European Review for Medical and Pharmacological Sciences 2019; 22: 699-707

MiR-431 suppresses proliferation and metastasis of lung cancer via down-regulating DDX5

C.-M. XU, L.-X. CHEN, F. GAO, M.-F. ZHU, Y. DAI, Y. XU, W.-X. QIAN

Department of Respiratory, The First People Hospital of Zhangjiagang City, Soochow University, Zhangjiagang, China

Chunming Xu and Lixiu Chen contributed equally to this work

Abstract. – OBJECTIVE: We aimed to detect the role and function of microRNA-431 (miR-431) in lung cancer, and to investigate the underlying mechanism in regulating the development of lung cancer.

PATIENTS AND METHODS: Quantitative Real-time polymerase chain reaction (qRT-PCR) was utilized to measure the relative expression level of miR-431 in lung cancer tissues and cell lines. Cell counting kit-8 (CCK-8) and colony formation assays were employed to measure the proliferative ability of lung cancer cells. Meanwhile, transwell assay was recruited to detect the invasive and migratory abilities of lung cancer cells. Furthermore, dual-luciferase reporter gene assay was designed to verify the target gene of miR-431. Western blot assay was used to gauge the protein level of DDX5 (DEAD box polypeptide 5).

RESULTS: MiR-431 expression was significantly lower in 122 lung cancer tissue samples or cell lines compared to the adjacent normal tissues or lung bronchial epithelial cell line, respectively. Over-expression of miR-431 significantly inhibited proliferation, invasion and migration of A549 cells. Down-regulation of miR-431 accelerated cell growth and metastasis of H1650 cells. DDX5 was proved to be a direct target for miR-431 in lung cancer.

CONCLUSIONS: MiR-431 expression decreased in lung cancer tissues and cells. MiR-431 suppressed proliferation, invasion and migration of lung cancer cells via inhibiting the expression of DDX5. Our study might provide a novel target for the biological therapy of lung cancer.

Key Words:
miRNA, miR-431, Lung cancer, DDX5.

Introduction

According to the latest global tumor data, the global morbidity and mortality rate of lung cancer still ranked the first place[1]. Cancer statistics of US in 2018 showed that lung cancer accounted for the second highest rate of cancer incidence in the United States. However, it was slightly lower than that in 2017, but its mortality rate was still the first in malignant tumors[2]. These data indicated that improvement of diagnosis and treatment of lung cancer are urgently needed. With the gradual realization of genetic engineering, many non-coding RNAs including microRNAs (miRNAs) have been found to be involved in the regulation of several biological behaviors of tumors. MiRNAs are small RNAs with about 19-22 nucleotides in length that do not have protein-encoding function[3]. They could inhibit the expressions of their target genes *via* combining with the 3'-untranslated region (3'-UTR) of them, which in turn affect cell proliferation, apoptosis, autophagy, and metastasis[4]. For examples, miR-1258 was up-regulated and inhibited cell proliferation though directly repressing E2F8 in colorectal cancer[5]. In gastric cancer, miR-487a directly targeted TIA1 to promote tumor progression[6]. In ovarian cancer, epithelial mesenchymal transition (EMT) was inhibited by miR-122 *via* regulating P4HA1[7]. In breast cancer, tumor invasion and migration were initiated by miR-10b[8]. Also, miRNAs have been found to participate in the tumorigenesis and progression of lung cancer. Over-expressed miR-143/145 promoted neoangiogenesis of lung cancer[9]. MiR-494, which was regulated by ERK1/2, down-regulated BMI expression and modulated TRAIL-induced cell apoptosis of non-small-cell lung cancer (NSCLC)[10]. MiRNA-431 was identified as a tumor suppressor in several tumors. For example, miR-431 inhibited cell invasion and its expression was negatively correlated to lymph node metastasis in papillary thyroid carcinoma[11]. In colorectal cancer, miR-431 decreased cell metastasis by repressing CUL4B[12]. In hepatocellular carcinoma, miR-431 reduced the ZEB1-mediated EMT, thus inhibiting cell invasion and migration

Corresponding Author: Wenxia Qian, BM; e-mail: qwx1208.happy@163.com

and was correlated with clinicopathological significance[13,14]. However, the expression level of miR-431 in lung cancer has not been mentioned and its mechanism in lung cancer still remains to be understood. Herein, we first examined the expression level of miR-431 in 122 paired lung cancer tissues and adjacent normal tissues. We found that the expression level of miR-431 in lung cancer tissues was remarkably lower than the matched normal tissues. At the same time, the expression levels of miR-431 in four different lung cancer-derived cell lines (A549, SPCA1, H1650, H1299) significantly decreased comparing to the normal lung bronchial epithelial cell line (BEAS-2B) as well. The influences of miR-431 on *in vitro* proliferation, invasion and migration of lung cancer cells were subsequently explored through a series of functional experiments. Furthermore, we found that miR-431 exerted its anti-cancer effect in lung cancer by inhibiting the expression of DDX5 (DEAD box polypeptide 5).

Patients and Methods

Clinical Species

All 122 pairs of lung cancer and adjacent normal tissues were derived from lung cancer patients who underwent surgery at The First People Hospital of Zhangjiagang City from 2010 to 2015. None of enrolled patients received preoperative radiotherapy or chemotherapy prior to surgery. Surgical specimens were stored directly in liquid nitrogen before use. Both the patients and their families signed an informed consent form and the experiment was approved by the First People Hospital of Zhangjiagang City Ethics Committee (Zhangjiagang, China).

