Original Article

LncARSR promotes non-small-cell lung cancer progression via regulating PTEN/Akt

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Abstract: LncRNAs have been suggested to be key modulators in many biological and pathological processes. LncARSR, a recently identified IncRNA, plays crucial roles in the progression of several cancers. However, the role of LncARSR in NSCLC is uninvestigated. In the present study, it was demonstrated that lncARSR expression was higher in NSCLC tissues than in noncancerous tissues. The expression of lncARSR was higher in four NSCLC cell lines than in a normal lung bronchial epithelial line. Further investigation demonstrated that increased lncARSR expression promoted NSCLC cell migration and growth and induced epithelial-mesenchymal transition in A549 cells. Moreover, ectopic expression of lncARSR suppressed PTEN expression and induced Akt phosphorylation in A549 cells. The expression level of PTEN was higher in NSCLC samples than in adjacent non-tumor specimens. PTEN expression was negatively correlated with lncARSR in NSCLC specimens. Furthermore, we demonstrated that overexpression of lncARSR induced NSCLC cell growth and migration via regulating the PTEN/Akt signaling pathway. These results suggest that lncARSR acts as an oncogene in NSCLC development and could serve as a new potential therapeutic target.

Keywords: NSCLC, LncARSR, PTEN, Akt

Introduction

Lung tumors rank as the leading cause of tumor-related deaths worldwide [1-4]. NSCLC accounts for approximately 85% of lung tumor cases [5-7]. Despite developments in chemotherapeutic and surgical interventions, the five-year survival rate for NSCLC patients remains unsatisfactory, and the recurrence rate in these patients is high due to tumor metastasis and chemoresistance development [4, 8-10]. Many genome-wide studies on NSCLC have been performed, such as gene expression profiling, copy number analysis and DNA sequencing [11-15]. However, the detailed mechanisms of NSCLC remain poorly understood. Thus, it is crucial to seek novel therapeutic targets for NSCLC.

LncRNAs are more than two hundred nucleotides (nts) in length with no or limited protein-coding capacity, and they regulate gene expression at the post-transcriptional level [16-22]. LncRNAs play roles in biological and pathological processes, such as development, metastasis, angiogenesis, proliferation, metabolism and invasion [23-28]. Deregulated IncRNA expression has been identified in almost all types of human malignancies, such as gastric cancer, urinary bladder carcinoma, glioblastoma, osteosarcoma, and NSCLC [19, 20, 27, 29-31]. Recent evidence has shown that lncARSR plays a role in the progression of several cancers [32-35]. Qu et al. [35] first indicated that lncARSR was associated with a poor sunitinib reaction in renal carcinoma. Overexpression of lncARSR induced sunitinib resistance by binding miR-449/miR-34 to induce c-MET and AXL expression in renal cell carcinoma cells. Moreover, Qu and colleagues found that lncARSR was overexpressed in renal tumor-initiating cells (T-ICs) and that knockdown of lncARSR abolished the tumorigenicity, metastasis and self-renewal of these renal tumor-initiating cells. Forced expression of lncARSR promoted T-IC characteristics in RCC cells by combining with YAP to impede LATS1-induced YAP phosphorylation.
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phosphorylation and induce YAP nuclear translocation [34]. However, the role of IncARSR in NSCLC remains uninvestigated.

In the present study, we found that IncARSR was overexpressed in NSCLC tissues. Further investigation indicated that ectopic IncARSR expression induced NSCLC cell migration, growth and EMT.

Materials and methods

Specimens and cell transfection

NSCLC and matched non-tumor samples were collected from 40 NSCLC patients at the Fifth Hospital of Harbin Medical University and Daqing People’s Hospital. These samples were immediately snap-frozen. None of the patients received any radiotherapy or chemotherapy. Written informed consent was obtained from the patients, and our research was approved by the Clinical Ethics Committee of the Fifth Hospital of Harbin Medical University and Daqing People’s Hospital. Lung bronchial cells (16HBE) and four lung tumor cell lines (SPC-A1, A549, H23 and H1299) were purchased from the Chinese Academy of Sciences of Shanghai (Shanghai). pcDNA-IncARSR and scrambled vector were obtained from GenePharma (Shanghai, China). A549 cell transfection was performed with Lipofectamine 2000 following the manufacturer’s instructions.

