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Original Article

LncRNA MNX1-AS1 contributes to lung adenocarcinoma progression by targeting the miR-34a/SIRT1 axis

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Abstract: LncRNA MNX1-AS1 is known to be involved in progression of several tumor types. However, few studies have investigated the molecular mechanism of MNX1-AS1 in lung adenocarcinoma (LAC). To explore the function of MNX1-AS1 in the pathogenesis of LAC, qRT-PCR was performed to show MNX1-AS1 expression. MNX1-AS1 expression in LAC cells was suppressed by siRNA to detect the biologic behavior. The relationships among miR-34a, MNX1-AS1 and SIRT1 were confirmed by pull-down and dual-luciferase reporter assay. Whether MNX1-AS1 was involved in LAC by targeting miR-34a/SIRT1 axis was verified. MNX1-AS1 was up-regulated in LAC, and over-expression of MNX1-AS1 was significantly associated with lymph node metastasis and poor prognosis. In A549 and H1299 cells, cell proliferation, migration, and invasion were suppressed, the cell cycle was regulated, as well as apoptosis was increased after silencing MNX1-AS1. Mechanistically, MNX1-AS1 served as a ceRNA of miR-34a to down-regulate miR-34a expression. SIRT1 is targeted by miR-34a and its expression is regulated by MNX1-AS1 and miR-34a. Up-regulation of SIRT1 salvaged the effect of silencing MNX1-AS1 on A549 and H1299 cells, to some extent. These results showed that MNX1-AS1 contributes to LAC progression by targeting the miR-34a/SIRT1 axis.

Keywords: Lung adenocarcinoma, MNX1-AS1, miR-34a, SIRT1

Introduction

Lung cancer has the highest incidence and mortality in the world, with 1.8 million deaths estimated in Global Cancer Statistics 2018 [1]. 85% lung cancer cases are diagnosed as non-small cell lung cancer (NSCLC) [2]. Lung adenocarcinoma (LAC) is a predominant pathologic type of NSCLC, accounting for nearly 50% [3]. Although therapeutic strategies have made advances, the 5-year survival rate of lung cancer remains poor [4]. Thus, to understand the molecular mechanisms is crucial to explore effective therapeutic targets, and improve the prognosis.

LncRNA is a member of the non-coding RNA family, and the length is >200 nucleotides. Aberrant expression of many lncRNAs plays a crucial role in cancers [5-7]. Emerging evidence reveals that lncRNA can serve as a proto-oncogene or tumor suppressor gene to regulate the growth, survival and metastasis of various cancers. LncRNA DANCR plays a critical role in gastric or cervical cancer progression, and can enhance cancer cell proliferation, migration, and invasion [8, 9]. DGCR5 targets the miR-211-5p/EPHB6 axis to restrain cell growth and invasion in NSCLC [10]. LncRNA MNX1-AS1, known as MNX1 antisense RNA1, is located in the cytoplasm and has been shown to participate in the progression of ovarian cancer [11], breast cancer [12], and other cancers [13]. For example, MNX1-AS1 promotes the aggressiveness of hepatocellular carcinoma (HCC) cells by mediating miR-218-5p, and downstream copper metabolizes the MURR1 domain 8 [14]. MNX1-AS1 is over-expressed in NSCLC according to a previous study, and MNX1-AS1 could enhance the cell growth, migration and invasion [15]. However, the function of MNX1-AS1 in the molecular pathogenesis of LAC remained unknown.

In this study, MNX1-AS1 expression in LAC tissues was detected and its correlation with the

Table 1. Characteristics of LAC patients [n (%)]

Gender	Male	74 (62.71)
	Female	44 (37.29)
Age (years, $\bar{x}\pm s$)		63.28±9.08
Smoke	Yes	55 (46.61)
	No	63 (53.39)
Operation mode	Thoracoscope	64 (54.24)
	Open	54 (45.76)
Tumor size	<3 cm	60 (50.85)
	≥3 cm	58 (49.15)
Differentiation grade	Poor	52 (44.07)
	Moderate	34 (28.81)
	Well	32 (27.12)
TNM stage	I+II	37 (31.36)
	III+IV	81 (68.64)
Lymph node metastasis	N0	57 (48.31)
	N1	61 (51.69)
Adjuvant chemotherapy	Yes	61 (51.69)
	No	57 (48.31)
Outcomes	Death	70 (59.32)
	Survival	48 (40.68)

clinical pathologic features and prognosis was analyzed. The function of MNX1-AS1 in LAC cells was explored, and the mechanism whereby MNX1-AS1 is involved in LAC progression was investigated through targeting the miR-34a/SIRT1 axis. This might be a novel signaling pathway for LAC progression and a novel therapeutic target.

Materials and methods

Clinical tissues samples and data collection

Data of 118 LAC patients who visited the No. 988 Hospital of PLA Joint Logistic Support Force from Jan, 2015 to Dec, 2018 were collected. The LAC tissues and the paired adjacent (≥1 cm) tissues were obtained during the resection and deposited into liquid nitrogen. All patients were diagnosed by imaging and pathology, and without receiving chemotherapy or radiotherapy treatment before surgery. All patients signed informed consent and received regular follow-up, up to Jun, 2020. Survival time refers to the time from the operation to death. The clinicopathologic and follow-up data were statistically analyzed (**Table 1** and Supplementary Data 1). This research was approved by the Ethics Committee of No. 988 Hospital of PLA Joint Logistic Support Force (No. 988YY20042LLSP).

Prediction of MNX1-AS1 expression in LAC

MNX1-AS1 expression in tumor and normal tissue, and the association between MNX1-AS1 and overall survival of LAC, were analyzed based on data obtained from the Cancer Genome Altas (TCGA) database.

Cell culture and transfection

A549, H1299, H1650, and HCC827 cells (human LAC cell lines) and BEAS-2B cells were purchased from the Beina Chuanglian Biotechnology Research Institute (Beijing, China). Two small interfering RNAs (siRNA) of MNX1-AS1 (Sequences: 5'-GAACAACGCAGACAACAUA-3', or 5'-CUGCCUGCAUGCUUUACCA-3'), the corresponding scramble siRNA (si-NC), pcDNA™ 3.1, and pcDNA3.1-SIRT1 overexpression vector were constructed by Shanghai GenePharma Co., Ltd.