RNA Isolation and Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

All RNAs in lung cancer tissues and cells were extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). After extraction, the concentration of RNA was detected and reverse transcription was done using Reverse Transcription Kit (TaKaRa, Otsu, Shiga, Japan). Thereafter, SYBR Green Master Mix II (TaKaRa, Otsu, Shiga, Japan) was used to measure the relative expression of miR-431 using ABI 7600 (ABI, Foster City, CA, USA). U6 was used as an internal control. Expression level of miR-431 was calculated using the $2^{-\Delta\Delta t}$ method. Primer sequences used in this study were as follows: miR-431, 5'- CAGGCCGTCATGCAAA-3'; U6: F: 5'-GCTTCGGCAGCACATATACTAAAAT-3', R: 5'-CGCTTCAGAATTTGCGTGTCAT-3.

Cell Culture

All cell lines, including four lung cancer cell lines (H1650, A549, SPCA1, H1299) and one normal lung bronchial epithelial cell line (BEAS-2B), were purchased from the Shanghai Academy of Sciences (Shanghai, China). The cells were maintained in Roswell Park Memorial Institute 1640 (RPMI-1640) medium (Gibco, Rockville, MD, USA) containing 10% fetal bovine serum (FBS) (Gibco, Rockville, MD, USA) and 1% streptomycin (Gibco, Rockville, MD, USA). Cells were cultured in an incubator at 37°C and containing 5% carbon dioxide in humidity air.

Cell Transfection

The miR-431 mimics, negative control (NC), inhibitor, inhibitor negative control (INC) used in transfected cells was purchased from Ribobio Co. (Guangzhou, China). Cell transfection was carried out using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) following the instructions. After transfection, transfection efficiency was detected by qRT-PCR. The plasmid over-expressing DDX5 (pcDNA-DDX5) and its control were transfected into the cells in the same manner.

Cell Counting Kit-8 (CCK-8) Assay

Cell Counting Kit-8 (Dojindo, Tokyo, Japan) was used to detect cell proliferative ability. Treated cells were plated into 96-well plates at a density of 1000 cells per well and cultured in 100 μL of medium. Prior to observation at 0, 1, 2 and 3 days after culture, each well was added with 10 μL of CCK-8 reagent. The absorbance at 470 nm was determined and recorded after 2 hours. Each experiment was repeated at least three times.

Colony Formation Assay

For colony formation assay, a total of 1×10^3 transfected cells were stained with crystal violet staining solution (Beyotime, Shanghai, China) for 20 minutes, and colonies containing more than 50 cells were measured. Each experiment was repeated at least three times.

Transwell Assay

The effect of miR-431 on invasive and migratory abilities was detected using Transwell assay. The 8-μm chambers (Millipore, Billerica, MA,

USA) were bought to carry out the transwell assay. For migration assay, a total of 1×10^5 cells suspended in serum-free Roswell Park Memorial Institute-1640 (RPMI-1640) medium were plated in the top chamber of the insert. RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) was added in the lower chamber. Then, cells were incubated for 48 hours. Cells that failed to pass through the membrane in the upper layer of the inserts were wiped off using a cotton swab. Cells migrating through membrane to the other side of the insert were stained with crystal violet and counted in 6 random visions per well (magnification × 100). For invasion, the inserts were coated by matrigel (BD, Franklin Lakes, NJ, USA), and the other steps were the same with the migration assay.

Dual-Luciferase Reporter Gene Assay

Dual-Luciferase Reporter Assay System Kit (Promega, Madison, WI, USA) was bought to verify the potential target of miR-431 according to the manufactures' instructions. Two specific luciferase reporter vectors inserting with the region of the DDX5-3′-UTR carrying the mutant or wild type miR-431 binding site were constructed, respectively. A549 cells were used to complete the assay using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA). The luciferase activity was measured and the experiment was repeated for three times.

Western-Blot Assay

Radioimmunoprecipitation assay (RIPA) (Beyotime, Shanghai, China) was used to measure protein expression levels after cells were washed with phosphate-buffered saline (PBS). A bicinchoninic acid (BCA) protein assay kit (Beyotime, Shanghai, China) was utilized to calculate the concentration of collected protein. Protein samples were separated with a 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). The membranes were then immersed in the 5% bovine serum albumin (BSA) (Sigma-Aldrich, St. Louis, MO, USA) overnight at 4°C containing the specific primary antibody against DDX5 (Abnova, Taiwan) or glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Abcam, Cambridge, MA, USA) relatively, after blocking non-specific protein sites. Next, the membranes were cultured with horseradish peroxide (HRP) conjugated secondary antibody (Abnova, Taiwan) for 2 hours after washed using Tris-buffered saline-tween (TBST) buffer for 10 min × 3 times. At last, the membranes were detected using enhanced chemiluminescence (ECL) Kit (Millipore, Billerica, MA, USA) following the instructions.

Statistical Analysis

STATA 13.0 and Statistical Product and Service Solutions (SPSS) 20.0 (IBM, Armonk, NY, USA) software were employed for statistical analysis. The independent samples *t*-test was used to measure statistical analysis. Data were showed using GraphPad prism 5.0 software (La Jolla, CA, USA) and presented as mean ± SD. $p<0.05$ was considered having significant difference.

