17 , respectively, which were both obviously higher than those in A549 and Control Groups ($P<0.001$).

Analysis of LC invasion and transfer

The invasion and transfer of A549 cells, Control cells

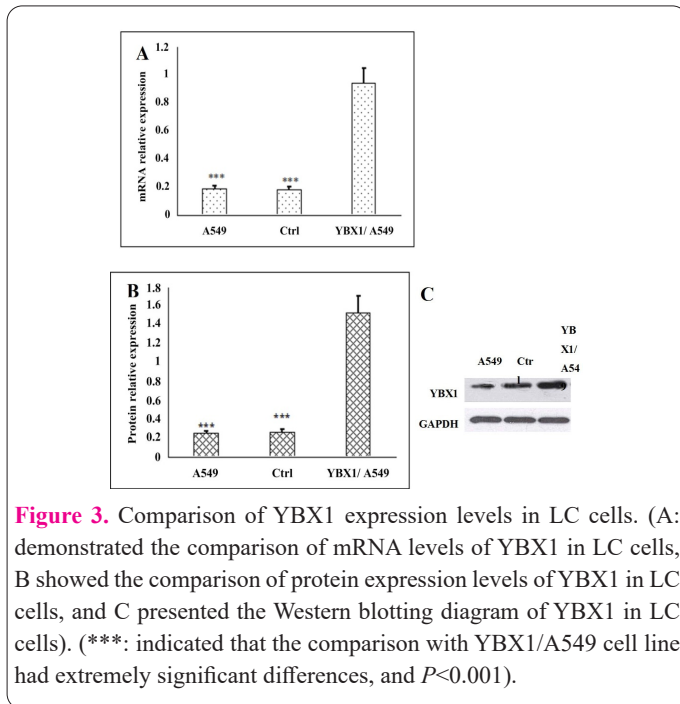


Figure 3. Comparison of YBX1 expression levels in LC cells. (A: demonstrated the comparison of mRNA levels of YBX1 in LC cells, B showed the comparison of protein expression levels of YBX1 in LC cells, and C presented the Western blotting diagram of YBX1 in LC cells). (***: indicated that the comparison with YBX1/A549 cell line had extremely significant differences, and $P<0.001$).

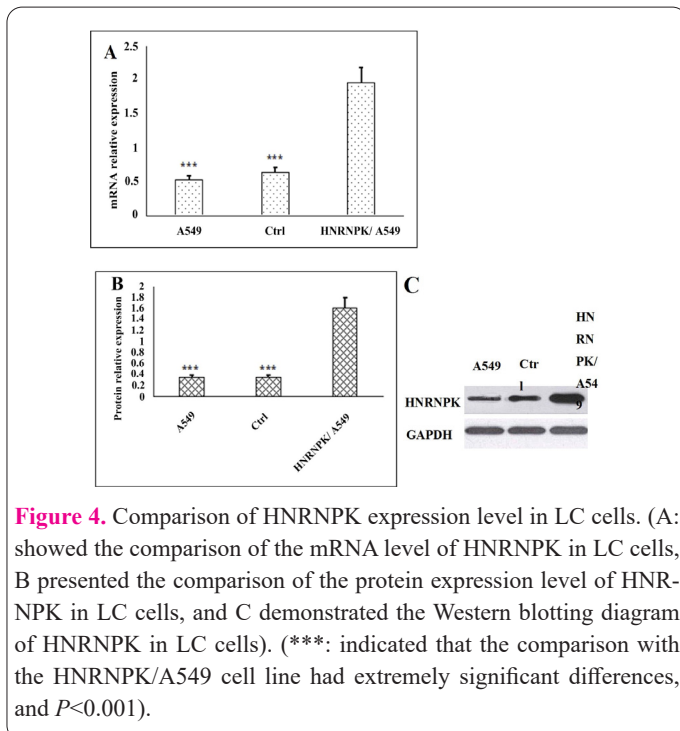


Figure 4. Comparison of HNRNPK expression level in LC cells. (A: showed the comparison of the mRNA level of HNRNPK in LC cells, B presented the comparison of the protein expression level of HNRNPK in LC cells, and C demonstrated the Western blotting diagram of HNRNPK in LC cells). (***: indicated that the comparison with the HNRNPK/A549 cell line had extremely significant differences, and $P<0.001$).