All cells were placed in RPMI-1640 medium (Thermo Fisher, USA) with 10% FBS and 1% streptomycin-penicillin (Gibco, USA) to culture in an incubator at 37°C and 5% CO_2. The transfection was completed following the instructions for Lipofectamine®RNAiMAX Reagent (Thermo Fisher, USA).

Real-time fluorescence quantitative PCR assays (qRT-PCR)

Total RNA was extracted by TRIzol reagent (Thermo Fisher, USA), and was reverse transcribed into cDNA using a PrimeScript™ RT reagent Kit (Takara, Japan). qRT-PCR assay was conducted to detect the levels of MNX1-AS1, miR-34a, and SIRT by a 7900HT Fast Real-Time PCR System (Applied Biosystems, USA) using cDNA as a template. Fast SYBR Green Master Mix with GAPDH or U6 as a reference gene, was used. The relative mRNA levels were calculated by using the $2^{-\Delta\Delta Ct}$ method.

CCK-8 assay

Each group of cells was incubated in 96-well plates with 5×10^3/well and three replicate wells. After being grown for 24, 48, or 72 h, 10 μl/well CCK-8 solution was diluted and added in, continuing incubation for 4 h. The optical

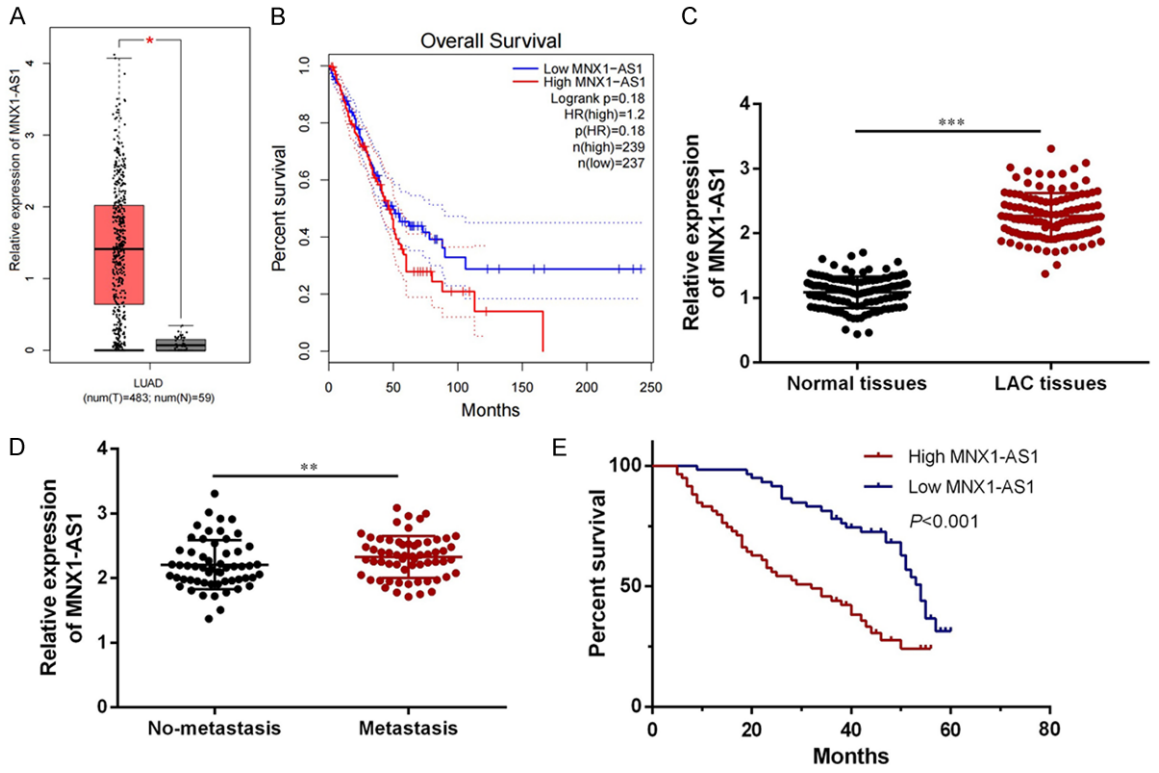

Figure 1. MNX1-AS1 was up-regulated in LAC tissues. A: MNX1-AS1 expression in the tumor tissue was higher than that in the normal tissues shown by the TCGA data. B: Kaplan-Meier survival plots showed that there was no correlation between MNX1-AS1 and prognosis according to TCGA data. C: MNX1-AS1 expressions in LAC tissues and adjacent normal tissues were detected by qRT-PCR. D: qRT-PCR was employed to detect MNX1-AS1 expression in LAC patients with lymph node metastasis compared to no lymph node metastasis. E: Kaplan-Meier curve analysis of the association between MNX1-AS1 expression and overall survival in LAC patients. *P<0.05, **P<0.01 and ***P<0.001.

Table 2. Associations between clinicapathological data and MNX1-AS1 in LAC patients

		MNX1-AS1 expression		t/χ^2	P
		High (n=59)	Low (n=59)		
Gender	Male	36 (61.02)	38 (64.41)	0.145	0.703
	Female	23 (38.98)	21 (35.59)		
Age (years, $\bar{x}\pm s$)		63.49±9.41	63.07±8.83	0.252	0.801
Smoke	Yes	31 (52.54)	24 (40.68)	1.669	0.196
	No	28 (47.46)	35 (59.32)		
Operation mode	Thoracoscope	31 (52.54)	33 (55.93)	0.137	0.712
	Open	28 (47.46)	26 (44.07)		
Tumor size	<3 cm	32 (54.24)	28 (47.46)	0.543	0.461
	≥3 cm	27 (45.76)	31 (52.54)		
Differentiation grade	Poor	28 (47.46)	24 (40.68)	0.550	0.759
	Moderate	16 (27.12)	18 (30.51)		
	Well	15 (25.42)	17 (28.81)		
TNM stage	I+II	16 (27.12)	21 (35.59)	0.984	0.321
	III+IV	43 (72.88)	38 (64.41)		
Lymph node metastasis	No	19 (32.20)	38 (64.41)	12.251	<0.001
	Yes	40 (67.80)	21 (35.59)		
Adjuvant chemotherapy	Yes	33 (55.93)	28 (47.46)	0.848	0.357
	No	26 (44.07)	31 (52.54)		

Table 3. Multivariate Cox regression analysis of prognostic factors for LAC patients

	HR	*95% CI*	P
TNM stage (III+IV *vs.* I+II)	2.647	1.417-4.948	0.002
MNX1-AS1 (High *vs.* Low)	2.776	1.685-4.573	0.000

HR, hazard ration; 95% CI, 95% confidence interval.

density (OD) at 450 nm on the microplate reader (Thermo Fisher, USA) was measured.