Results

MiR-431 was Down-Regulated in Lung Cancer Tissue Samples and Cell Lines

To investigate the expression of miR-431 in lung cancer, we used qRT-PCR to detect the level of miR-431 in 122 cases of lung cancer tissues and their adjacent normal tissues. We found that the expression of miR-431 in lung cancer tissues was significantly lower than that in adjacent normal tissues ($p<0.001$) (Figure 1A). At the same time, we also found the expression of miR-431 in four-lung cancer cell lines (A549, SPCA1, H1650, H1299) decreased compared with the normal lung bronchial epithelial cell line BEAS-2B (Figure 1B). These results indicated that miR-431 might play a role as a tumor-suppressor gene in lung cancer. To further investigate the specific function of miR-431 in lung cancer, overexpression and knockdown of miR-431 were performed in A549 cells and H1650 cells, respectively. MiR-431 level in A549 cells was significantly elevated after transfection of miR-431 mimics, while miR-431 expression in H1650 cells was significantly reduced by transfection of miR-431 inhibitor (Figure 1C, 1D).

MiR-431 Inhibited Cell Proliferation of Lung Cancer

To examine the function of miR-431 on the proliferation of lung cancer cells, CCK-8 assay and colony formation assay were conducted. Up-regulation of miR-431 markedly inhibited proliferation of A549 cells, while knockdown of miR-431 significantly promoted proliferation of H1650 cells comparing with their respecti-

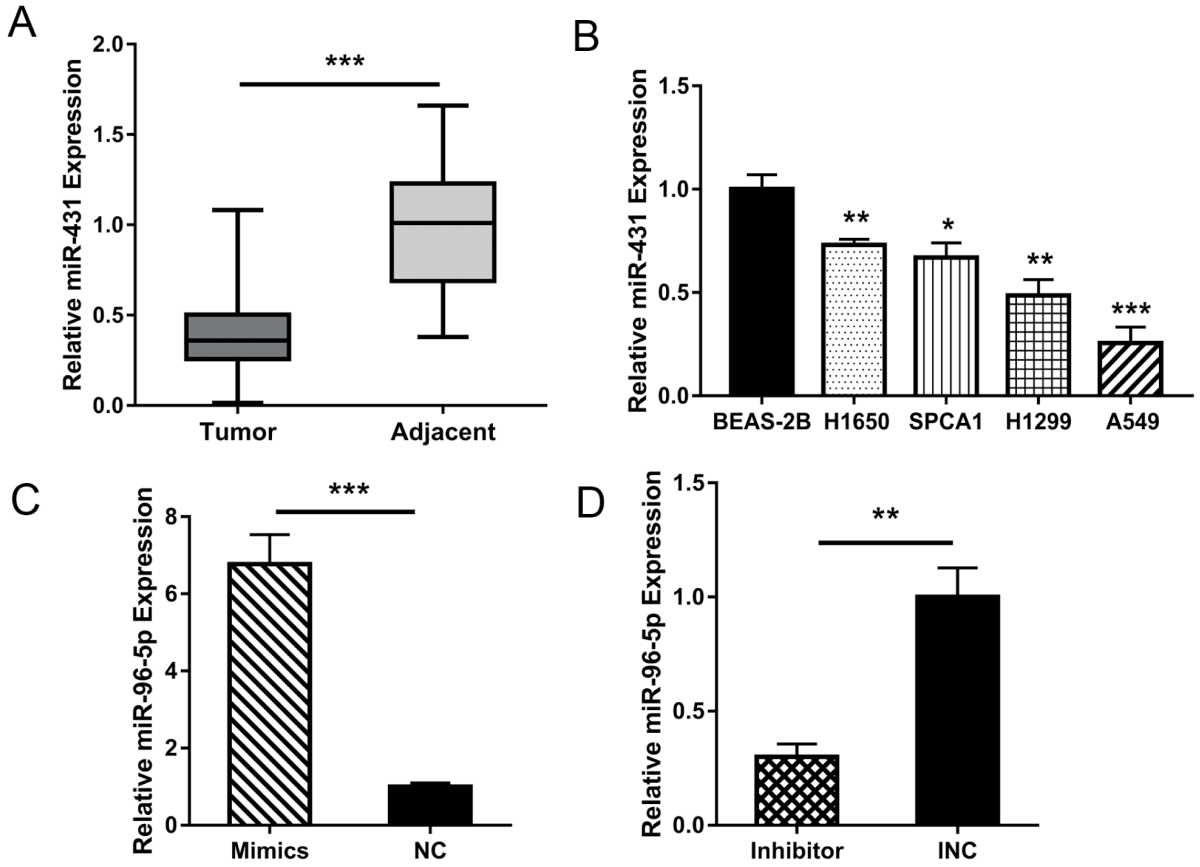

Figure 1. MiR-431 expression decreased in lung cancer tissues and cell lines. ***A,*** Analysis of the expression level of miR-431 in 122 lung cancer samples and adjacent normal tissues. ***B,*** Analysis of miR-431 expression level in lung cancer cell lines (A549, SPCA1, H1299, H1650) and human normal lung bronchial epithelial cell line BEAS-2B. ***C,*** Expression of miR-431 in A549 cells transfected with miR-431 mimics. ***D,*** Expression of miR-431 in H1650 cells transfected with miR-431 inhibitor. *p<0.05, **p<0.01, ***p<0.001.

ve control groups (Figure 2A, 2B). Similarly, the number of colonies formed by A549 cells transfected with miR-431 mimics was significantly reduced, while H1650 cells showed more colonies after transfection of miR-431 inhibitor compared with the respective control groups (Figure 2C, 2D, 2E). These results indicated that miR-431 reduced cell proliferative ability of lung cancer.

