

Original Article

LncRNA MALAT1 inhibits the proliferation and invasiveness of laryngeal squamous cell carcinoma Hep-2 cells by modulating miR-362-3p

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Abstract: Objective: To investigate the mechanism of lncRNA MALAT1 (MALAT1) inhibiting the proliferation and invasiveness of laryngeal squamous cell carcinoma (LSCC) Hep-2 cells by modulating miR-362-3p. Methods: We collected the expression profile of lncRNAs and miRNAs in LSCC downloaded from The Cancer Genome Atlas (TCGA) database as well as LSCC tissue samples and adjacent normal counterparts resected from LSCC patients in Lvliang People's Hospital and First Hospital of Shanxi Medical University between January 2018 and June 2020 for analysis. Human LSCC Hep-2 cells were selected for experiments. The expression of miR-362-3p and MALAT1 was detected by quantitative real-time polymerase chain reaction (qRT-PCR). Cells were subsequently transfected to knock out MALAT1, and the growth, metastasis and invasiveness of cells were evaluated by CCK-8 assay, plate clone formation, wound healing, and Transwell invasion assays respectively. The binding of MALAT1 to miR-362-3p was verified by RNA pull-down, RNA binding protein immunoprecipitation (RIP), and dual-luciferase reporter assays. Results: MALAT1 was highly expressed while miR-362-3p was lowly expressed in both LSCC tissues and cells compared with normal counterparts. MALAT1 knockdown inhibited the viability of Hep-2 cells, reducing the number of plate clone-forming cells as well as the number of migrated and invaded cells. Transfection of miR-362-3p inhibitor into Hep-2 cells treated by si-MALAT1 reversed the inhibition of si-MALAT1 on the proliferation of Hep-2 cells, and promoted cell invasiveness and migration. MALAT1 can sponge miR-362-3p and inhibit its expression. Conclusions: Knockdown of MALAT1 can inhibit Hep-2 cell proliferation and reduce its invasiveness and migration by modulating miR-362-3p.

Keywords: LncRNA MALAT1, miR-362-3p, laryngeal squamous cell carcinoma, proliferative activity, invasive ability

Introduction

Laryngeal carcinoma (LC) is the most common malignancy among head and neck tumors [2]. Laryngeal malignancies can be divided into primary and secondary tumors. The vast majority of primary tumors are laryngeal squamous cell carcinoma (LSCC), which accounts for the majority of laryngeal cancers and is the second most common tumor of the head and neck worldwide [3, 4]. According to the data of the World Cancer Report, there were 184,615 new laryngeal cancer patients and 99,840 associated deaths worldwide in 2020 [5]. The fatality rate of the disease is high, with a recurrence rate of about 50% in locally advanced patients despite aggressive treatment such as surgery,

radiotherapy and chemotherapy [6]. LSCC usually presents with persistent pain, cough, halitosis, and dysphagia. The disease is difficult to detect in the early stage due to the narrow laryngopharyngeal cavity, relatively hidden anatomical location, and inconspicuous early symptoms. Most hypopharyngeal carcinoma occurs in the pyriform fossa, followed by the posterior wall of hypopharynx, and the postcricoid area [7, 8]. Laryngopharyngeal carcinoma is relatively aggressive, with local infiltration and early cervical lymph node metastasis. Currently, the treatments for early LC include surgical treatment, chemotherapy/radiotherapy, either alone or in combination [9-11]. Despite encouraging progress from the perspective of LSCC treatment, patient prognosis needs improvement.

This is particularly true for those with advanced or recurrent disease who present with a 5-year survival rate <50% [12]. Therefore, it is of utmost importance to seek new ideas and methods for improving the early diagnosis and treatment and enhancing the prognosis of patients with LC.

With the constant advances in the Human Genome Project and genetic engineering technology, many studies have confirmed that the human genome is highly extensive and dense in the transcription process; whereas, only about 1.5% of the genes can be transcribed into proteins, and more than 98% of the remaining cannot, that is to say, the vast majority of genes do not encode protein. This indicates that non-coding RNAs may have a wealth of undiscovered biologic functions in organisms [13-15]. Short non-coding RNA and long non-coding RNA (lncRNA) can be classified according to their nucleotide length [16]. Among them, lncRNA has become a research focus, which has aroused the discussion and attention of researchers. Relevant research results have confirmed that lncRNAs are essential for tumor pathogenesis, and even some specific ones can be used as tumor predictors [17]. Among them, lncRNA MALAT1 (MALAT1) is one of the earliest discovered long-chain non-coding RNAs, which was named because it was initially found in human NSCLC, also known as NEAT2 [17]. MALAT1 is evolutionarily conserved in sequence, and has highly homologous sequences among species and genera, which suggests that it plays an important role in carcinogenesis and progression [18]. Although MALAT1 was first found in NSCLC, years of study have confirmed that it is also highly expressed in many other tumor tissues such as esophageal, nasopharyngeal, liver, oral squamous cell, and cervical carcinoma [19-23].

In addition, lncRNA and microRNA (miRNA) can regulate each other and affect the occurrence and development of tumors. Studies have shown that miR-362-3p is closely associated with the migration and invasiveness of renal cell carcinoma, breast cancer, vascular smooth muscle cells, and gastric cancer cells [24-26]. A study [27] reported that MALAT1 knockdown can reduce drug resistance of renal cell carcinoma to shunitinib by modulating miR-362-3p-mediated G3BP1. However, whether miR-362-3p and MALAT1 interact with each other to affect the proliferation and invasiveness of LSCC cells has not been reported. Therefore,

this study intended to detect the expression of MALAT1 and miR-362-3p in LC tissues and their impact on cancer cell proliferation and invasiveness by use of molecular biological techniques, so as to provide clues for further study on the interaction and molecular mechanism of MALAT1 and miR-362-3p in LC.