Transwell assay

Cell migration was assayed using Transwell chambers without Matrigel (Corning, USA). For invasion measurement, the Transwell chambers were coated with Matrigel. 1×10^5 cells were seeded in the upper chamber with 1 ml RPMI-1640 with free serum. RPMI-1640 complete medium with 20% FBS was put into the lower chamber. After being cultured for 24 h, the cells attached on the lower chamber were fixed and stained. The stained cells were counted and photographed under a microscope, in five random views for each group.

Flow cytometry assay

For cell cycle: Each group of cells was suspended and adjusted to 1×10^6/ml. After being with PBS, the cells were fixed at least for 2 h by 70% ethanol at -20°C. After being washed with PBS for twice, the cells were incubated with 500 μl PI in the darkness for 30 min at room temperature, and analyzed by flow cytometry (FACS Calibur, BD, Shanghai, China), immediately.

For apoptosis: Each group of cells was collected and suspended in Binding buffer, adjusted to 1×10^5/ml. Then the cells were stained with 5 μl Annexin V-APC for 15 min and 10 μl 7-ADD for 5 min at 4°C in the dark, using Annexin V-APC/7-ADD apoptosis kit (Abnova, AmyJet Scientific Inc, Wuhan). The amount of apoptotic cells was detected by flow cytometry.

RNA pull down assay

LAC cells were transfected with biotinylated miR-34a-wild type (bio-miR-34a-wt), biotinylated miR-34a-mutation (bio-miR-34a-mut) or biotinylated-NC (bio-NC) (Guangzhou RiboBio Co., Ltd.), respectively. After 48 h, the transfected cells were treated with lysis buffer. The cell lysate was incubated with M-280 Streptaidin magnetic beads (Invitrogen, USA), according to protocol. After elution, the bound RNAs were purified using Trizol and we measured MNX1-AS1 levels by qRT-PCR.

Dual-luciferase reporter assay

Bioinformatic databases (TargetScan or StarBase V2.0) were utilized to predict the miRNA targeted by MNX1-AS1 and the mRNA targeted by miR-34a.

The wt or mut fragments of MNX1-AS1 or SIRT1 3'-UTR containing the miR-34a binding sites were cloned into the pmiR-GLO (Invitrogen, USA) respectively, named MNX1-AS1-wt, MNX1-AS1-mut, SIRT1-wt and SIRT1-mut. The recombinant pmiR-GLO and miR-34a mimic or miR-NC (Guangdong, RiboBio Co., Ltd.) were co-transfected into 293T cells. 48 h after transfection, the Dual-Luciferase® Reporter (DLR™) Assay system (Promega, USA) was used to caculate the relative luciferase activity.

Western blotting

Proteins were extracted with RIPA lysis buffer with 0.1 mM phenylmethylsulfonyl fluoride (Beyotime), and separated using 10% SDS-PAGE. After being transferred and blocked, the primary antibodies to SIRT1 and β-actin (1:1000, Abcam, Britain) were incubated over night at 4°C. The secondary antibody was added and incubated for 1 h at room temperature after washing for 3 times. Then, chemiluminesence reagent (Cell Signaling Technology, USA) was employed to observe the protein bands. Image J (National Institutes of Health, USA) was used for quantitative analysis, to calculate SIRT1 relative expression, with β-actin as the internal reference.

Statistical analysis

All data were analyzed by SPSS Statistics software (V.19.0, IBM). All data that coincided with a normal distribution were expressed as ($\bar{x}\pm s$). Differences between two groups were analyzed by Student *t*-test or χ^2 test. The differences in MNX1-AS1 in LAC and adjacent tissues were measured by paired *t*-test. The survival of LAC patients in the two groups was compared and