Abnormal Expression of miR-431 Influenced Cell Invasion and Migration of Lung Cancer

Further, we explored the effect of miR-431 on invasive and migratory abilities of lung cancer cells. Transwell invasion assay showed that after over-expressing miR-431, the number of A549 cells passing through the transwell chamber was significantly less than the control group, while knockdown of miR-431 in H1650 cells showed a completely opposite trend (Figure 3A). On the other hand, the migratory ability of A549 cells was also remarkably inhibited by transfection of miR-431 mimics, whereas it was significantly enhanced in H1650 cells transfected with miR-431 inhibitor (Figure 3B). The above results indicated that miR-431 could inhibit the invasion and migration of lung cancer cells.

DDX5 Functioned as a Direct Target of miR-431 in Lung Cancer

We have discovered that miR-431 could affect the proliferation and metastasis of lung cancer cells. Next, we further explored the mechanism of its anti-cancer effect. Since miR-

NAs could function by binding to the 3'-UTR of their target genes, we found DDX5 as a target gene of miR-431 through searching several databases (PiTar, miRase, TargetScan) (Figure 4A). To confirm our hypothesis, we constructed two luciferase vectors to execute the dual-luciferase reporter gene assay and found that the activity of the mutant DDX5 3'-UTR group had no change, while the wild type group was markedly lower than the control group (Figure 4B). It indicated that miR-431 could directly bind to DDX5 to play its biological function. Next, we examined the protein level of DDX5 in established cells, and found that the protein expression of DDX5 in A549 cells over-expressing miR-431 significantly decreased when comparing to the NC group. DDX5 level in H1650 cells knocking down miR-431 was higher than that of the INC group (Figure 4C, 4D). These data suggested DDX5 was a direct downstream target for miR-431 in lung cancer.

MiR-431 Functioned as a Tumor Suppressor in Lung Cancer Via Down-Regulating DDX5

To further validate the conformability of DDX5 as a miR-431 target gene, we constructed a DDX5 over-expression plasmid (pcDNA-DDX5) to implement the rescue test. Figure 5A showed that DDX5 expression significantly increased in A549 cells co-transfected with miR-431 mimics and pcDNA-DDX5 compared to the miR-431 mimics