Cell invasion, cycle and growth assays

For the cell growth assay, cells were grown in 96-well plates. Cell proliferation was detected using a CCK-8 kit (Beyotime, China). The proliferation rate was measured at 0, 1, 2, and 3 days post-transfection, and the absorbance (450 nM) was measured on a microtiter reader. For the cell cycle assay, cells were collected, washed with PBS and fixed overnight with ethyl alcohol at 4°C. RNase A was added to the cells, which were then washed with cold PBS and incubated with propidium iodide. The signal was measured by FACS. For the invasion analysis, a Transwell chamber with Matrigel (Bioscience, CA, USA) was used. The cells were cultured in the upper transwell chamber with no serum, and complete medium was added to the lower chambers. After 24 hours of incubation, the non-invasive cells were removed with a cotton swab, and the invasive cells were fixed with ethanol and stained with crystal violet. The invaded cells were captured by photomicrograph and counted.

qRT-PCR

Total RNA from NSCLC specimens and cell lines was acquired with a Trizol kit (Invitrogen, Technologies). qRT-PCR was performed to analyze the expression of IncRNA, miRNA and mRNA with SYBR PCR reagent (Takara, Dalian, China) on a Bio-Rad PCR system following the manufacturer’s instructions. For gene expression quantification, the relative CT method (2^{ΔΔCT} method) was used.

Western blot analysis

Cells and specimens were collected and lysed in RIPA buffer with a proteinase inhibitor (Beyotime, China), and the protein concentrations were assessed using a BCA kit (Beyotime, China). Equal protein amounts were separated by 12% SDS PAGE and then transferred to PVDF membranes (Millipore, USA). After blocking with milk, the membranes were incubated with primary antibodies (N-cadherin, E-cadherin and vimentin), followed by hybridization with an HRP-conjugated antibody. The protein signals were visualized using enhanced chemiluminescence (ECL) reagents (Pierce, USA).

Statistical analysis

The data are shown as the mean ± SD (standard deviation) and were obtained at least in triplicate. The data were measured and analyzed with SPSS (SPSS Inc., Chicago, IL). One-way ANOVA or Student’s t-test was performed for data analyses. P < 0.05 was set to indicate statistical significance.

Results

LncARSR expression was upregulated in NSCLC tissues

As demonstrated in Figure 1A, IncARSR expression was higher in the cancerous tissues of 31 NSCLC patients (31/40, 77.5%) than in the adjacent noncancerous tissues. In general, the expression level of IncARSR was higher in NSCLC samples than in adjacent noncancerous samples (Figure 1B). In addition, we showed that IncARSR expression was higher in four NSCLC cell lines (A549, H23, H1299 and SPC-A1) than in 16HBE cells (Figure 1C).
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Ectopic IncARSR expression promoted NSCLC cell migration and growth

To evaluate the effect of IncARSR on the growth and migration of NSCLC cells, IncARSR was overexpressed in A549 cells by transfection with pcDNA-IncARSR (Figure 2A). CCK-8 assays indicated that IncARSR overexpression induced A549 cell proliferation (Figure 2B). In line with this finding, IncARSR upregulation increased the expression levels of cyclin D1 (Figure 2C) and Ki-67 (Figure 2D) in A549 cells. We used a wound healing migration assay and found that IncARSR overexpression induced A549 cell migration (Figure 2E).

LncARSR overexpression induced epithelial-mesenchymal transition (EMT): Furthermore, we found that IncARSR overexpression inhibited the expression of the epithelial marker E-cadherin (Figure 3A). The expression levels of mesenchymal markers, vimentin and N-cadherin, were increased in A549 cells after treatment with pcDNA-IncARSR (Figure 3B and 3C). In sum, we confirmed that E-cadherin was downregulated and that N-cadherin and vimentin were upregulated in A549 cells after incubation with pcDNA-IncARSR (Figure 3D).