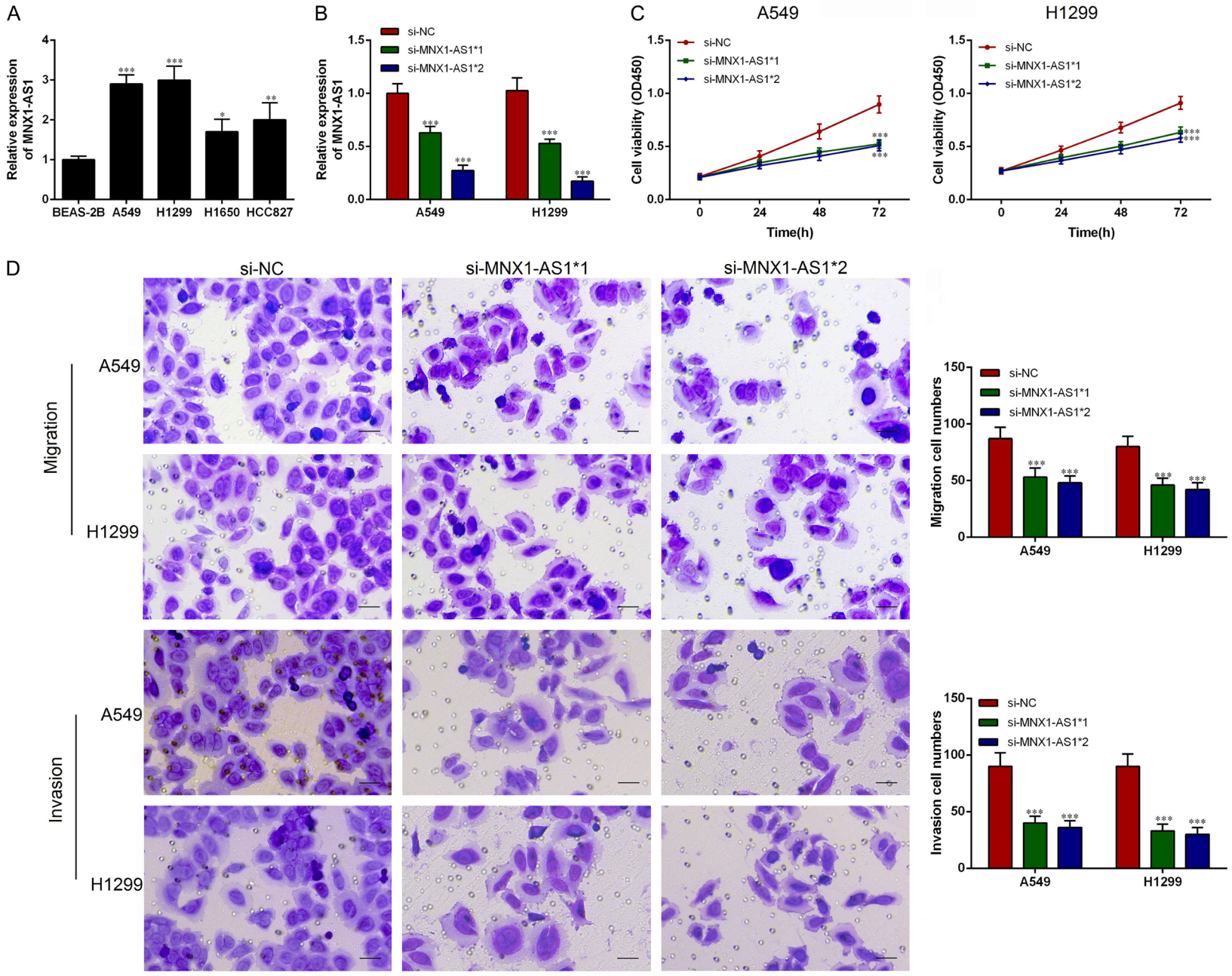
A
Relative expression of MNX1-AS1
BEAS-2B
A549
H1299
H1650
HCC827
B
si-NC
si-MNX1-AS1*1
si-MNX1-AS1*2
Relative expression of MNX1-AS1
A549
H1299
C
A549
H1299
Cell viability (OD450)
Time(h)
D
si-NC
si-MNX1-AS1*1
si-MNX1-AS1*2
Migration
Invasion
A549
H1299
Migration cell numbers
Invasion cell numbers

Figure 2. MNX1-AS1 promoted proliferation, migration, and invasion of A549 and H1299 cells. A: MNX-AS1 expression in LAC cell lines and a normal cell line, BEAS-2B, were analyzed by qRT-PCR. B: MNX1-AS1 expression was estimated by qRT-PCR in A549 and H1299 cells after transfection with two siRNAs targeting MNX1-AS1 (si-MNX1-AS1*1 and si-MNX1-AS1*2). C: The effect of MNX1-AS1 knockdown on the proliferation of A549 and H1299 was analyzed by CCK8 assay. D: Transwell assay was used to detect the effect of MNX1-AS1 knockdown on the migration and invasion of A549 and H1299 cells. Bar =100 μm. $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$.

analyzed by Kaplan-Meier curve and Cox regression. $P<0.05$, was considered significant.

Results

MNX1-AS1 is up-regulated in LAC tissues

The TCGA cohort data showed that MNX1-AS1 expression in tumor tissues was higher than that in normal tissue, with a significant difference (**Figure 1A**). MNX1-AS1 was not associated with overall survival according to Kaplan-Meier plots from the TCGA data (**Figure 1B**). It was also found that MNX1-AS1 was up-regulated in LAC tissues (**Figure 1C**). Low MNX1-AS1 and high MNX1-AS1 groups were divided according to the median (2.23) of MNX1-AS1 expression, to estimate the correlation between MNXS-AS1 and clinicopathologic data (**Table 2**). There was a difference in MXN1-AS1 expression between metastasis and non-metastasis groups, and the MNX1-AS1 expression was elevated in the metastasis group (**Figure 1D**). Furthermore, LAC patients with high MNX1-AS1 had a lower survival rate (**Figure 1E**). MNX1-AS1 was an independent prognostic factor for LAC, and the risk of poor prognosis for LAC patients with high MNX1-AS1 was 2.776 fold higher than for those with low MNX1-AS1, as shown by Cox-regression analysis in **Table 3**. The results suggest that MNX1-AS1 is a prognostic indicator in LAC.

MNX1-AS1 promotes proliferation, migration, and invasion of LAC cells

Similar to the clinical result, MNX1-AS1 was also up-regulated in LAC cells, compared to the BEAS-2B, with the most significance in A549 and H1299 (**Figure 2A**). To explore the effect of MNX1-AS1 on the biologic behavior of LAC cells, two siRNAs against MNX1-AS1 were made to restrain MNX1-AS1 expression in A549 and H1299 cells, respectively (**Figure 2B**). As shown in **Figure 2C**, si-MNX1-AS1 suppressed cell proliferation. si-MNX1-AS1 markedly suppressed migration and invasion, both in A549 and H1299 cells (**Figure 2D**). The results revealed that MNX1-AS1 enhanced the proliferation, migration, and invasion of both A549 and H1299 cells.

MNX1-AS1 regulates the cell cycle, and inhibits apoptosis in LAC cells

The cell cycle was analyzed to verify whether MNX1-AS1 could regulate it to affect cell proliferation or apoptosis. The results showed that si-MNX1-AS1 blocked the cells in the G1 phase (**Figure 3A**). si-MNX1-AS1 promoted cell apoptosis, in both A549 and H1299 cells (**Figure 3B**). Thus, it was concluded that MNX1-AS1 regulates the cell cycle and inhibits apoptosis in A549 and H1299 cells.

MNX1-AS1 functions as a miRNA sponge for miR-34a

A growing body of research suggests that lncRNA, as a miRNA sponge regulates miRNA expression and biological functions [16, 17]. miR-34a expression was lower in LAC tissues than in normal tissues, and was negatively correlated with MNX1-AS1, shown in **Figure 4A**, **4B**. The bioinformatic databases predicted that miR-34a was targeted by MNX1-AS1, and the binding site is shown in **Figure 4C**. The luciferase activity of MNX1-AS1-wt was inhibited by a miR-34a mimic (**Figure 4D**). Compared to the bio-miR-34a-mut and bio-NC, MNX1-AS1 expression in bio-miR-34a-wt was increased, suggesting that bio-miR-34a-wt directly interacts with miR-34a (**Figure 4E**). Moreover, miR-34a expression was up-regulated by si-MNX1-AS1 in A549 and H1299 cells (**Figure 4F**). The above data suggest that miR-34a is targeted and regulated by MNX1-AS1 in A549 and H1299 cells.