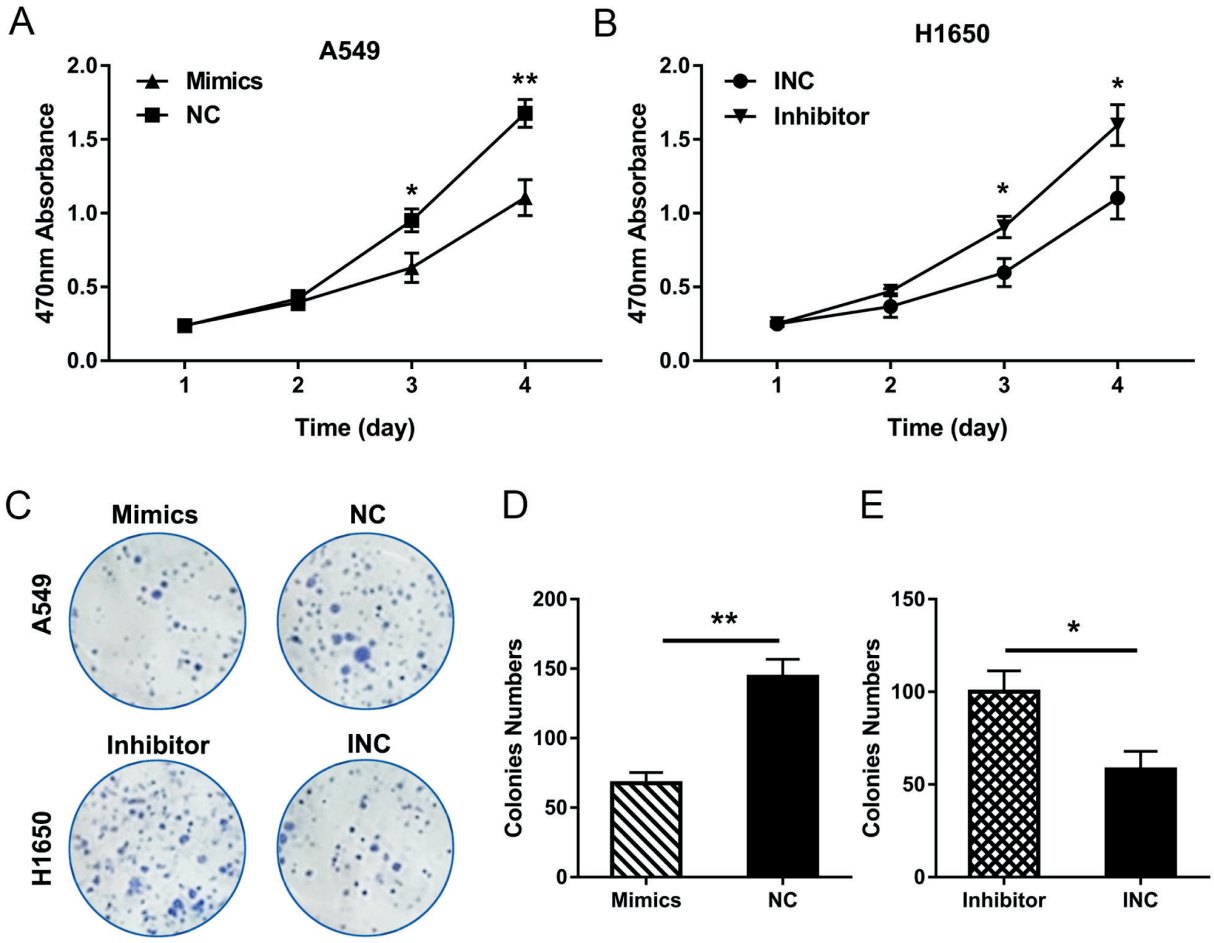

Figure 2. MiR-431 affected the proliferation of lung cancer cells. ***A, B,*** CCK-8 assay was performed to determine the proliferation of A549 (***A***) or H1650 (***B***) cells transfected with miR-431 mimics or inhibitors compared to each negative control. ***C, D, E,*** Colony formation assay was performed to determine the growth of A549 (***C, D***) or H1650 (***C, E***) cells transfected with mimics or inhibitor, respectively. *p<0.05, ** p<0.01, ***p<0.001.

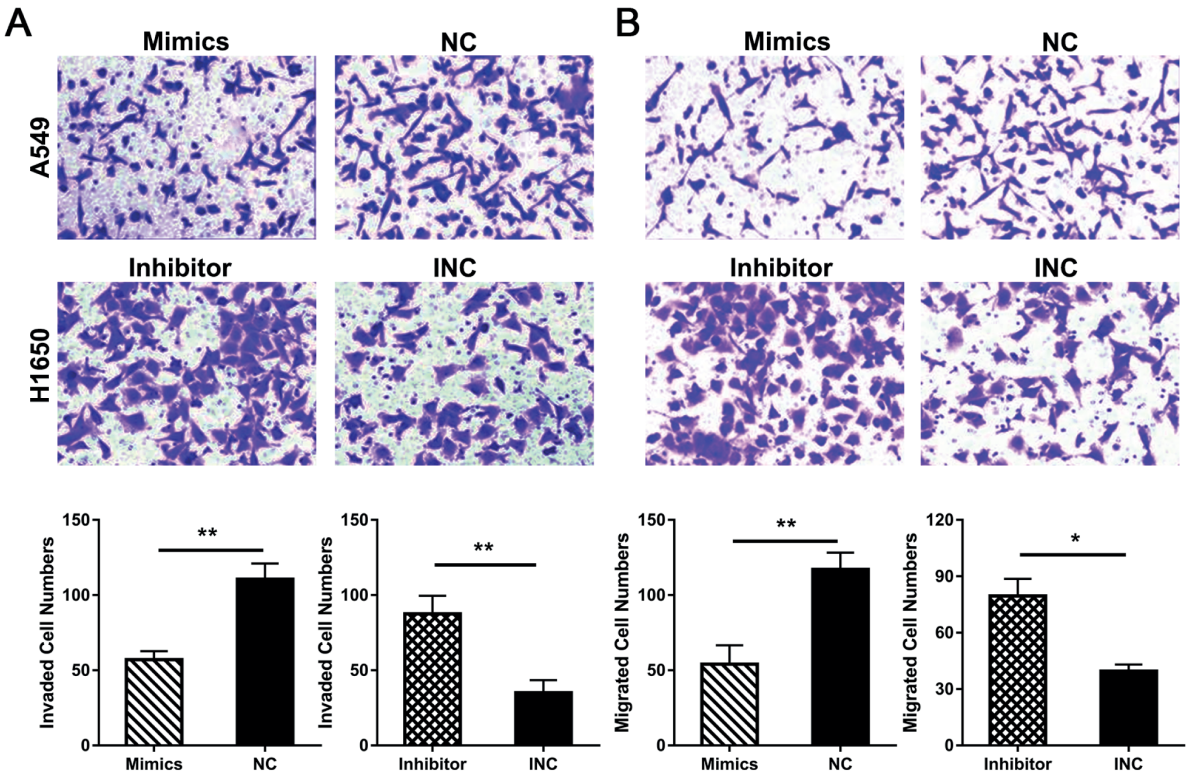

Figure 3. MiR-431 affected the invasion and migration of lung cancer cells. ***A,*** Transwell invasion assay was used to detect the invasive ability of miR-431 in A549 cells and H1650 cells. ***B,*** Transwell migration assay was used to detect the migration ability of miR-431 in A549 cells and H1650 cells. Data were presented as the mean ± SD of three independent experiments. *$p<0.05$, **$p<0.01$.

group. Thereafter, by the CCK8 assay, we found that the cell proliferative ability inhibited by miR-431 was significantly restored by over-expression of DDX5 (Figure 5B). Transwelll assay also demonstrated that overexpression of DDX5 significantly impaired the ability of miR-431 overexpression to inhibit cell metastasis (Figure 5C). These results indicated that miR-431 exerted its anti-cancer effect by inhibiting the expression of DDX5.