LncARSR activated AKT signaling in NSCLC cells: As indicated in Figure 4A, ectopic expression of IncARSR suppressed the mRNA expression of PTEN. In line with this finding, ectopic expression of IncARSR decreased PTEN protein expression in A549 cells (Figure 4B). Furthermore, increased expression of IncARSR induced Akt phosphorylation in A549 cells (Figure 4C).

LncARSR expression was negatively correlated with PTEN expression

We first measured the expression of PTEN in NSCLC samples. As demonstrated in Figure 1, LncARSR expression was upregulated in NSCLC tissues. A. LncARSR expression levels in NSCLC tissues and adjacent noncancerous tissues were detected by qRT-PCR. The data are shown as log2. B. The expression level of IncARSR was higher in NSCLC samples than in adjacent noncancerous samples. C. LncARSR expression levels in four NSCLC cell lines (H1299, H23, A549 and SPC-A1) and a lung bronchial epithelial cell line (16HBE) were determined using qRT-PCR. ***P < 0.001.
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5A. PTEN expression was lower in the cancerous tissues of 30 NSCLC patients (30/40, 75%) than in their adjacent noncancerous tissues. In general, the expression level of PTEN was lower in NSCLC samples than in adjacent noncancerous samples (Figure 5B). Moreover, the expression level of PTEN was negatively correlated with lncARSR in the NSCLC specimens (Figure 5C).

The Akt signaling pathway was responsible for lncARSR-induced proliferation and migration in NSCLC cells

To further study whether the PTEN/Akt signaling pathway participated in lncARSR-induced proliferation and migration in NSCLC cells, we treated lncARSR-overexpressing A549 cells with the PI3K/Akt inhibitor LY294002. CCK-8...
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analysis indicated that cell proliferation was suppressed after treating LncARSR-overexpressing A549 cells with LY294002 (Figure 6A). Treatment with LY294002 reversed the increases in cyclin D1 and Ki-67 induced by LncARSR overexpression (Figure 6B and 6C).

Figure 3. LncARSR overexpression induced epithelial-mesenchymal transition (EMT). A. LncARSR overexpression inhibited the expression of the epithelial marker E-cadherin. B. The mRNA expression of N-cadherin was detected by qRT-PCR. C. The mRNA expression of vimentin was detected by qRT-PCR. D. The protein expression of E-cadherin was decreased, and that of N-cadherin and vimentin was increased in A549 cells after treatment with pcDNA-LncARSR. GAPDH was used as a loading control.

Figure 4. LncARSR activated AKT signaling in NSCLC cells. A. Ectopic expression of LncARSR suppressed the mRNA expression of PTEN. B. The protein expression of PTEN was detected by Western blotting. C. Increased expression of LncARSR induced Akt phosphorylation in A549 cells. GAPDH was used as a loading control.
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Furthermore, overexpression of lncARSR increased A549 cell migration, which was abolished by LY294002 (Figure 6D).

Discussion

Our findings demonstrated that lncARSR expression was higher in NSCLC tissues than in non-tumor tissues. LncARSR expression was also higher in four NSCLC cells (A549, H23, H1299 and SPC-A1) than in lung 16HBE cells. Further investigation suggested that IncARSR upregulation promoted NSCLC cell proliferation and migration and induced EMT in A549 cells. We found that ectopic lncARSR expression suppressed PTEN expression and induced Akt

Figure 5. Negative correlation between lncARSR and PTEN expression in NSCLC tissues. A. PTEN expression was downregulated in the cancerous tissues of 30 NSCLC patients (30/40, 75%) compared to that in the adjacent noncancerous tissues. B. The expression level of PTEN was lower in NSCLC samples than in adjacent noncancerous samples. C. The PTEN expression level was negatively correlated with the lncARSR expression level in NSCLC tissues.

