Biomedicine & Pharmacotherapy 116 (2019) 109029

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

LncRNA MNX1-AS1 promotes progression of esophageal squamous cell carcinoma by regulating miR-34a/SIRT1 axis

Jie Chu[a,1], Hongle Li[b,1], Yurong Xing[c], Jinlin Jia[a], Jinxiu Sheng[a], Lijun Yang[a], Kaiyan Sun[a], Yunhui Qu[a], Yan Zhang[a], Huiqing Yin[a], Junhu Wan[a,*], Fucheng He[a,*]

[a] Department of Medical Laboratory, The First Affiliated Hospital, Zhengzhou University, Zhengzhou, Henan Province, China
[b] Department of Molecular Pathology, The Affiliated Cancer Hospital, Zhengzhou University, Zhengzhou, Henan Province, China
[c] Department of Physical Examination, The First Affiliated Hospital, Zhengzhou University, Zhengzhou, Henan Province, China

ARTICLE INFO

Keywords:
Esophageal squamous cell carcinoma
Long non-coding RNA
MNX1-AS1
MiR-34a
SIRT1

ABSTRACT

Background: Long non-coding RNAs (lncRNAs) are powerful factors influencing the tumorigenesis and metastasis of multiple carcinomas. LncRNA MNX1-AS1 plays critical roles in the progression of tumor formation according to recent research, while its roles in esophageal squamous cell carcinoma (ESCC) remains unknown.
Methods: The expression levels of lncRNA MNX1-AS1 were examined in ESCC tissues by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR). The role of lncRNA MNX1-AS1 was performed by WST-1 proliferation assays, migration and invasion assays. Besides, the molecular mechanism of lncRNA MNX1-AS1 was verified by online bioinformatics, qRT-PCR and rescue assays.
Results: MNX1-AS1 was signifcantly upregulated in ESCC tissues. It was conformed that high MNX1-AS1 expression was associated with ESCC lymph node metastasis. Moreover, we found that knockdown of MNX1-AS1 apparently suppressed the cell proliferation, migration, and invasion capacity. Flow cytometry analysis showed MNX1-AS1 regulated ESCC cell cycle and apoptosis progression. Mechanism analysis revealed that miR-34a inhibitor could rescue the influence of inhibiting MNX1-AS1 on ESCC cells migration by serving as competing endogenous RNA (ceRNAs). Furthermore, we found that miR-34a specifically targeted SIRTI.
Conclusions: Taken together, we demonstrated that lncRNA MNX1-AS1/miR-34a/SIRT1 regulatory axis could play an important role in ESCC progression, and MNX1-AS1 may act as a novel potential biomarker for esophageal squamous cell carcinoma.

1. Introduction

Esophageal cancer (EC) is one of the most serious cancers, leading to cancer-related death all over the world [1,2]. Esophageal cancer contains two major cancer types, esophageal squamous cell carcinoma (ESCC) and esophageal adeno carcinoma (EAC) [3]. Patients diagnosed at advanced-stage ESCC, often have distant metastasis and drug resistance, causing poor 5-year survival rates [4,5]. Therefore, developing a diagnostic biomarker for early detection of ESCC will improve clinical diagnosis and treatment.

Long noncoding RNAs (lncRNAs) are a subgroup of RNA molecules which are longer than 200 nucleotides without protein-coding ability [6,7]. Recently, numerious lncRNAs have been identified crucial in various cancers, such as breast cancer, lung cancer and gastric cancer [8–10]. Accumulating evidence reveals that lncRNAs are often dysregulated expression leading to carcinoma's progression and metastasis through diverse mechanisms, such as epigenetic modifications, transcriptional interference and transcriptional activation [11–13]. Therefore, lncRNAs may serve as tumor suppressors or oncogenes to regulate the developmen of multiple cancers. For example, lncRNA DANCR was found to be upregulated in non-small cell lung cancer (NSCLC), and it could facilitate malignancy through a positive feedback loop of DANCR/miR-138/Sox4 in NSCLC [14]. In addition, lncRNA XIAP-AS1 was significantly increased in colorectal cancer (CRC). Besides, knockdown XIAP-AS1 evidently suppressed CRC cell proliferation and arrested the cell cycle by regulating the expression of EMT markers [15].

* Corresponding authors.
E-mail addresses: 504017950@qq.com (J. Chu), llhl73@163.com (H. Li), 405274775@qq.com (Y. Xing), 2632943581@qq.com (J. Jia), 18537732817@163.com (J. Sheng), 18638949686@163.com (L. Yang), 1348580389@qq.com (K. Sun), zdqyh09@126.com (Y. Qu), 252531992@qq.com (Y. Zhang), yinhuiqing12@126.com (H. Yin), wanjh2009@163.com (J. Wan), hefucheng@zzu.edu.cn (F. He).
[1] Contributed equally.

https://doi.org/10.1016/j.biopha.2019.109029
Received 10 April 2019; Received in revised form 21 May 2019; Accepted 21 May 2019

MNX1-AS1, known as Motor neuron and pancreas homeobox 1-antisense RNA1, is located at chromosome 7q36.3 and is 992 bp long [16]. The lncRNA MNX1-AS1 was reported to be upregulated in colon adenocarcinoma, and MNX1-AS1 could facilitate colon adenocarcinoma progression through regulating miR-218-5p/SEC61A1 axis [17]. In addition, lncRNA MNX1-AS1 was found to induce epithelial–mesenchymal transition through activating AKT/mTOR pathway in breast cancer [18]. However, the biological roles of MNX1-AS1 in ESCC has not been studied.

In our study, we demonstrated that MNX1-AS1 is upregulated in ESCC, and high expression of MNX1-AS1 is associated with ESCC lymph node metastasis. Moreover, we confirmed that MNX1-AS1 promotes the cell proliferation, migration, and invasion capacity, and MNX1-AS1 reguletes ESCC cell cycle and apoptosis progression. Besides, MNX1-AS1 promotes progression of esophageal squamous cell carcinoma by regulating miR-34a/SIRT1 axis.

2. Materials and methods

2.1. Clinical specimens and data collection

Totally 45 pairs of fresh frozen ESCC tissues and corresponding adjacent non-tumorous tissues were obtained from the First Affiliated Hospital of Zhengzhou University between April 2016 and May 2017. All of patients didn't receive chemotherapy or radiotherapy before surgery and all clinical specimens were diagnosed by the department of pathology. Furthermore, every sample immediately stored at −80 °C after taken from surgery operation, and the clinical characteristics of all patients were inquired from the patients' electronic medical records. Written informed consents were received from all patients before implementation, and this study was approved by the Ethics Committee of the First affiliated hospital of Zhengzhou University.

2.2. Cell lines and culture

ESCC cell lines, KYSE30, KYSE150 were purchased from Shanghai Institute of Life Sciences cell bank center (Shanghai, China), and were maintained in Roswell Park Memorial Institute-1640 medium (RPMI-1640, Hyclone, UT, USA) supplemented with 10% fetal bovine serum (FBS), and 1% penicillin-streptomycin. All cell lines were cultured in an atmosphere at 37 °C with 5% CO2.