SIRT1 is targeted by miR-34a

Early existing evidence confirms that SIRT1 is a target of miR-34a in cancer [18, 19]. There is a binding site for miR-34a on the SIRT1 3'-UTR (**Figure 5A**). **Figure 5B** shows that miR-34a mimic inhibited luciferase activity in SIR-

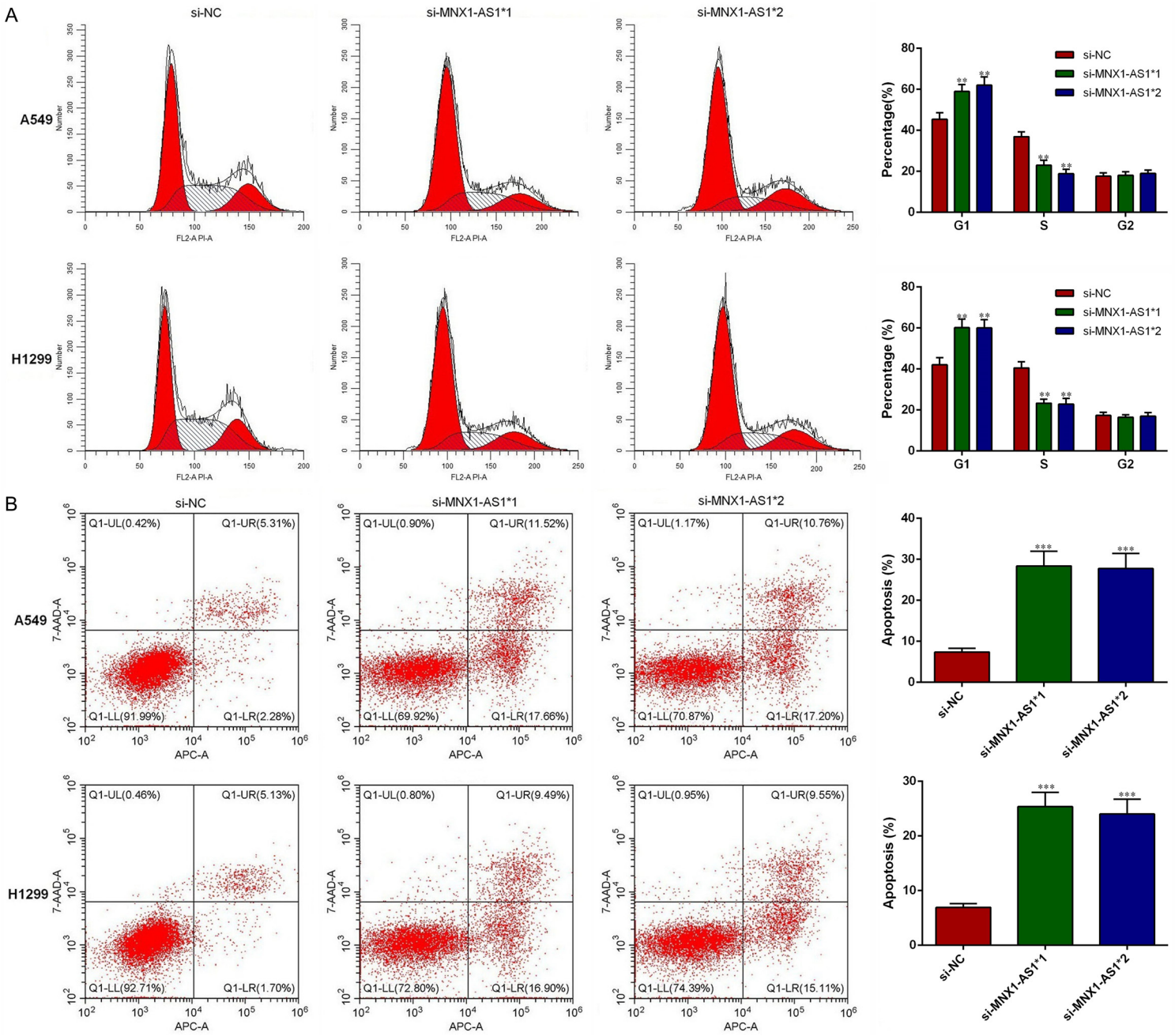
A
si-NC
si-MNX1-AS1*1
si-MNX1-AS1*2
A549
H1299
Number
FL2-A PI-A
Percentage(%)
Percentage (%)
G1
S
G2
B
Q1-UL(0.42%)
Q1-UR(5.31%)
Q1-LL(91.99%)
Q1-LR(2.28%)
Q1-UL(0.90%)
Q1-UR(11.52%)
Q1-LL(69.92%)
Q1-LR(17.66%)
Q1-UL(1.17%)
Q1-UR(10.76%)
Q1-LL(70.87%)
Q1-LR(17.20%)
Q1-UL(0.46%)
Q1-UR(5.13%)
Q1-LL(92.71%)
Q1-LR(1.70%)
Q1-UL(0.80%)
Q1-UR(9.49%)
Q1-LL(72.80%)
Q1-LR(16.90%)
Q1-UL(0.95%)
Q1-UR(9.55%)
Q1-LL(74.39%)
Q1-LR(15.11%)
7-AAD-A
APC-A
Apoptosis (%)

Figure 3. MNX1-AS1 regulates the cell cycle and inhibits apoptosis of A549 and H1299 cells. A: Flow cytometry assay was performed to analyze that the effect of MNX1-AS1 knockdown on the cell cycle of A549 and H1299 cells. B: Flow cytometry assay was employed to detect the effect of MNX1-AS1 knockdown on the apoptosis of A549 and H1299 cells. **P<0.01 and ***P<0.001.