Figure 6. The Akt signaling pathway was responsible for lncARSR-induced proliferation and migration in NSCLC cells. A. CCK-8 assays were performed to measure cell proliferation. B. The mRNA expression of cyclin D1 was detected by qRT-PCR. C. The mRNA expression of Ki-67 was measured by using qRT-PCR. D. Ectopic expression of lncARSR promoted A549 cell migration, which was abolished by LY294002. The relative ratio of wound closure is shown. *P < 0.05, **P < 0.01 and ***P < 0.001.
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phosphorylation in A549 cells. Moreover, the expression level of PTEN was higher in NSCLC samples. The PTEN expression level was negatively correlated with IncARSR expression in NSCLC specimens. Furthermore, we demonstrated that ectopic IncARSR expression promoted NSCLC cell migration and proliferation via regulating the PTEN/Akt signaling pathway. These results suggest that IncARSR plays an oncogenic role in NSCLC development and might serve as a new potential therapeutic target.

Recent evidence has demonstrated that IncARSR plays crucial roles in the development of several tumors [32-37]. Qu et al. [35] first demonstrated that IncARSR was associated with a poor chemoresponse to sunitinib treatment in renal cancer. Overexpression of IncARSR induced sunitinib resistance through binding to miR-449/miR-34, thus promoting c-MET and AXL expression in renal cell carcinoma cells. In addition, Qu and colleagues indicated that IncARSR was overexpressed in renal tumor-initiating cells (T-ICs) and that IncARSR knockdown abolished tumorigenicity, metastasis and self-renewal in renal tumor-initiating cells. Forced expression of IncARSR promoted T-IC properties in RCC cells through binding with YAP to inhibit LATS1-induced YAP phosphorylation and induce YAP nuclear translocation [33, 34]. Li and colleagues indicated that IncARSR was overexpressed in HCC. Increased expression of IncARSR induced HCC cell resistance to doxorubicin treatment via regulating the PTEN-PI3K/Akt pathway. Shu et al. [32] proved that IncARSR expression was increased in ovarian cancer and associated with lymph node metastasis, poor survival, FIGO (International Federation of Gynecology and Obstetrics) staging and histological grade. Overexpression of IncARSR induced cell progression via the β-catenin/Wnt pathway in ovarian cancer. Our research indicated that IncARSR expression was upregulated in NSCLC samples and NSCLC cell lines. Further study showed that ectopic IncARSR expression enhanced NSCLC cell proliferation and induced EMT progression in NSCLC cells.

LncRNAs are important posttranscriptional modulators of the majority of human genes [38-40]. Previous evidence suggested that ectopic IncARSR expression induced chemoresistance to doxorubicin in HCC cells via PTEN-PI3K/Akt signaling pathway regulation [33]. The PTEN gene, located on chromosome 10q, consists of 9 exons and a 5-Kb variable exon [41]. PTEN was found to act as a tumor suppressor gene in the progression of several tumors [42-44]. PTEN was also shown to be involved in cell migration, proliferation, invasion and metabolism [45]. Loss of the PTEN gene in the PI3K/Akt pathway is frequently found in NSCLC [46-48]. We demonstrated that increased expression of IncARSR inhibited PTEN expression and induced Akt phosphorylation in A549 cells. Moreover, we reported that PTEN expression was higher in NSCLC samples than in adjacent noncancerous specimens. PTEN expression was negatively correlated with IncARSR expression in NSCLC samples. Furthermore, we demonstrated that ectopic IncARSR expression induced cell migration and proliferation via regulating the PTEN/Akt signaling pathway.

In summary, we showed that the expression level of IncARSR was higher in NSCLC specimens and cells and that ectopic IncARSR expression induced NSCLC cell proliferation, migration and invasion via the PTEN/Akt pathway. These results suggest that IncARSR plays an oncogenic role in the initiation and progression of NSCLC and might serve as a novel therapeutic target.

Disclosure of conflict of interest
None.