2.3. RNA extraction and quantitative RT-PCR

Total RNA was isolated from tissues and cell lines using TriZol reagent (TakaRa, Dalian, China), and then were reverse-transcribed reaction using the PrimeScript™RT reagent Kit with gDNA Eraser (TakaRa, Dalian, China). Quantitative RT-PCR was performed by the kit specification of SYBR® Premix Ex Taq™ II (TakaRa, Dalian, China) on LightCycler 480 II Real-Time PCR System (Roche, Basel, Switzerland). The primers for MNX1-AS1, were designed as follows: MNX1-AS1 forward prime: 5′-GCTCTGCAGGTCGAACCTTA-3′, reverse: 5′- CCCGCAG GCTAGTGTCTATC -3′; miR-34a forward prime: 5′- CGCGTGGCAGTG TCTTAGCT-3′; reverse: 5′-

AGTGCAGGGTCCGAGGTATT-3′; miR-34a RT primer: 5′- GTCGTA TCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACACAACC-3′; SIRT1 forward prime: 5′-TGCTGGCCTAATAGAGTGGCA-3′; reverse: 5′-CTCAGCGCCATGGAAAATGT-3′. Relative expression levels of mRNA or miRNA were normalized to GAPDH gene or U6 gene.

2.4. Cell transfection

Small interfering RNA targeting MNX1-AS1 (siMNX1-AS1), scramble siRNA of MNX1-AS1 (siRNA control) were constructed by RiboBio Co., Ltd (Guangzhou, China), and transfected inhibitor using RFect sRNA Transfection Reagent (Changzhou Biogenerating Biotechnologies Co.,Ltd). The sequences of siRNAs used in this study as follows: si-MNX1-AS1#1: 5′-GAACAACGCAGACAACATA-3′; si-MNX1-AS1#2: 5′-CTGCCTGCATGCTTTACCA-3′.The ESCC cell lines were cultured in 6-well plates with 5 μl siRNA, and the concentration of siRNAs transfected was 50 nM. Then cells were collected and used for further experiments after transfection 48 h. Inhibiting miR-34a was transfected of miR-34a inhibitor (RiboBio, Guangzhou, China). MicroRNA inhibitor imitates the absence of microRNAs, and inhibitor is a complementary chain of microRNAs that competes with microRNAs through a single chain.

2.5. WST-1 cell proliferation assay

Cell proliferation assays were performed by WST-1 Assay Kit (Beyotime Biotechnology, Shanghai, China) as described before [19,20]. Primarily ESCC cells transfected with siRNA were seeded into 96-well plates at a density of 2000 cells per well. Then 10 μl WST-1 reagent (Roche) was added into relevant well after culturing 24, 48, 72, 96 h. After a continuous incubation for 2 h at 37 °C, the absorbance was measured at 450 nm by using Molecular Devices (SpectraMax M5, America).

2.6. Cell migration and invasion assays

For migration and invasion, 24-well chambers (Corning, NY, USA) with or without Matrigel was used to perform the ability of cell migration and invasion. Transfected cells (1 × 105 cells/ml) treated with mitomycin C (Genview, Florida, USA) in 200 μl of serum-free 1640 media were added into the upper chamber and meanwhile 650ul medium containing 15% FBS was seeded into at the lower chamber. After a further incubation for 24 h at 37 °C, cells in the upper chamber can migrated through the 8-mm pore membranes. Then cells were cleared away at the upper chambers with a medical cotton swab and were fixed through 4% paraformaldehyde and stained by crystal violet. Thereafter, cell numbers were counted and imaged in five random fields by microscope.

2.7. Flow cytometry assay

For cell cycle analysis, transfected cells were trypsinized and the cell suspension were collected containing 1×10^6 cells as described before [21]. Then the cells were washed twice by PBS and fixed with 1 ml 70% absolute ethanol at 4 °C overnight, following by washing thrice with PBS. ESCC cells were incubated for at least 30 min with 1 ml of propidium iodide (PI) dyeing liquid (Genview, Florida, USA), and analyzed by flow cytometry (FACScan; BD Biosciences, Shanghai, China). For apoptosis analysis, transfected cells were digested with trypsin without EDTA and the cell suspension were collected and washed twice by PBS. Then adding AnnexinV/PI double staining and measured by flow cytometry according to the manufacturer.

2.8. Statistical analysis

The SPSS 21.0 software (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 5 (GraphPad Software Inc., La Jolla, CA, USA) were conducted data analysis. The data of experiments, which were performed at least three times, were presented as mean ± standard deviation. The differences between groups were analyzed by Student's t-test, Chi-square tests for two groups and one-way analysis of variance (ANOVA) for three or more groups. The survival curves were drawn using $P < 0.05$ was considered statistical significance.

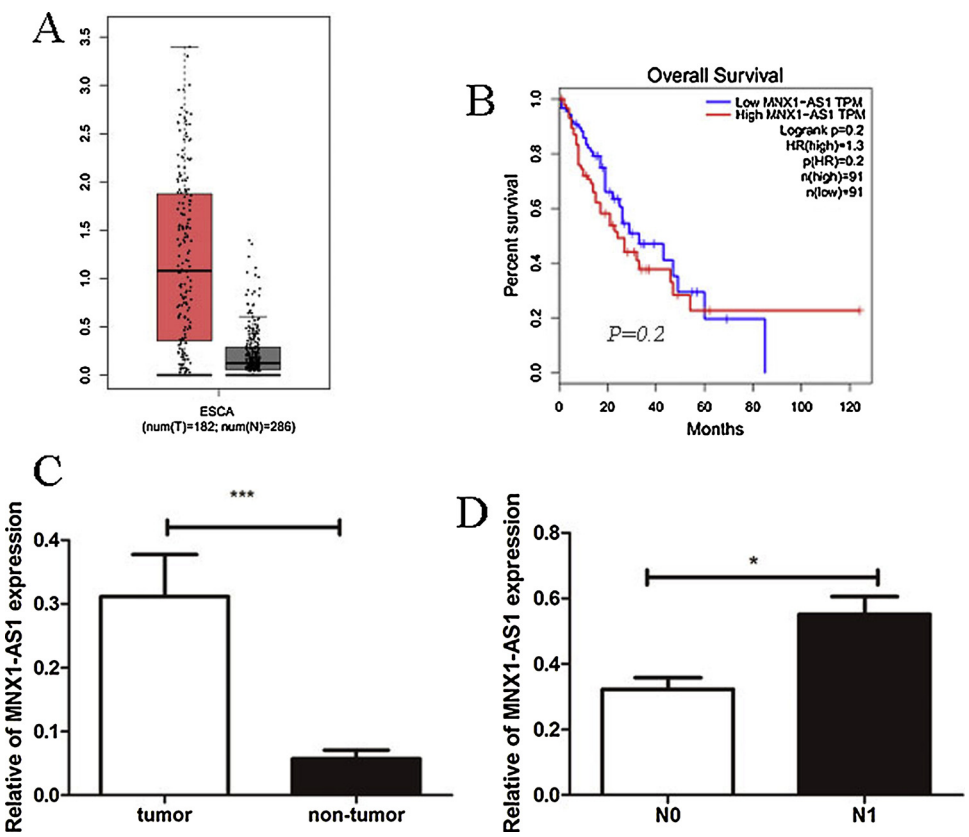

Fig. 1. The expression level of lncRNA MNX1-AS1. (A, B) The expression of MNX1-AS1 in primary esophageal carcinoma (ESCA) compared with normal tissues from GEPIA database, and the Kaplan-Meier plot of MNX1-AS1 expression in ESCA from GEPIA database. (C) The expression of MNX1-AS1 in ESCC tumor tissues compared with corresponding tumor-adjacent tissues. (D) Relative expression of MNX1-AS1 in patients with lymph node metastasis compared with patients without lymph node metastasis. * P < 0.05, ***P < 0.001.