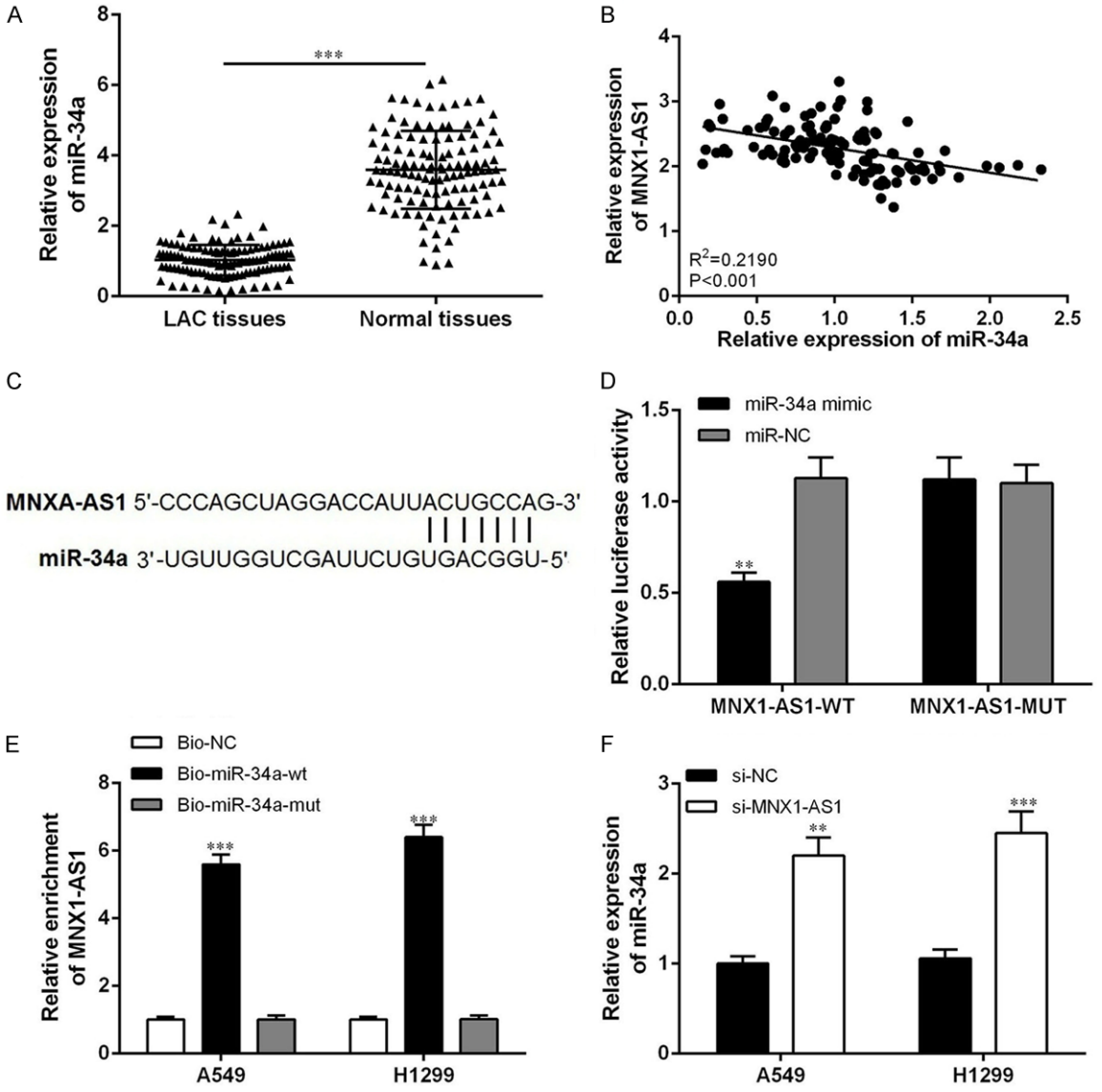

Figure 4. MNX1-AS1 functions as a sponge of miR-34a. A: miR-34a expression in the LAC tissues and adjacent normal tissues were analyzed by qRT-PCR. B: Negative correlation between MNX1-AS1 and miR-34a in LAC tissues was measured by Pearson analysis. C: Potential binding sites for miR-34a on MNX1-AS1 were predicted by bioinformatics. D: Luciferase reporter assay was performed to verify the predicted binding sites between MNX1-AS1 and miR-34a. E: RNA pulldown assay was employed to detect the direct interaction between MNX1-AS1 and miR-34a in A549 and H11299 cells. F: qRT-PCR was used to detect miR-34a expression in A549 and H1299 cells after transfection with si-MNX1-AS1 or si-NC. **P<0.01 and ***P<0.001.

T1-wt, while SIRT1-mut abolished this effect. Meanwhile, si-MNX1-AS1 and miR-34a mimic suppressed SIRT1 expression, in both A549 and H1299 cells (**Figure 5C-F**). Thus, SIRT1 is a target gene of miR-34a and is regulated by miR-34a and MNX1-AS1.

MNX1-AS1 contributes to LAC progression by mediating the miR-34a/SIRT1 axis

SIRT1 expression was restored in A549 and H1299 cells co-transfected with si-MNX1-AS1 and oe-SIRT1 in **Figure 6A**, **6B**. Up-regulation of