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References


[26] LncARSR promotes progression of NSCLC.
noncoding RNA lncARSR promotes hepatic li-
Pogenesis via Akt/SREBP-1c pathway and con-
tributes to the pathogenesis of nonalco-
holic steatohepatitis. Biochem Biophys Res
Commun 2018; 499: 66-70.
[37] Huang JB, Chen SJ, Cai DL, Bian DQ and Wang
FL. Long noncoding RNA IncARSR promotes
hepatic cholesterol biosynthesis via modula-
ting Akt/SREBP-2/HMGCR pathway. Life Sci
2018; 203: 48-53.
Q, Li CY, Zhang PJ, Cui FA, Chen WW and Jiang
AL. Long non-coding RNA LOC283070 medi-
ates the transition of LNCalp cells into andro-
gen-independent cells possibly via CAMK1D.
[39] Zhang XY, Zhang LX, Tian CJ, Tang XY, Zhao LM,
Guo YL, Cheng DJ, Chen XL, Ma LJ and Chen
ZC. LncRNAs BCYRN1 promoted the prolifera-
tion and migration of airway smooth mus-
cle cells in asthma via upregulating the expres-
sion of transient receptor potential 1. Am J
Transl Res 2016; 8: 3409-3418.
[40] Zhang XY, Tang XY, Ma LJ, Guo YL, Li XS, Zhao
LM, Tian CJ, Cheng DJ, Chen ZC and Zhang LX.
Schisandrin B down-regulated lncRNA BCYRN1
expression of airway smooth muscle cells by
improving miR-150 expression to inhibit the
proliferation and migration of ASMC in as-
[41] Phin S, Moore M and Cotter P. Genomic rearr-
rangements of pten in prostate cancer. Front
[42] Li L, Sun JX, Wang QX, Liu XK, Chen XX, Zhang
B, He ZD, Liu DZ, Chen LX, Wang LW and Huang
Z. Notoginsenoside R7 suppresses cervical
cancer via PI3K/PTEN/Akt/mTOR signaling.
Oncotarget 2017; 8: 109487-109496.
[43] Zhao YF, Wang YH and Wang YQ. Up-regulated
miR-500a enhances hepatocarcinoma metas-
tasis by repressing PTEN expression. Biosci
Rep 2017; 37.
[44] Szarzyńska-Zawadzka B, Kunz JB, Sędęk Ł,
Kosmalska M, Zdon K, Bieccek P, Bandapalli
OR, Kraszewska-Hamilton M, Jaksik R, Dro-
bna M, Kowalczyk JR, Szczepański T, Van
Vlierberghe P, Kuloski AE, Witt M, Dawidowska
M. PTEN alterations are related to unfavorable
prognosis in children with T-Cell acute lympho-
blastic leukemia treated according to ALL IC-
BFM protocols. Am J Hematol 2019; 94:
118; 4498-4507.
[45] Zhang M, Chi XM, Qu N and Wang CY. Long
noncoding RNA IncARSR promotes hepatic li-
invasion by association with HuR and miR-200
[46] Li YL, Ye Y, Feng BM and Qi Y. Long noncoding
RNA lncARSR promotes sunitinib resistance in
renal cancer and regulates YAP activity promotes
tumor growth and tumor angiogenesis via modu-
lating miR-326, is over-expressed and predicts sur-
vival in glioblastoma. Biochem Cell Biol 2018;
[47] Chen C, Chen J, He L and Stiles B. PTEN: tumor
suppressor and metabolic regulator. Front
Endocrinol (Lausanne) 2018; 9: E93-E96.
[48] Liu J, Xing Y and Rong L. miR-181 regulates
expression of airway smooth muscle cells by
improving miR-150 expression to inhibit the
proliferation and migration of airway smooth mus-
cle cells in asthma via upregulating the expres-
sion of transient receptor potential 1. Am J
Transl Res 2016; 8: 3409-3418.
MS, Darwish MK, Abdel-Daim MM, Toraiha EA.
Long non-coding RNA H19, and not microRNA
miR-326, is over-expressed and predicts sur-
vival in glioblastoma. Biochem Cell Biol 2018;
[50] Zhu HX, Li XC, Song YR, Zhang P, Xiao YJ and
Xing YF. Long non-coding RNA ANRIL is up-reg-
ulated in bladder cancer and regulates bladder
cancer cell proliferation and apoptosis through the
intrinsic pathway. Biochem Biophys Res Comm-
unn 2015; 467: 223-228.
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