3. Results

3.1. Upregulated expression of MNX1-AS1 was observed in ESCC tissues

To investigate the roles of MNX1-AS1 in ESCC tissues, the expression levels of 45 pairs of ESCC and corresponding tumor-adjacent tissues were detected by qRT-PCR. The result indicated that MNX1-AS1 was significantly upregulated in ESCC tissues compared with the corresponding tumor-adjacent tissues (Fig. 1C). Besides, the web-based tools of Gene Expression Profiling Interactive Analysis (GEPIA, http://gepia.cancer-pku.cn/) was used to analyse MNX1-AS1 expression levels and prognosis of patients. The results showed that MNX1-AS1 was significantly upregulated in many malignant tumor tissues compared to the corresponding tumor-adjacent tissues (Fig. 1A), while the survival analysis showed patients with high MNX1-AS1 expression had similar prognosis compared with patients with low MNX1-AS1 expression (Fig. 1B).

Furthermore, to further estimate the clinicopathological features of MNX1-AS1 level in ESCC patients, the MNX1-AS1 expression level was divided into two groups according to the median. We found that high MNX1-AS1 level was correlated with ESCC lymph node metastasis (Table1), and the expression level of MNX1-AS1 in metastasis group was apparently increased compared with that in non-metastasis group (Fig. 1D).

Table 1
The correlation between MNX1-AS1 expression and clinicopathological factors of ESCC patients.

Variable		N	MNX1-AS1 expression		P-value
			High(23)	Low(22)	
Gender	Male	23	13	10	0.5559
	Female	22	10	12	
Age	< 65years	19	11	8	0.5499
	≥ 65years	26	12	14	
Tumor size	≤ 3cm	19	7	12	0.1362
	> 3cm	26	16	10	
Differentiation grade	Poor	12	6	6	1.000
	Well/ moderate	33	16	17	
TNM stage	I + II	13	7	6	0.3368
	III + IV	32	16	16	
Lymphatic metastasis	N0	23	4	19	< 0.0001
	N1	**22**	**19**	**3**	

3.2. MNX1-AS1 promoted ESCC cell proliferation, migration and invasion

Considering the high expression level of MNX1-AS1 in ESCC tissues, we first investigated the biological roles of MNX1-AS1 in ESCC cells. We designed two siRNAs targeting MNX1-AS1 to inhibit the MNX1-AS1 expression in KYSE30 and KYSE150 cells. The two siRNAs apparently decreased MNX1-AS1 expression levels (Fig. 2A). WST-1 proliferation assays showed that knockdown of MNX1-AS1 apprently suppressed the proliferation of KYSE30 and KYSE150 cells compared to the siRNA negative control (Fig. 2B, C). Furthermore, we performed transwell assays to measure the ESCC cell migration and invasion mediated by MNX1-AS1. We found that cell migration and invasion ability were decreased after knockdown of MNX1-AS1 in KYSE30 and KYSE150 cells (Fig. 3A–D). Thus, these findings indicated that MNX1-AS1 could promote ESCC cell proliferation, migration and invasion.

3.3. MNXI-AS1 regulated ESCC cell cycle and apoptosis progression

To further confirm the effect of MNX1-AS1 expression on ESCC cell proliferation, we analyzed cell cycle distribution. The flow cytometry assay indicated that inhibiting MNX1-AS1 expression in KYSE30 cells increased the percentage of cells in the G1 phase and decreased the percentage of cells in the S phase (Fig. 4A–D). Additionally, we performed flow cytometry analysis to assess the impact of MNX1-AS1 on

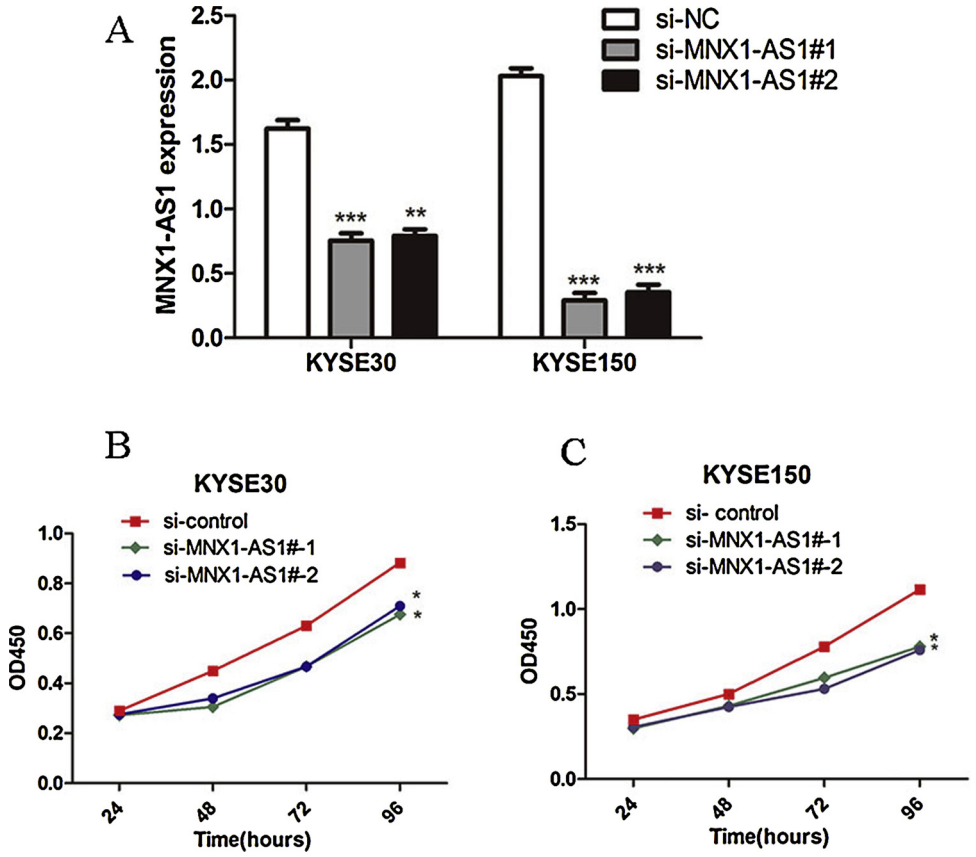

Fig. 2. MNX1-AS1 regulated ESCC cell proliferation. (A) The ESCC cell lines KYSE30 and KESE150 were transfected with two different MNX1-AS1 siRNA. (B, C) The effect of MNX1-AS1 on cell proliferation was performed in KYSE30 and KYSE150 cells. * P < 0.05, **P < 0.01, ***P < 0.001.