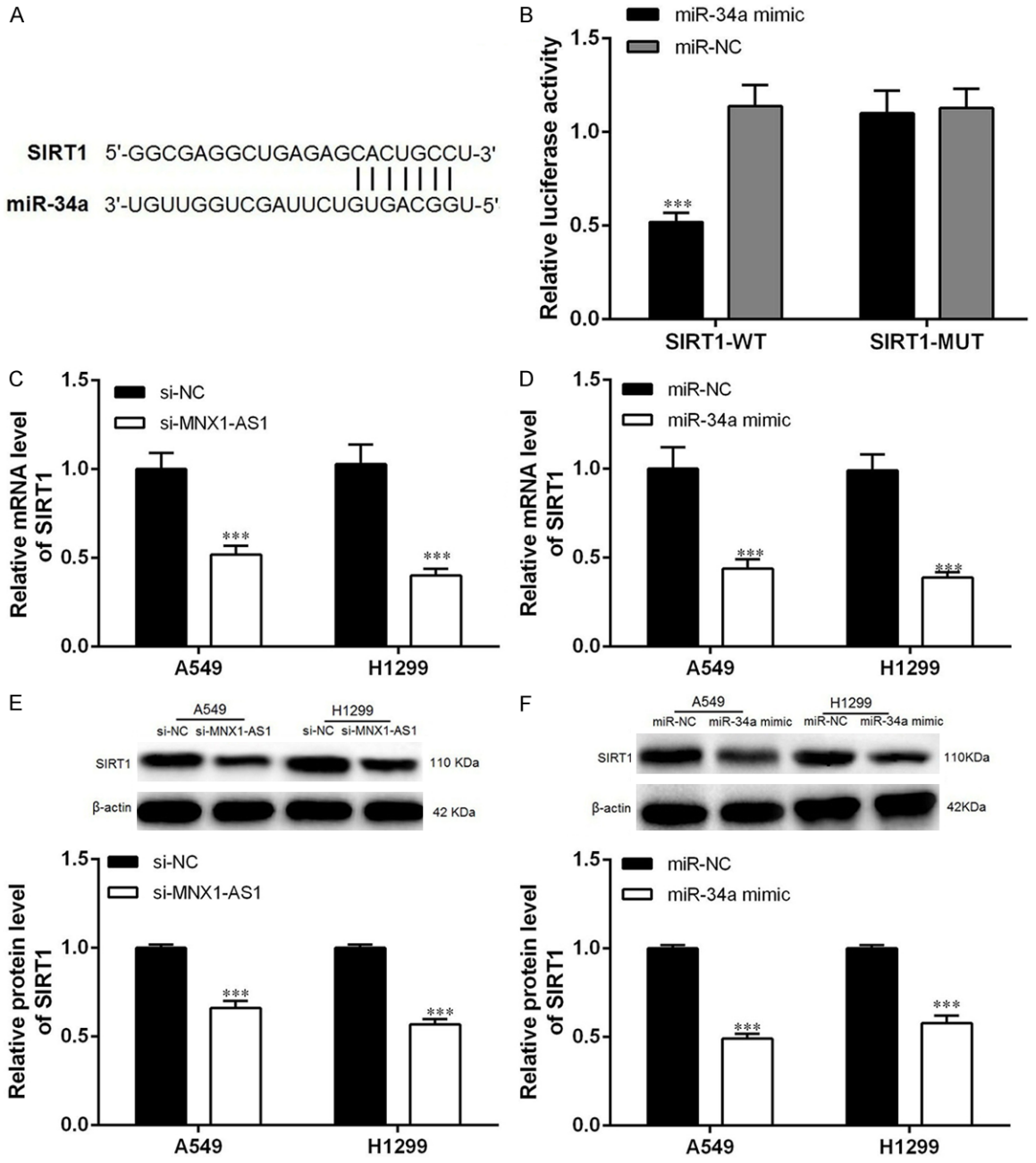

Figure 5. SIRT1 was a target gene of miR-34a. A: Potential binding sites for miR-34a on SIRT1 3'-UTR were predicted by bioinformatics. B: Luciferase reporter assay was performed to verify the predicted binding sites between miR-34a and SIRT1. C: SIRT1 mRNA expression in A549 and H1299 cells were analyzed by qRT-PCR after transfection with si-MNX1-AS1 or si-NC. D: SIRT1 mRNA expression in A549 and H1299 cells were analyzed by qRT-PCR after transfection with miR-34a mimic or miR-NC. E: SIRT1 protein expression in A549 and H1299 cells were analyzed by western blotting after transfection with si-MNX1-AS1 or si-NC. F: SIRT1 protein expression in A549 and H1299 cells were analyzed by western blotting after transfection with miR-34a mimic or miR-NC. ***P<0.001.

SIRT1 salvaged the proliferation, migration, invasion, and apoptosis of MNX1-AS1-silenced cells (**Figure 6C-E**). These data suggest that MNX1-AS1 contributes to LAC progression by up-regulating SIRT1 by inhibiting miR-34a.

Discussion

Recent research showed that lncRNA represents a crucial position in the development and progression of LAC. High expression of lncRNA