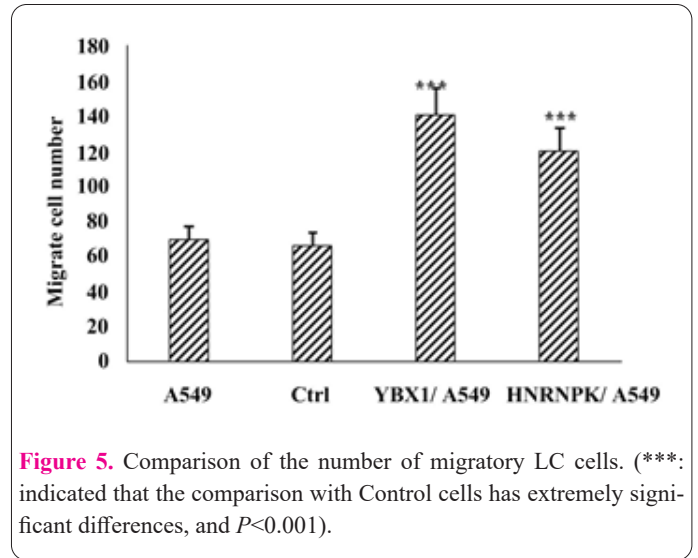


Figure 5. Comparison of the number of migratory LC cells. (***: indicated that the comparison with Control cells has extremely significant differences, and $P<0.001$).

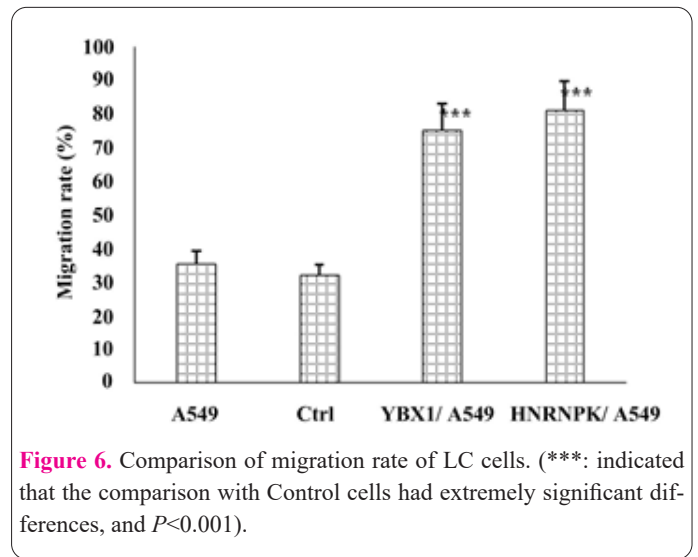


Figure 6. Comparison of migration rate of LC cells. (***: indicated that the comparison with Control cells had extremely significant differences, and $P<0.001$).

of transfected blank plasmid, YBX1/A549 cells, and HNRNPK/A549 cells were compared and analyzed (Figures 5 and 6). According to Figures 5 and 6, the number of migratory YBX1/A549 and HNRNPK/A549 cells was increased compared with A549 cells and Control cells of transfected blank plasmid ($P<0.001$). Besides, the migration rate of YBX1/A549 and HNRNPK/A549 cells was also enhanced compared with that of A549 cells and Control cells of transfected blank plasmid ($P<0.001$).

Interaction between YBX1 and HNRNPK in LC cells

RT-PCR and WB methods were adopted to analyze the relative expression levels of YBX1 and HNRNPK in YBX1/A549 and HNRNPK/A549 cell lines (Figures 7 and 8). The mRNA level and protein expression level of YBX1 in the YBX1/A549 cell line was increased obviously compared with those in the Control Group ($P<0.01$). The comparison of the mRNA level and protein expression level of HNRNPK in the YBX1/A549 cell line with those in Control Group had no statistical differences ($P>0.05$), and the mRNA level and protein expression level of HNRNPK in HNRNPK/A549 cell line was enhanced significantly compared with those in Control Group ($P<0.01$). Besides, the YBX1 level and protein expression level in the HNRNPK/A549 cell line.

The interaction between YBX1 and HNRNPK in LC cells was further verified by co-immunoprecipitation, and the expression of HNRNPK in YBX1/HNRNPK

compounds was examined by beads embedded in YBX1 antibody and IgG antibody (negative control) (Figure 9). HNRNPk protein was abundant in YBX1/HNRNPk compounds.