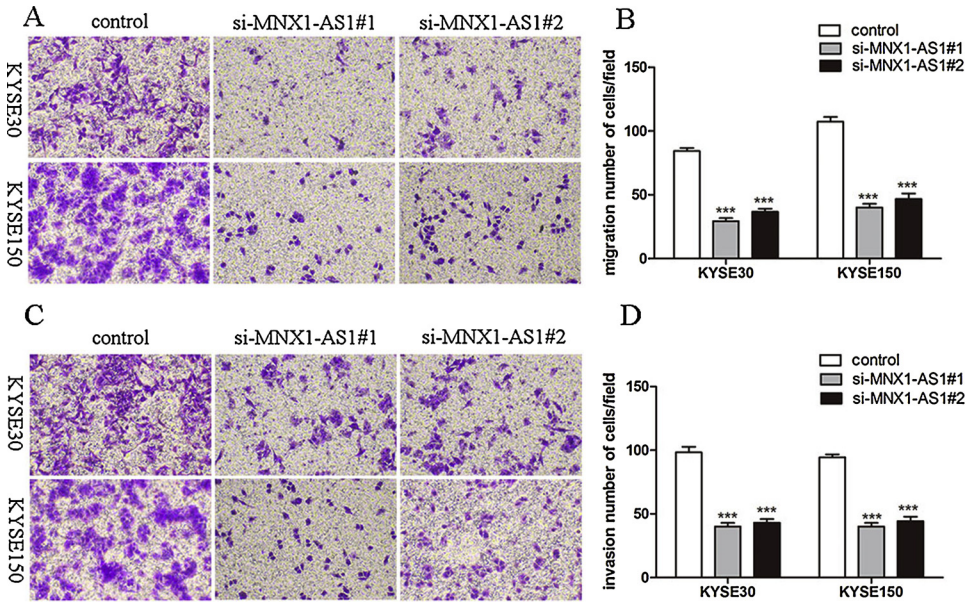

Fig. 3. MNX1-AS1 promoted ESCC cell migtation and invasion. (A–D) Transwell assays were performed to confirm the effect of MNX1-AS1 on cell migration and invasion in KYSE30 and KYSE150 cells. Representative images of cell migration and invasion were shown (A, C), and data was exhibited the capacity of cell migration and invasion using statistical analysis (B, D). ***P < 0.001.

cell apoptosis. The results showed that the number of apoptotic cells was increased after knockdown of MNX1-AS1 in KYSE30 cells (Fig. 5A–D). Therefore, these results manifested that MNX1-AS1 promoted cell proliferation by regulating ESCC cell cycle and apoptosis.

3.4. MNX1-AS1 functioned as competitive endogenous RNA for miR-34a in ESCC cells

Recently, increasing evidences have indicated that lncRNAs can serve as a competing endogenous RNA to regulate the expression and biological functions of miRNA [22–24]. Using the bioinformatics databases (Annolnc, http://annolnc.cbi.pku.edu.cn/), we found that miR-34a was predicted to have the numerous probability of binding to

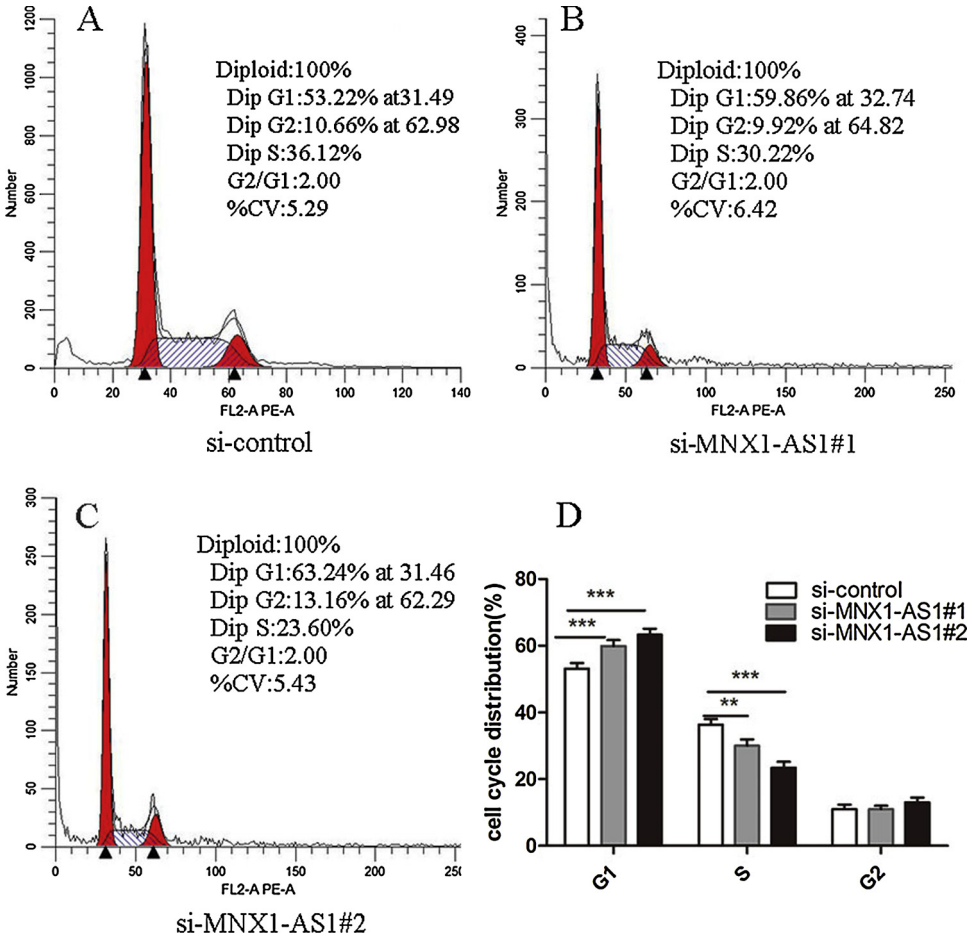

Fig. 4. MNX1-AS1 regulated ESCC cell cycle progression. (A–C) Flow cytometry assay was performed to analyse the effect of MNX1-AS1on cell cycle distribution in KYSE30 cells. (D) The experiment was repeat triple, and data was exhibited from three independent experiments using statistical analysis. **P < 0.01, ***P < 0.001.

MNX1-AS1 (Fig. 6A). To investigate whether miR-34a is a direct target gene of MNX1-AS1, we performed qRT-PCR to measure the expression level of miR-34a in KYSE 30 cells transfected with siRNA MNX1-AS1 or control. The results showed that the expression level of miR-34a was significantly increased after inhibiting MNX1-AS1 expression (Fig. 7A). In addition, qRT-PCR confirmed that miR-34a was downregulated in ESCC tissues and the expression of miR-34a was evidently negatively correlated with MNX-AS1 expression in ESCC tissues (Fig. 6B, C).These data indicated that MNX1-AS1 could regulate miR-34a expression.