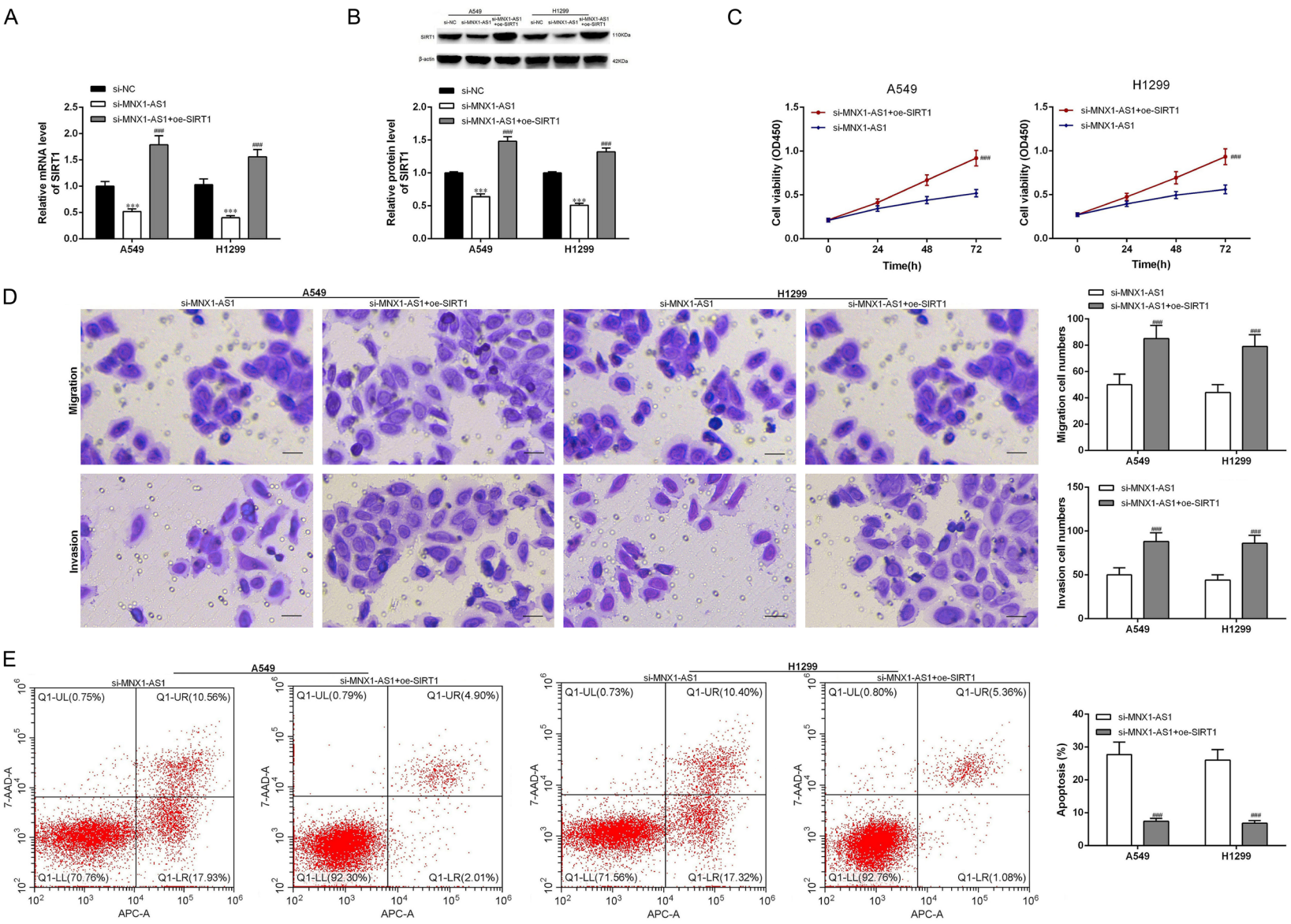

Figure 6. Overexpression of SIRT1 rescued the effect of MNX1-AS1 silencing in LAC cells. A: SIRT1 mRNA expression was analyzed by qRT-PCR in A549 and H1299 cells transfection with si-MNX1-AS1 and pcDNA3.1-SIRT1. B: Western blotting was used to analyze SIRT1 protein expression in A549 and H1299 cells transfection

with si-MNX1-AS1 and pcDNA3.1-SIRT1. C: CCK8 assay was used to detect the effect of SIRT1 on proliferation in MNX1-AS1-silenced A549 and H1299 cells. D: Transwell assay was used to detect the effect of SIRT1 on the migration and invasion analysis in MNX1-ASA1 silenced A549 and H1299 cells. Bar =100 μm. E: The effect of SIRT1 on the apoptosis of MNX1-ASA1 silenced A549 and H1299 cells were analyzed by flow cytometry assay. ***P<0.001 *vs.* si-NC, and $^{###}$P<0.001 *vs.* si-MNX1-AS1.

MIR31HG was found in LAC tissues, and it was associated with clinical staging, degree of differentiation and unfavorable prognosis. Knockdown of MIR31HG inhibited proliferation and blocked the cell cycle in LAC cells [20]. LncRNA DANCR was also reported to be up-regulated in LAC, and DANCR, as an oncogenic lncRNA, promoted tumor cell viability and metastasis, and inhibited cell apoptosis by directly targeting miR-496 [21]. Our previous study found that MNX1-AS1 was overexpressed and promoted the malignancy of tumor cells in NSCLC [15]. Herein, the results also demonstrated that MNX1-AS1 was up-regulated and associated with lymph node metastasis and poor prognosis in LAC, which was not consistent with the TCGA data. In *vitro*, si-MNX1-AS1 suppressed the cell proliferation, migration and invasion, regulated the cell cycle, and promoted apoptosis in LAC cells. Many researchers have verified that MNX1-AS1 is a dormant oncogene in various cancers. In cervical cancer, MNX1-AS1 was up-regulated, and promoted cell survival and viability and suppressed apoptosis through activating the MAPK signaling pathway [22]. In breast cancer, MNX1-AS1 was also up-regulated and activated the Akt-mTOR pathway to promote cell viability, and induce migration and invasion [23]. Lv et al. [24] indicated that MNX1-AS1 was over-expressed in ovarian cancer patients, and silencing its expression could suppress proliferation, migration, block the cell cycle at G0/G1 phase, and enhance apoptosis by regulating CDK4, cyclin D, Bax and Bcl-2. Gao et al. [25] also indicated that MNX1-AS1 enhanced giloblastoma cell proliferation and stimulated migration and invasion by inhibiting miR-4443. Yet the function of MNX1-AS1 in the molecular pathogenesis of LAC remained largely unclear.

Many studies have demonstrated that lncRNAs regulate cancer progression, as competing endogenous RNA (ceRNA) [26, 27]. MNX1-AS1 interacts with miR-218-5p to promote SEC61A1 or COMMD8 expression to enhance the progression of colonic adenocarcinoma or hepatocellular carcinoma had been reported [14, 28]. In lung cancer, MNX1-AS1, as an oncogene, competes with miR-527 to activate BRF2 [29]. In the present study, miR-34a was down-regulated, and negatively correlated with MNX1-AS1 in LAC. Furthermore, MNX1-AS1 was shown to be a ceRNA of miR-34a and negatively regulated miR-34a expression in LAC cells. Sirtuin1 (SIRT1), a NAD^+-dependent deacetylase, had been confirmed to be involved in cell proliferation, autophagy, and apoptosis, affecting cancer development and progression [30]. Recent data suggest that SIRT1 is a target of miR-34a, and miR-34a negatively targeted SIRT1 and inhibited cell proliferation, in tumor progression [31, 32]. In this study, SIRT1 was targeted by miR-34a, and regulated by miR-34a and MNX1-AS1 in LAC cells. Up-regulation of SIRT1 salvaged the influence of silencing MNX1-AS1 on the proliferation, migration, invasion, and apoptosis of LAC cells. Thus, it was suggested that MNX1-AS1 may promote LAC progression by targeting the miR-34a/SIRT1 axis.

Conclusion

MNX1-AS1 promotes LAC progression by targeting the miR-34a/SIRT1 axis, which may be a new mechanism of MNX1-AS1 involvement in LAC. The results provide a novel biomarker or therapeutic target for LAC. However, there are some limitations: First, the association of MNX1-AS1 with prognosis needs more investigation. Second, the function of MNX1-AS1 on lung cancer growth in *vivo* should be further verified by xenograft experiments. Whether MNX1-AS1 participates in LAC progression by targeting other target genes also remains to be further studied.

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Disclosure of conflict of interest

None.

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