Discussion

Lung cancer is a kind of high-grade malignant tumor. The occurrence and progression of lung cancer are complex processes involving multiple genetic changes and multiple steps[2]. The specific molecular mechanism of its development is still unclear. In recent years, with the development of epigenetics, the role of miRNA in diseases has attracted more and more attention[15, 16]. Researches[17,18] have revealed that miRNAs are abnormally expressed in many types of tumors, and they play important roles in the tumorigenesis and progression of malignant tumors. Current studies found that the abnormal expression of miR-431 was closely involved in the incidence and process of malignant tumors. However, few studies have been conducted to analyzing the relationship between miR-431 expression and the development of lung cancer. In this study, miR-431 expression in 122 cases of lung cancer tissues and adjacent normal tissues, four human lung cancer-derived cell lines (H1650, A549, SPCA1, H1299) and human normal lung bronchial epithelial cell line BEAS-2B was detected by qRT-PCR. The expression level of miR-431 significantly decreased in lung cancer

tissues and lung cancer cell lines. Meanwhile, functions and mechanisms of miR-431 on the proliferation and metastasis of lung cancer cells have not been reported. In order to study the influence of miR-431 on the proliferation of lung cancer cells, miR-431 mimics or inhibitor was successfully transfected into A549 or H1650 cells, respectively. Functional experiments showed that up-regulation of miR-431 significantly inhibited the proliferation and metastasis of A549 cells, while down-regulation of miR-431 accelerated cell growth, invasion, and migration of H1650 cells, indicating that miR-431 belonged to the "tumor-suppressor gene" of lung cancer. One miRNA could be involved in the regulation of multiple target genes, and each target gene could in turn be regulated by multiple miRNAs. The roles of miRNAs in tumors depend primarily on the biological effects of their downstream target genes[19, 20]. To investigate the possible mechanism by which miR-431 inhibited proliferation and metastasis of lung cancer cells, several target gene prediction databases were searched. Combined with functional analysis of genes, DDX5 was predicted to be the possible target gene of miR-431. DDX5 (DEAD-box helicase 5), also named p68, participates in the process transcription to translation, RNA decay, and miRNA processing. Several studies[21-23] have identified DDX5 as a tumor-promoting factor. For example, DDX5 regulated mTOR signaling pathway to promote *in vitro* and *in vivo* cell growth of gastric cancer[24]. In esophageal cancer, down-regulation of DDX5 inhibited the growth and tumorigenesis[25]. In lung cancer, DDX5 promoted cell proliferation and development though activating β-catenin signaling axis[26]. We showed that miR-431 could specifically bind to the 3'-UTR region of DDX5 by dual-luciferase reporter gene assay. Western blot analysis showed that over-expression of miR-431 could down-regulate the expression of DDX5, indicating that miR-431 negatively regulated its target gene DDX5. We demonstrated that miR-431 was down-regulated in lung cancer tissues and cells, and DDX5 was a direct regulatory target of miR-431. Further-

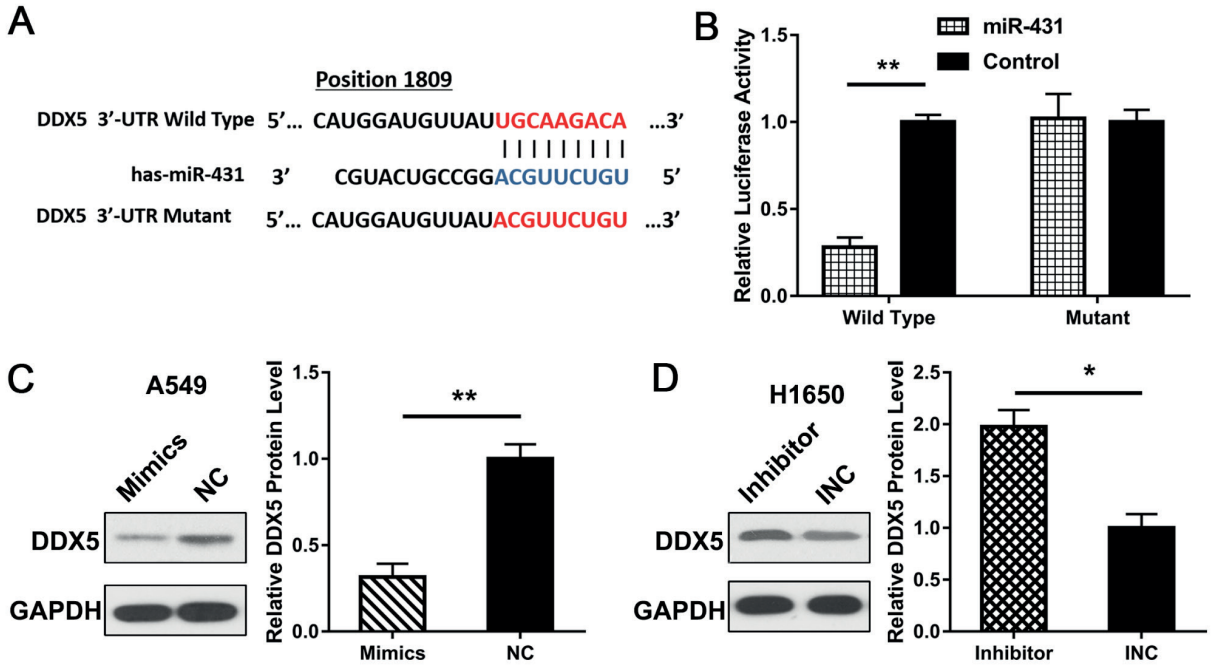

Figure 4. DDX5 was the direct target of miR-431. ***A,*** The predicted binding sites of miR-431 in the 3'-UTR of DDX5. ***B,*** Dual-luciferase reporter gene assay was used to determine the binding site. ***C, D,*** Levels of DDX5 and GAPDH in A549 cells overexpressing miR-431 (***C***) and H1650 cells knocking down miR-431 (***D***)were measured by Western-blot. The relative protein level of DDX5 was compared to GAPHD. Data were presented as the mean ± SD of three independent experiments. *$p<0.05$, **$p<0.01$, ns: non-sense.