3.5. Inhibiting miR-34a rescued the ability of MNX1-AS1 in enhancing cancer cell migration

Previous studies revealed miR-34a serves as a tumor suppressor in multifarious cancers including ESCC [25,26]. To further explore the role of MNX1-AS1 in regulating ESCC cell migration ability mediated by miR-34a, we designed miR-34a inhibitor and negative control in KYSE30 cells. The inhibitor apparently decreased miR-34a expression level (Fig. 7B). Moreover, the expression level of miR-34a was increased in KYSE30 cells transfected with siRNA MNX1-AS1 compared to the control group, and this upregulation was abolished by miR-34a inhibitor (Fig. 7C). Furthermore, the transwell assays showed that cell migration ability was decreased after knockdown MNX1-AS1, and miR-34a inhibitor rescued the ability of MNX1-AS1 in enhancing KYSE30 cells migration (Fig. 7D, E). Therefore, these findings indicated miR-34a was a significant downstream effector of MNX1-AS1 in regulating ESCC cell migration.

3.6. MNX1-AS1 regulated SIRT1 expression via miR-34a

Increasing evidence indicated that SIRT1 could serve as a target of miR-34a in cancer [27,28]. However, the biological roles of between miR-34a and SIRT1 in ESCC has not been studied. We analyzed SIRT1 expression in GEPIA database, and we found that SIRT1 mRNA levels was significantly upregulated in ESCC tissues compared with corresponding tumor-adjacent tissues (Fig. 8A). High SIRT1expression level in tumor tissue was related to poor overall survival rate than those with low SIRT1 expression (Fig. 8B).

Moreover, we demonstrated whether MNX1-AS1 can regulate SIRT1 expression via miR-34a. We performed qRT-PCR to measure the expression level of SIRT1, and the rusults showed the expression level of SIRT1 was decreased in KYSE 30 cells transfected with si-RNA MNX1-AS1 (Fig. 8C), while the expression level of SIRT1 was increased after transfecting with miR-34a inhibitor in KYSE 30 cells (Fig. 8D). Therefore, these findings suggested that MNX1-AS1 promoted ESCC progression via regulating miR-34a/ SIRT1 axis.

4. Discussion

Esophageal cancer has been regarded as a serious malignancy owing to poor prognosis and high mortality rate [29]. Therefore, effective therapeutic targets and intervention strategies will help improve the prognosis of ESCC patients, and this emphasizes the need for understanding the molecular mechanisms in ESCC progression. Recently, mounting evidences have revealed that lncRNAs took part in the development of ESCC progression. For instance, Sun et al. found

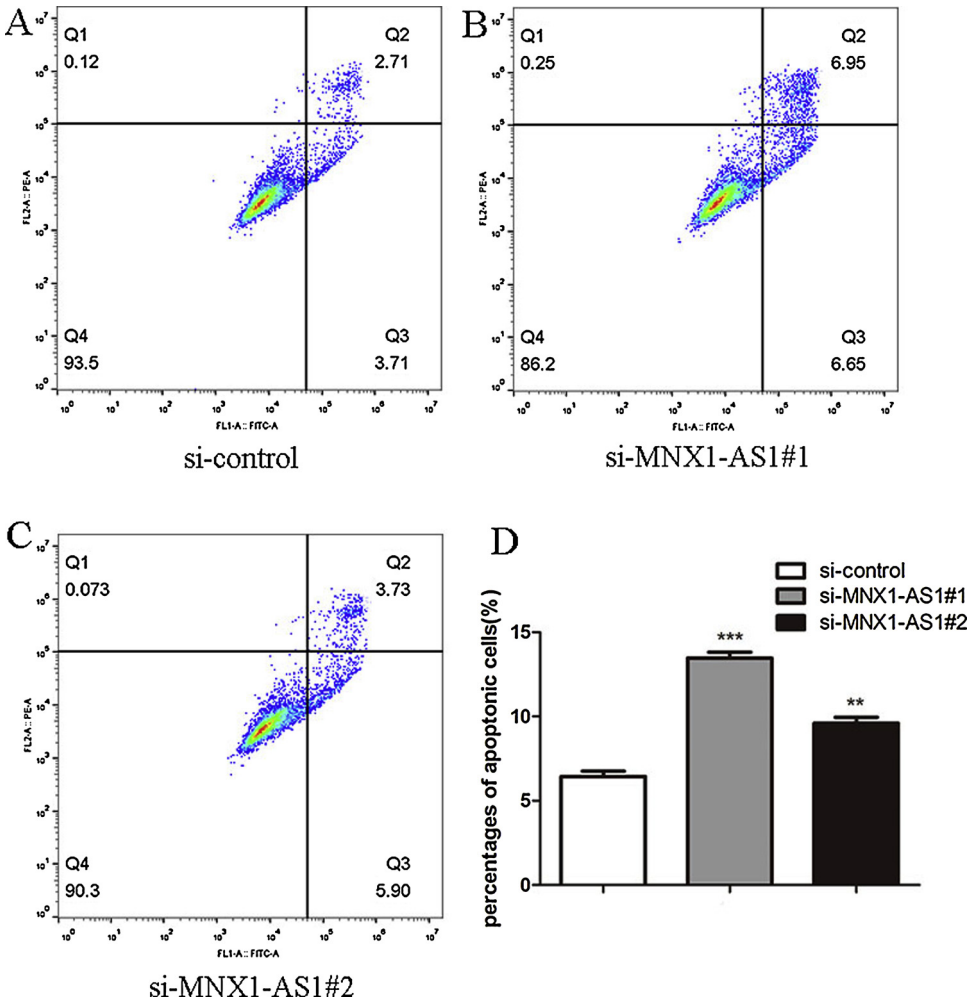

Fig. 5. MNX1-AS1 promoted ESCC cell apoptosis progression. (A–C) The KYSE30 cells were transfected with MNX1-AS1 siRNA. The effect of MNX1-AS1 on cell apoptosis was determined by flow cytometry. (D) The experiment was repeat triple, and data was exhibited from three independent experiments using statistical analysis. **P < 0.01, ***P < 0.001.

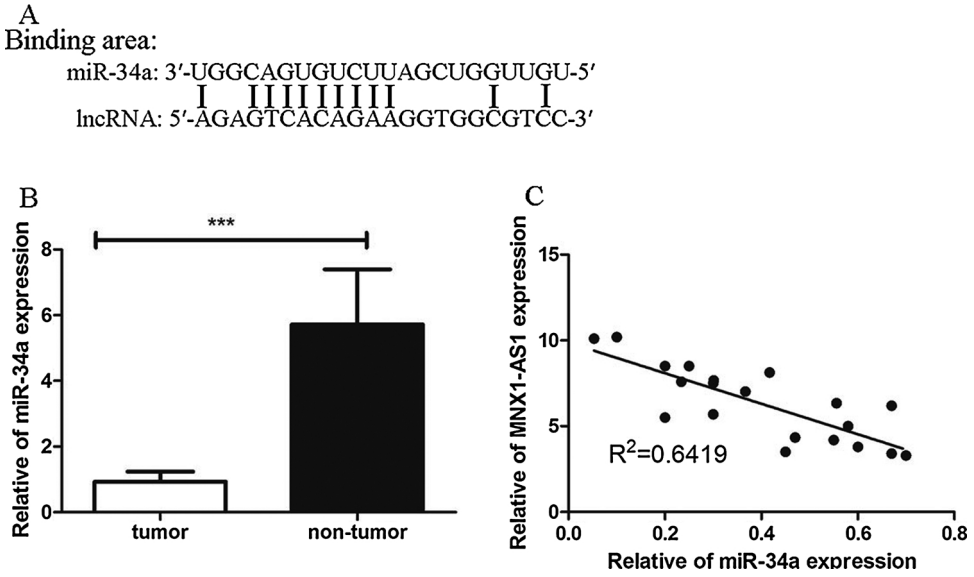

Fig. 6. MNX1-AS1 negatively regulated miR34a expression in ESCC. (A) Potential binding site for miR-34a and MNX1-AS1. (B) The expression of miR34a in ESCC tumor tissues compared with corresponding tumor-adjacent tissues. (C) Correlation between MNX1-AS1 expression and miR34a expression in ESCC tissues.