Influences of interaction between YBX1 and HNRNPk in cell migration through CDC25

RT-PCR and WB methods were adopted to analyze CDC25 relative expression levels in YBX1/A549 and HNRNPk/A549 cell lines (Figure 10). The mRNA levels and protein expression levels of YBX1 and HNRNPk in YBX1/A549 and HNRNPk/A549 cell lines were obviously enhanced compared with those in Control Group ($P < 0.001$), while the comparison of the mRNA level and protein expression level of CDC25 with those in Control Group showed no statistical differences ($P > 0.05$).

The mRNA level and protein expression level of CDC25a in A549 cells (YBX1/HNRNPk/A549) which expressed YBX1 and HNRNPk proteins simultaneously were further compared (Figure 11). The mRNA level and protein expression level of CDC25a in YBX1/HNRNPk/A549 were both significantly higher than those in the YBX1/A549 cell line and HNRNPk/A549 and the comparison of the mRNA level and protein expression level

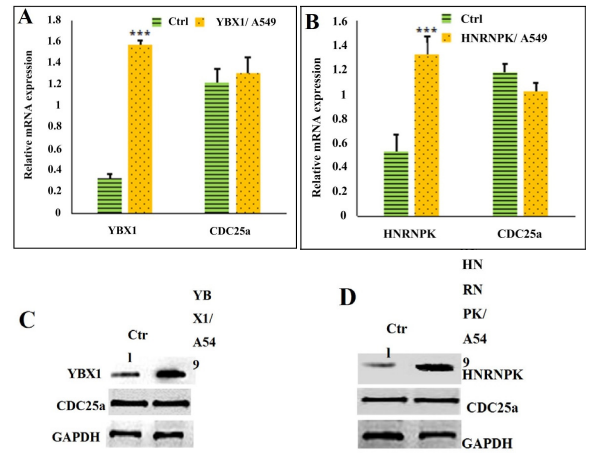


Figure 10. Influences of interaction between YBX1 and HNRNPk in CDC25. (A: showed the comparison between mRNA levels of YBX1 and CDC25 in A549-sh YBX1 cells. B demonstrated the comparison between mRNA levels of HNRNPk and CDC25 in the A549-sh HNRNPk cell line. C illustrated A549-sh YBX1 cell line protein Western blotting diagram. D presented A549-sh HNRNPk cell line protein Western blotting diagram). (***: indicated the comparison with the Control Group had extremely significant differences, and $P < 0.001$).

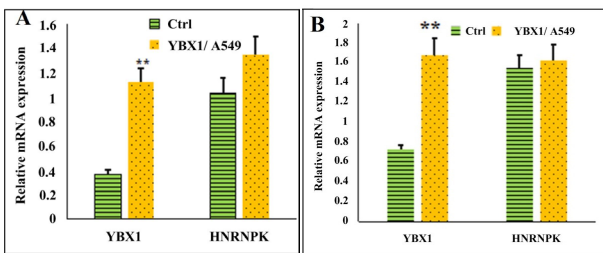


Figure 7. Interaction between YBX1 and HNRNPk in LC cells. (A: showed the comparison between mRNA levels of YBX1 and HNRNPk in YBX1/A549 cells. B demonstrated the comparison between protein levels of YBX1 and HNRNPk in YBX1/A549 cells.) (**: meant that the comparison with the Control Group had significant differences, and $P < 0.01$).

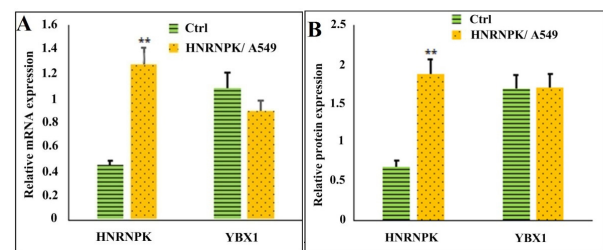


Figure 8. Interaction between YBX1 and HNRNPk in LC cells. (A: illustrated the comparison of mRNA levels in the HNRNPk/A549 cell line. B showed the comparison of protein levels in the HNRNPk/A549 cell line). (**: meant that the comparison with the Control Group had significant differences, and $P < 0.01$).

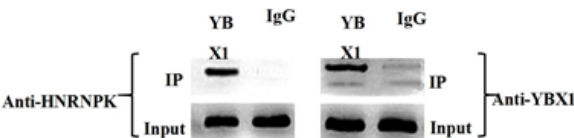


Figure 9. Analysis of results of co-immunoprecipitation.