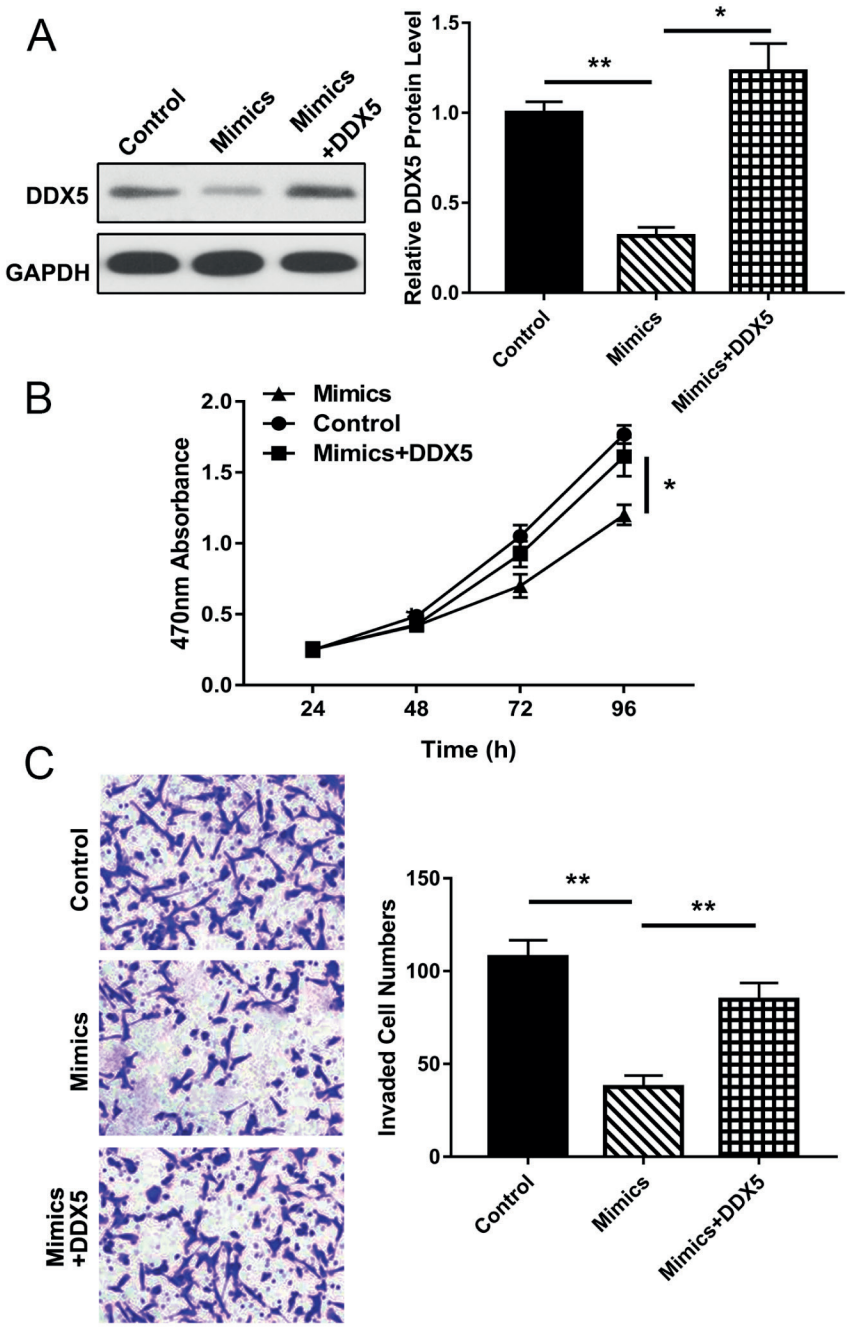

Figure 5. DDX5 rescued the effects of miR-431 mimics in A549 cells. ***A,*** Western-blot analyses of DDX5. GAPDH was used as an internal control. ***B,*** Analysis of the cell proliferation ability by CCK-8 assay in control, mimics, or mimics+DDX5 treated A549 cells; ***C,*** Cell invasion ability was measured by Transwell assay; Data were represented as the mean ± SD of three replicates. *p<0.05, **p<0.01, ***p<0.001.

more, miR-431 inhibited the proliferation and metastasis of lung cancer cells by negatively regulating the expression of DDX5. Our results provided a new research target for treatment of lung cancer and might become a potential target for future lung cancer gene therapy.

Conclusions

We found that miR-431 inhibited lung cancer by decreasing the expression of DDX5. Our study might provide a novel target for the treatment of lung cancer.

Conflict of Interest

The Authors declare that they have no conflict of interest.