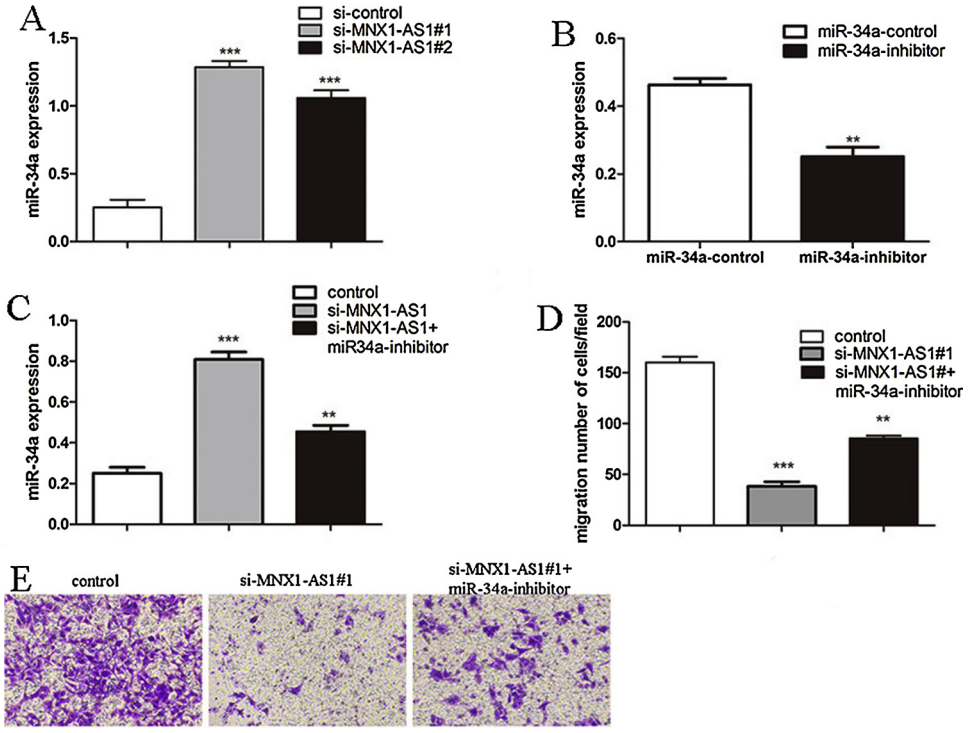

Fig. 7. MNX1-AS1 functioned as a ceRNA for miR34a and inhibiting miR-34a rescued the ability of MNX1-AS1 in enhancing cancer cell migration. (A) Real-time PCR was performed to analyse the expression of miR-34a after knockdown MNX1-AS1 in KYSE30 cells. (B) The KYSE30 cells were infected with miR-34a inhibitor. (C) The expression of miR-34a was measured by qRT-PCR after KYSE30 cells transfected with MNX1-AS1 siRNA or miR-34a inhibitor. (D, E) The KYSE30 cells were infected with MNX1-AS1 siRNA or miR-34a inhibitor, following with cell migration assays. $^{**}P < 0.01$, $^{***}P < 0.001$.

LINC00657 could facilitate ESCC progression by targeting miR-615-3p and JunB [30]. In addition, Wu et al found that CASC9 was relevant to tumor size and TNM stage by suppressing PDCD4 expression through recruiting EZH2 [31].

MNX1-AS1 is a antisense transcript of MNX1, of which originates from near the promoter [32]. The role of MNX1-AS1 was complicated in the progression of tumors, and MNX1-AS1 was found overexpressed and functioned as an oncogene in colon adenocarcinoma [17], breast cancer [18], gastric cancer [33]and lung cancer [34]. In this study, we found the expression level of MNX1-AS1 in ESCC was higher than

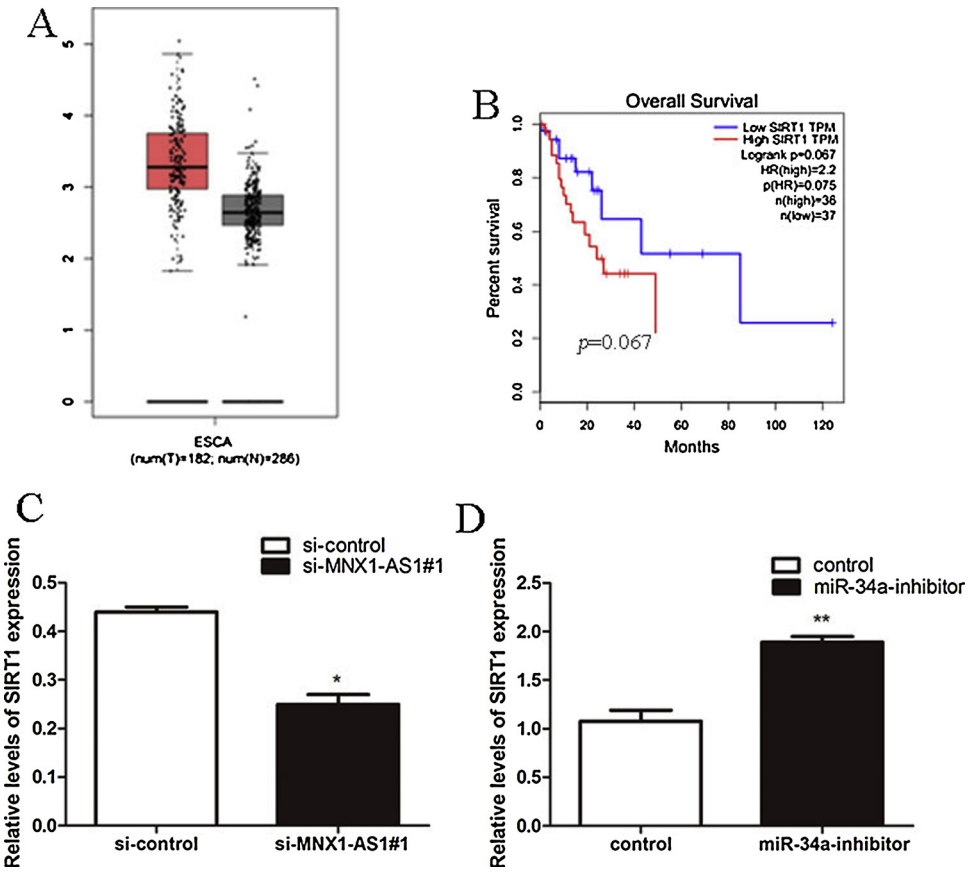

Fig. 8. MNX1-AS1 promoted ESCC progression via regulating miR-34a/ SIRT1.
(A, B) The expression of SIRT1 compared with normal tissues in ESCA from GEPIA database, and the Kaplan-Meier analysis of SIRT1 expression in ESCA from GEPIA database. (C, D) The expression of SIRT1 was measured by qRT-PCR after KYSE30 cells transfected with MNX1-AS1 siRNA or miR-34a inhibitor.