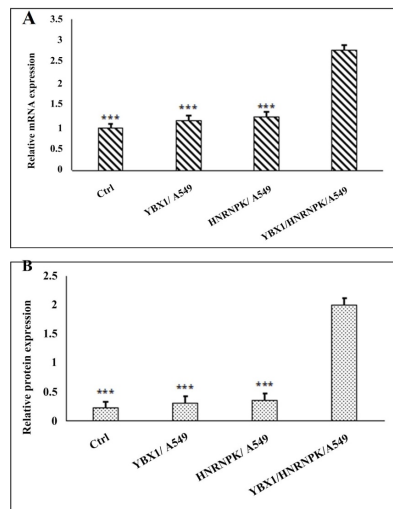


Figure 11. Comparison of CDC25a in different LC cell lines. (***: indicated that the comparison with YBX1/HNRNPk/A549 cells had extremely significant differences, and $P < 0.001$).

of CDC25a between them both had extremely significant differences ($P < 0.001$).

CDC25a was induced by YBX1, HNRNPk, and YBX1/HNRNPk composite, respectively, and cell migration was detected and analyzed by cell migration experiment (Figure 12). With being induced by YBX1 or HNRNPk, the number of migratory CDC25/A549 cells was obviously enhanced compared with that in the Control Group. The comparison of the number of migratory CDC25/A549 cells with that in the Control Group both had statistical differences ($P < 0.05$). Besides, the mRNA level and protein expression level of CDC25a in YBX1/HNRNPk/A549 were both significantly higher than those in YBX1/A549 cell line and HNRNPk/A549 and the comparison of mRNA level and protein expression level of CDC25a between YBX1/HNRNPk/A549 and YBX1/A549 cell line as well as HNRNPk/A549 both showed extremely

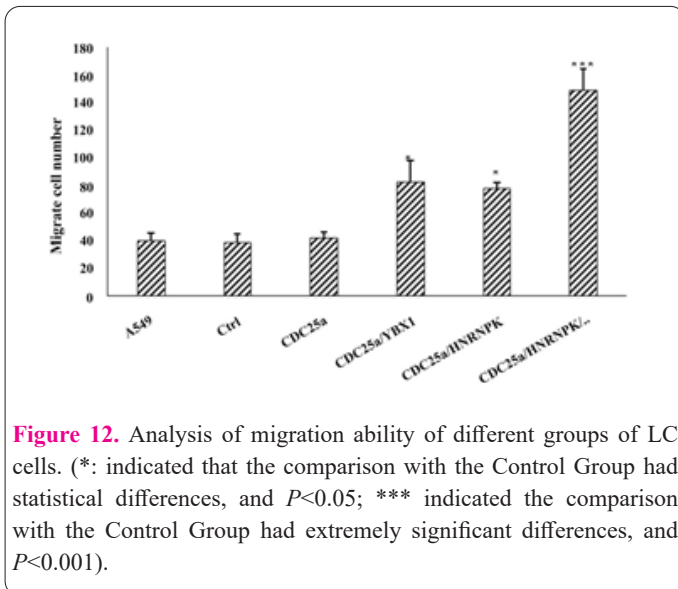


Figure 12. Analysis of migration ability of different groups of LC cells. (*: indicated that the comparison with the Control Group had statistical differences, and $P < 0.05$; *** indicated the comparison with the Control Group had extremely significant differences, and $P < 0.001$).

significant differences ($P < 0.001$).

Discussion

YBX1 is one of the Y-box binding protein family members which was discovered earliest, and the earliest discovery is related to gene transcription regulation (13). The cooperation of YBX1 with other regulatory factors exerts influences on multiple important cell functions (14). Studies in recent years found that the YBX1 protein got involved in each phase of tumor occurrence and development. It not only promoted excessive proliferation and anti-apoptosis of cancer cells but also assisted in tumor diffusion and metastasis. Besides, it directly engaged in the occurrence of chromosomal abnormalities and cancer tissue drug resistance (15). Among a large number of human malignant tumor cells, the expression level of the YBX1 protein was significantly increased, which was closely related to the biological features of the tumor and its prognosis (16). The results of this research found that the YBX1 protein expression level in LC metastasis tissues was significantly higher than that in non-metastasis LC tissues ($P < 0.001$), and the HNRNPK protein expression level in LC metastasis tissues was obviously higher than that in non-metastasis LC tissues ($P < 0.01$). Besides, the CDC25a protein expression level in LC metastasis tissues was higher than that in non-metastasis LC tissues ($P < 0.05$). The above results demonstrated that the expression levels of YBX1, HNRNPK, and CDC25a in LC metastasis tissues were all enhanced compared with those in non-metastasis LC tissues, which was consistent with the results of current studies. YBX1 was significantly related to the occurrence and development of tumors. YBX1 reduced the proliferation, migration, and invasion ability of cancer cells obviously (17). In addition, the results of the research revealed that the mRNA level and protein expression level of YBX1 in A549/YBX1 were both obviously increased ($P < 0.001$) and the number of migratory cells significantly grew ($P < 0.001$), which demonstrated that the migration ability of LC cells was remarkably enhanced after YBX1 high expression, and YBX1 might play a significant role in LC metastasis. Heterogeneous nuclear ribonucleoproteins (hnRNPs) are a kind of RNA-binding protein existing in the cell nucleus, and it is a super-protein family