References

1) Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015; 65: 87-108.
2) Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. CA Cancer J Clin 2018; 68: 7-30.
3) Ameres SL, Zamore PD. Diversifying microRNA sequence and function. Nat Rev Mol Cell Biol 2013; 14: 475-488.
4) Bartels CL, Tsongalis GJ. MicroRNAs: novel biomarkers for human cancer. Clin Chem 2009; 55: 623-631.
5) Zhang Z, Li J, Huang Y, Peng W, Qian W, Gu J, Wang Q, Hu T, Ji D, Ji B, Zhang Y, Wang S, Sun Y. Upregulated miR-1258 regulates cell cycle and inhibits cell proliferation by directly targeting E2F8 in CRC. Cell Prolif 2018: e12505.
6) Yang X, Wang M, Lin B, Yao D, Li J, Tang X, Li S, Liu Y, Xie R, Yu S. miR-487a promotes progression of gastric cancer by targeting TIA1. Biochimie. 2018 Aug 23;154:119-126. doi: 10.1016/j.biochi.2018.08.006. [Epub ahead of print]
7) Duan Y, Dong Y, Dang R, Hu Z, Yang Y, Hu Y, Cheng J. MiR-122 inhibits epithelial mesenchymal transition by regulating P4HA1 in ovarian cancer cells. Cell Biol Int. 2018 Aug 23. doi: 10.1002/cbin.11052. [Epub ahead of print]
8) Ma L, Teruya-Feldstein J, Weinberg RA. Tumour invasion and metastasis initiated by microRNA-10b in breast cancer. Nature 2007; 449: 682-688.
9) Dimitrova N, Gocheva V, Bhutkar A, Resnick R, Jong RM, Miller KM, Bendor J, Jacks T. Stromal expression of miR-143/145 promotes neoangiogenesis in lung cancer development. Cancer Discov 2016; 6: 188-201.
10) Romano G, Acunzo M, Garofalo M, Di Leva G, Cascione L, Zanca C, Bolon B, Condorelli G, Croce CM. MiR-494 is regulated by ERK1/2 and modulates TRAIL-induced apoptosis in non-small-cell lung cancer through BIM down-regulation. Proc Natl Acad Sci U S A 2012; 109: 16570-16575.
11) Liu Y, Li L, Liu Z, Yuan Q, Lu X. Downregulation of MiR-431 expression associated with lymph node metastasis and promotes cell invasion in papillary thyroid carcinoma. Cancer Biomark 2018; 22: 727-732.
12) Su WB, Liu ZY. MiR-431 inhibits colorectal cancer cell invasion via repressing CUL4B. Eur Rev Med Pharmacol Sci 2018; 22: 3047-3052.
13) Pan L, Ren F, Rong M, Dang Y, Luo Y, Luo D, Chen G. Correlation between down-expression of miR-431 and clinicopathological significance in HCC tissues. Clin Transl Oncol 2015; 17: 557-563.
14) Sun K, Zeng T, Huang D, Liu Z, Huang S, Liu J, Qu Z. MicroRNA-431 inhibits migration and invasion of hepatocellular carcinoma cells by targeting the ZEB1-mediated epithelial-mensenchymal transition. FEBS Open Bio 2015; 5: 900-907.
15) Zhang B, Pan X, Cobb GP, Anderson TA. microRNAs as oncogenes and tumor suppressors. Dev Biol 2007; 302: 1-12.
16) Beermann J, Piccoli MT, Viereck J, Thum T. Non-coding RNAs in development and disease: background, mechanisms, and therapeutic approaches. Physiol Rev 2016; 96: 1297-1325.
17) Ling H, Fabbri M, Calin GA. MicroRNAs and other non-coding RNAs as targets for anticancer drug development. Nat Rev Drug Discov 2013; 12: 847-865.
18) Berindan-Neagoe I, Monroig PC, Pasculli B, Calin GA. MicroRNAome genome: a treasure for cancer diagnosis and therapy. CA Cancer J Clin 2014; 64: 311-336.
19) Iorio MV, Croce CM. MicroRNAs in cancer: small molecules with a huge impact. J Clin Oncol 2009; 27: 5848-5856.
20) Slack FJ, Weidhaas JB. MicroRNA in cancer prognosis. N Engl J Med 2008; 359: 2720-2722.
21) Dai L, Pan G, Liu X, Huang J, Jiang Z, Zhu X, Gan X, Xu Q, Tan N. High expression of ALDOA and DDX5 are associated with poor prognosis in human colorectal cancer. Cancer Manag Res 2018; 10: 1799-1806.
22) Sha M, Lin M, Wang J, Ye J, Xu J, Xu N, Huang J. Long non-coding RNA MIAT promotes gastric cancer growth and metastasis through regulation of miR-141/DDX5 pathway. J Exp Clin Cancer Res 2018; 37: 58.
23) Su LJ, Chang CW, Wu YC, Chen KC, Lin CJ, Liang SC, Lin CH, Whang-Peng J, Hsu SL, Chen CH, Huang CY. Selection of DDX5 as a novel internal control for Q-RT-PCR from microarray data using a block bootstrap re-sampling scheme. BMC Genomics 2007; 8: 140.
24) Du C, Li DQ, Li N, Chen L, Li SS, Yang Y, Hou MX, Xie MJ, Zheng ZD. DDX5 promotes gastric cancer cell proliferation in vitro and in vivo through mTOR signaling pathway. Sci Rep 2017; 7: 42876.
25) Ma Z, Feng J, Guo Y, Kong R, Ma Y, Sun L, Yang X, Zhou B, Li S, Zhang W, Jiang J, Zhang J, Qiao Z, Cheng Y, Zha D, Liu S. Knockdown of DDX5 Inhibits the Proliferation and Tumorigenesis in Esophageal Cancer. Oncol Res 2017; 25: 887-895.
26) Wang Z, Luo Z, Zhou L, Li X, Jiang T, Fu E. DDX5 promotes proliferation and tumorigenesis of non-small-cell lung cancer cells by activating beta-catenin signaling pathway. Cancer Sci 2015; 106: 1303-1