corresponding tumor-adjacent tissues. Furthermore, the expression of MNX1-AS1 was correlated with ESCC lymph node metastasis, and konckdown MNX1-AS1 with siRNA apprently suppressed the ability of cell proliferation, migration and invasion. Inhibiting MNX1-AS1 increased the percentage of cells in the G1 phase and decreased the percentage of cells in the S phase by flow cytometry analysis. In addition, knockdown of MNX1-AS1 increased the number of apoptotic cells. These data can partly explicate the reason of MNX1-AS1 oncogenic role in ESCC progression.

Recently, increasing evidences have indicated that lncRNAs regulated cancer progression through acting as competing endogenous RNA. For example, lncRNA ZNFX1-AS1 was found to promote colorectal cancer progression and metastasis by functioning as a ceRNA of miR-144 to regulate EZH2 expression [35]. However, the mechanism of MNX1-AS1 in ESCC progression remains unknown. In this sthdy, we found miR-34a was a direct target gene of MNX1-AS1, and the expression of miR-34a was increased after knockdown MNX1-AS1. Furthermore, inhibiting miR-34a rescued the ability of MNX1-AS1 in enhancing cancer cell migration. These findings indicate miR-34a was a significant downstream effector of MNX1-AS1 in regulating ESCC cell migration.

Sirtuin 1, also known as SIRT1, was reported involved in cancer development and progression [36]. SIRT1 could serve as a target of miR-34a in tumor progression according to previous studies [27,28]. In this study, we found that SIRT1 mRNA levels was significantly upregulated in ESCC tissues, and high SIRT1 was relevant to poor overall survival of ESCC patients. In addition, qRT-PCR showed that knockdown MNX1-AS1 decreased SIRT1 expression, while miR-34a inhibitor increased SIRT1 expression. These findings suggested that MNX1-AS1 promoted ESCC progression via regulating miR-34a/ SIRT1 axis.

5. Conclusion

In conclusion, we have confirmed that the roles of lncRNA MNX1-AS1 in ESCC, and MNX1-AS1 might work as a ceRNA for miR-34a to upregulate SIRT1 expression. Thus, MNX1-AS1 may function as a potential therapeutic target for ESCC.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Acknowledgments

This study was supported by grants from the Medical Scientific Research Foundation of Health Department of Henan Province of China (Grant No. 201403077), the National Natural Science Foundation of China grants (Grant No. 81702780), and the Science and Technology Project of Henan province of China (Grant No. 192102310133). We also thank for the support from Academy of Medical Sciences of Zhengzhou University Translational Medicine platform.