containing more than 20 kinds of proteins (18). hn-RNPs got involved not only in RNA metabolic transcription, but also in the splicing, processing, and maturity of mRNA precursors. Besides, it also engaged in the regulation of the stability of mRNA and the transfer from cell nucleus to cytoplasm (19). The hnRNP is a special member of the hnRNPs protein family and a kind of multi-functional protein molecule, which engages in DNA transcription, RNA processing, transport, and translation, as well as cell cycle and apoptosis adjustment. In addition, it also plays a role in signal transduction, which is close to the incidence and development of tumors (20). Related studies showed that the antibody 47 in expression cells could remove accumulations of hnRNP in the cytoplasm, which caused the loss of chemotaxis for fibronectin reaction. This result demonstrated that hnRNP could mediate signal cascade amplification effects, and then result in cell migration and metastasis (21). HNRNPK interacted with multiple protein kinases and protooncogenes to some extent (7). With being influenced by the external environment, phosphorylation occurred in HNRNPK protein in cells, which engaged in the proliferation, metastasis, and invasion of cancer cells by regulating CDC25a and other protein expressions (22). The results of this research found out that the mRNA level and protein expression level of YBX1 in the YBX1/A549 cell line was increased obviously compared with those in Control Group ($P < 0.01$), and the comparison of mRNA level and protein expression level of HNRNPK in YBX1/A549 cell line with those in Control Group had no statistical differences ($P > 0.05$). In addition, the mRNA level and protein expression level of HNRNPK in the HNRNPK/A549 cell line was obviously increased compared with those in the Control Group ($P < 0.01$). All the above results demonstrated that YBX1 and HNRNPK were both correlated with LC metastasis to some extent because the interaction between YBX1 and HNRNPK in LC cells might be involved in LC metastasis (23). YBX1 regulated the proliferation of LC by CDC25 (24). What's more, the detection results of co-IP showed that numerous HNRNPK protein was abundant in the YBX1/HNRNPK composite. The mRNA levels and protein expression levels of YBX1 and HNRNPK in YBX1/A549 and HNRNPK/A549 cell lines were obviously increased compared with those in Control Group ($P < 0.001$), while the comparison of mRNA level and protein expression level of CDC25 with those in Control Group showed no statistical differences ($P > 0.05$). Besides, the mRNA level and protein expression level of CDC25a in YBX1/HNRNPK/A549 were both higher than those in the YBX1/A549 cell line and HNRNPK/A549, and the comparison of the mRNA level and protein expression level of CDC25a between them both demonstrated extremely significant differences. The results of the comparison indicated that the interaction between YBX1 and HNRNPK regulated LC cell metastasis by the CDC25 signal pathway.

Conclusion

To discuss the correlation between the interaction between YBX1 and HNRNPK and the CDC25 signal pathway, YBX1 and HNRNPK over-expression systems were constructed and verified by co-IP. The results showed the correlation between the interaction between YBX1 and HNRNPK-regulated LC cell metastasis by the CDC25 signal pathway. However, the correlation between the inte-

reaction between YBX1 and HNRNPK and CDC25 signal pathway was preliminarily explored in this research without further analysis for the expression of CDC25 signal pathway-related factors. In future research, the correlation between the expression of factors in the CDC25 signal pathway and LC cell metastasis is to be further analyzed based on the interaction between YBX1 and HNRNPK. To conclude, the interaction between YBX1 and HNRNPK regulated LC cell metastasis by the CDC25 signal pathway, which offered new ideas for the diagnosis and treatment of LC metastasis.

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