References

[1] D.C. Codipilly, Y. Qin, S.M. Dawsey, J. Kisiel, M. Topazian, D. Ahlquist, P.G. Iyer, Screening for esophageal squamous cell carcinoma: recent advances, Gastrointest. Endosc. 88 (3) (2018) 413–426.
[2] J. Inadomi, H. Alastal, L. Bonavina, S. Gross, R.H. Hunt, H. Mashimo, M. di Pietro, H. Rhee, M. Shah, S. Tolone, D.H. Wang, S.H. Xie, Recent advances in Barrett's esophagus, Ann. N. Y. Acad. Sci. 1434 (1) (2018) 227–238.
[3] K. Sun, X. Zhao, J. Wan, L. Yang, J. Chu, S. Dong, H. Yin, L. Ming, F. He, The diagnostic value of long non-coding RNA MIR31HG and its role in esophageal squamous cell carcinoma, Life Sci. 202 (2018) 124–130.
[4] X. Liu, M. Song, P. Wang, R. Zhao, H. Chen, M. Zhang, Y. Shi, K. Liu, F. Liu, R. Yang, E. Li, A.M. Bode, Z. Dong, M.H. Lee, Targeted therapy of the AKT kinase inhibits esophageal squamous cell carcinoma growth in vitro and in vivo, Int. J. Cancer 176 (2019) 1619–1634.
[5] S. Qie, A. Yoshida, S. Parnham, N. Oleinik, G.C. Beeson, C.C. Beeson, B. Ogretmen, A.J. Bass, K.K. Wong, A.K. Rustgi, J.A. Diehl, Targeting glutamine-addiction and overcoming CDK4/6 inhibitor resistance in human esophageal squamous cell carcinoma, Nat. Commun. 10 (1) (2019) 1296.
[6] T.B. Nobel, A. Barbetta, M. Hsu, K.S. Tan, T. Pinchinat, F. Schlottmann, M.S. Bains, G.Y. Ku, A.J. Wu, M.G. Patti, D.R. Jones, D. Molena, Outcomes of radiation-associated esophageal squamous cell carcinoma: the MSKCC experience, J. Gastrointest. Surg. 23 (1) (2019) 11–22.
[7] L. Yang, K. Sun, J. Chu, Y. Qu, X. Zhao, H. Yin, L. Ming, J. Wan, F. He, Long non-coding RNA FTH1P3 regulated metastasis and invasion of esophageal squamous cell carcinoma through SP1/NF-kB pathway, Biomed. Pharmacother. 106 (2018) 1570–1577.
[8] X. Jin, X.E. Xu, Y.Z. Jiang, Y.R. Liu, W. Sun, Y.J. Guo, Y.X. Ren, W.J. Zuo, X. Hu, S.L. Huang, H.J. Shen, F. Lan, Y.F. He, G.H. Hu, G.H. Di, X.H. He, D.Q. Li, S. Liu, K.D. Yu, Z.M. Shao, The endogenous retrovirus-derived long noncoding RNA TROJAN promotes triple-negative breast cancer progression via ZMYND8 degradation, Sci. Adv. 5 (3) (2019) eaat9820.
[9] L. Li, Y. Wang, G. Song, X. Zhang, S. Gao, H. Liu, HOX cluster-embedded antisense long non-coding RNAs in lung cancer, Cancer Lett. 450 (2019) 14–21.
[10] M. Xie, T. Ma, J. Xue, H. Ma, M. Sun, Z. Zhang, M. Liu, Y. Liu, S. Ju, Z. Wang, W. De, The long intergenic non-protein coding RNA 707 promotes proliferation and metastasis of gastric cancer by interacting with mRNA stabilizing protein HuR, Cancer Lett. 443 (2019) 67–79.
[11] J. Carlevaro-Fita, R. Johnson, Global positioning system: understanding long non-coding RNAs through subcellular localization, Mol. Cell 73 (5) (2019) 869–883.
[12] S. Kumar, R.A. Boon, L. Maegdefessel, S. Dimmeler, H. Jo, Role of noncoding RNAs in the pathogenesis of abdominal aortic aneurysm, Circ. Res. 124 (4) (2019) 619–630.
[13] S.J. Liu, D.A. Lim, Modulating the expression of long non-coding RNAs for functional studies, EMBO Rep. 19 (12) (2018).
[14] Y. Bai, G. Zhang, H. Chu, P. Li, J. Li, The positive feedback loop of lncRNA DANCR/miR-138/Sox4 facilitates malignancy in non-small cell lung cancer, Am. J. Cancer Res. 9 (2) (2019) 270–284.
[15] X. Lu, Y. Yu, S. Tan, Long non-coding XIAP-AS1 regulates cell proliferation, invasion and cell cycle in colon cancer, Artif. Cells Nanomed. Biotechnol. 47 (1) (2019) 767–775.
[16] R. Yang, L. Wang, M. Han, MNX1-AS1 is a novel biomarker for predicting clinical progression and poor prognosis in lung adenocarcinoma, J. Cell. Biochem. 120 (2018) 7222–7228.
[17] Y. Ye, B. Gu, Y. Wang, S. Shen, W. Huang, E2F1-mediated MNX1-AS1-miR-218-5p-SEC61A1 feedback loop contributes to the progression of colon adenocarcinoma, J. Cell. Biochem. 120 (4) (2019) 6145–6153.
[18] Y. Cheng, Y. Pan, Y. Pan, O. Wang, MNX1-AS1 is a functional oncogene that induces EMT and activates the AKT/mTOR pathway and MNX1 in breast cancer, Cancer Manag. Res. 11 (2019) 803–812.
[19] J. Wan, H. Liu, Q. Feng, J. Liu, L. Ming, HOXB9 promotes endometrial cancer progression by targeting E2F3, Cell Death Dis. 9 (5) (2018) 509.
[20] J. Wan, H. Liu, L. Ming, Lysine crotonylation is involved in hepatocellular carcinoma progression, Biomed. Pharmacother. 111 (2019) 976–982.
[21] J. Wan, H. Liu, L. Yang, L. Ma, J. Liu, L. Ming, JMJD6 promotes hepatocellular carcinoma carcinogenesis by targeting CDK4, Int. J. Cancer 144 (10) (2019) 2489–2500.
[22] F. Gao, J. Feng, H. Yao, Y. Li, J. Xi, J. Yang, LncRNA SBF2-AS1 promotes the progression of cervical cancer by regulating miR-361-5p/FOXM1 axis, Artif. Cells Nanomed. Biotechnol. 47 (1) (2019) 776–782.
[23] X. Wang, K. Hu, Y. Chao, L. Wang, LncRNA SNHG16 promotes proliferation, migration and invasion of osteosarcoma cells by targeting miR-1301/BCL9 axis, Biomed. Pharmacother. 114 (2019) 108798.
[24] Y. Zhang, Y. Li, L. Han, P. Zhang, S. Sun, SUMO1P3 is associated clinical progression and facilitates cell migration and invasion through regulating miR-136 in non-small cell lung cancer, Biomed. Pharmacother. 113 (2019) 108686.
[25] P. Vaitkiene, A. Pranckeviciene, R. Stakaitis, G. Steponaitis, A. Tamasauskas, A. Bunevicius, Association of miR-34a expression with quality of life of glioblastoma patients: a prospective study, Cancers 11 (3) (2019).
[26] L. Yang, X. Song, J. Zhu, M. Li, Y. Ji, F. Wu, Y. Chen, X. Cui, J. Hu, L. Wang, Y. Cao, Y. Wei, W. Zhang, F. Li, Tumor suppressor microRNA-34a inhibits cell migration and invasion by targeting MMP-2/MMP-9/FNDC3B in esophageal squamous cell carcinoma, Int. J. Oncol. 51 (1) (2017) 378–388.
[27] L. Song, T.Y. Chen, X.J. Zhao, Q. Xu, R.Q. Jiao, J.M. Li, L.D. Kong, Pterostilbene prevents hepatocyte EMT in fructose-induced liver fibrosis through suppressing miR-34a/Sirt1/p53 and TGF-beta1/Smads signaling, Br. J. Pharmacol. 176 (2019) 1619–1634.
[28] C. Yao, J. Liu, X. Wu, Z. Tai, Y. Gao, Q. Zhu, J. Li, L. Zhang, C. Hu, F. Gu, J. Gao, S. Gao, Reducible self-assembling cationic polypeptide-based micelles mediate co-delivery of doxorubicin and microRNA-34a for androgen-independent prostate cancer therapy, J. Control. Release 232 (2016) 203–214.
[29] X. Huang, X. Zhou, Q. Hu, B. Sun, M. Deng, X. Qi, M. Lu, Advances in esophageal cancer: a new perspective on pathogenesis associated with long non-coding RNAs, Cancer Lett. 413 (2018) 94–101.
[30] Y. Sun, J. Wang, S. Pan, T. Yang, X. Sun, Y. Wang, X. Shi, X. Zhao, J. Guo, X. Zhang, LINC00657 played oncogenic roles in esophageal squamous cell carcinoma by targeting miR-615-3p and JunB, Biomed. Pharmacother. 108 (2018) 316–324.
[31] Y. Wu, L. Hu, Y. Liang, J. Li, K. Wang, X. Chen, H. Meng, X. Guan, K. Yang, Y. Bai, Up-regulation of lncRNA CASC9 promotes esophageal squamous cell carcinoma growth by negatively regulating PDCD4 expression through EZH2, Mol. Cancer 16 (1) (2017) 150.
[32] A. Goyal, K. Myacheva, M. Gross, M. Klingenberg, B. Duran Arque, S. Diederichs, Challenges of CRISPR/Cas9 applications for long non-coding RNA genes, Nucleic

Acids Res. 45 (3) (2017) e12.
[33] W. Zhang, L. Huang, X. Lu, K. Wang, X. Ning, Z. Liu, Upregulated expression of MNX1-AS1 long noncoding RNA predicts poor prognosis in gastric cancer, Bosn. J. Basic Med. Sci. 19 (2) (2019) 164–171.
[34] H. Liu, L. Han, Z. Liu, N. Gao, Long noncoding RNA MNX1-AS1 contributes to lung cancer progression through the miR-527/BRF2 pathway, J. Cell. Physiol. 234 (2019) 13843–13850.
[35] L. Shi, X. Hong, L. Ba, X. He, Y. Xiong, Q. Ding, S. Yang, G. Peng, Long non-coding RNA ZNFX1-AS1 promotes the tumor progression and metastasis of colorectal cancer by acting as a competing endogenous RNA of miR-144 to regulate EZH2 expression, Cell Death Dis. 10 (3) (2019) 150.
[36] A.L.C. Ong, T.S. Ramasamy, Role of Sirtuin1-p53 regulatory axis in aging, cancer and cellular reprogramming, Ageing Res. Rev. 43 (2018) 64–80.