Materials and methods

Data sources

The RNA-Seq and miRNA-Seq data of patients with head and neck squamous cell carcinoma (HNSCC) were downloaded from The Cancer Genome Atlas (TCGA) database (<https://portal.gdc.cancer.gov/>) for analysis. Data extraction and sorting, using the coding/non-coding classification supplemented by GENCODE/Ensembl, were performed by R software (v4.0.3), and those genes that produced only “antisense”, “lincRNA”, “non_coding” and “processed_transcript” transcripts were regarded as lncRNAs. The expression matrices of lncRNAs and miRNAs were obtained. Clinical materials were downloaded from the TCGA portal.

Specimen collection

Fifty-five cases of LSCC specimens and adjacent normal counterparts were collected from LSCC patients with surgical treatment in the Otolaryngology Department of our hospital during January 2018 and June 2020. The LC and hypopharyngeal cancer tissues were excised intraoperatively and immediately put into liquid nitrogen for later use. All postoperative specimens were confirmed by pathological examination, and the cancer tissues were divided into cancer-rich tissues and with no obvious necrotic tissue. All participants signed an informed consent form and agreed to donate laryngeal and laryngopharyngeal carcinoma tissues for related laboratory testing. The study was approved by the Medical Ethics Committee of our hospital (2019lyll-04).

Cell culture and transfection

Normal human nasopharyngeal epithelial NP-69 cells and human LC Hep-2 cells (Cell Bank, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences) were routinely cultured in Roswell Park Memorial Institute (RPMI) 1640 medium (Thermo Scientific, the States) + 10% fetal bovine serum (FBS; Thermo Scientific, the States) in a 37°C

Table 1. Primer sequences

Gene	Forward	Reverse
MALAT1	5'-AAAGTCCGCCATTTTGCCAC-3'	5'-AAAGTCCGCCATTTTGCCAC-3'
U6	5'-GACTGCGCAAGGATGACAC-3'	5'-CAGTGCCTGTCTGGAGTC-3'
miR-362-3p	5'-GCGCGAACACACCTATTCAG-3'	5'-AGTGCAGGGTCCGAGGTATT-3'
GAPDH	5'-ACAACCTTGGTATCGTGGAAGG-3'	5'-GCCATCACGCCACAGTTTC-3'

incubator with 5%CO₂ in air. On the day before transfection, Hep-2 cells during the logarithmic growth phase were seeded into 6-well plates, and then transfected with si-MALAT1, si-NC, miR-362-3p inhibitor, and inhibitor NC (all supplied by Shanghai GenePharma) when the cell density reached 60%-80%, strictly following the instructions of Lipofectamine 3000 (Invitrogen, Carlsbad, CA, the States). si-MALAT1: 5'-CCCUCAAUAAGGAAUAA-3', si-NC: 5'-UUCUCCGAA-CGUGUCACGU-3'. In the transfection process, serum-free and antibiotic-free RPMI1640 medium was used. After 6 h of transfection, the medium was replaced with RPMI1640 containing 10% FBS. Cells transfected for 24 h were gathered to perform experiments. Untreated Hep-2 cells were used as the control group.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Trizol-isolated (Invitrogen, Carlsbad, CA, the States) total RNA from tissues and cells was collected for reverse transcription into cDNA following the instructions of the PrimeScript RT Reagent Kit (Takara).

Using the cDNA as a template and with use of SYBR Premix Ex Taq II Kit (Takara), ABI Step One real-time PCR System (Thermo Fisher Scientific) was used for PCR reaction. The specific primers for qRT-PCR were synthesized by Shanghai Sangon Bio-tech (**Table 1**). The relative expression levels of MALAT1 and miR-362-3p relative to GAPDH and U6 respectively were calculated by $2^{-\Delta\Delta Ct}$. The average value was obtained from three repeated experiments.

Cell proliferation

The cell counting kit-8 (CCK-8) assay (Dojindo, JPN) was used to determine the effect of MALAT1 on the proliferation of Hep-2 cells. After transfection, Hep-2 cells were planted in 96-well plates to grow for 24 h, 48 h, 72 h and 96 h (37°C, 5%CO₂). This was followed by 2 h of culture after the addition of CCK-8 solution at

10 μ L/well. The absorbance was measured with use of a microplate reader, and the measuring wavelength indicating cell proliferation was 450 nm. The experiment was repeated three times

at different time points and the values were averaged.

Plate clone formation assay

Hep-2 cells (2×10^3 /well) after 24 h of transfection were inoculated in 6-well plates and cultured in the medium composed of DMEM + 10%FBS + 5%CO₂ for 2 weeks. Then, immobilized with paraformaldehyde (4%) and stained with crystal violet (0.1%) both for half an hour, the cells were microscopically counted for the number of colonies (≥ 50 cells).

Transwell

The effect of MALAT1 on the invasiveness of Hep-2 cells was detected by Transwell assay. First, Matrigel was synthesized and added into the upper chamber for 2 h of incubation (37°C). The transfected cells were then conventionally digested and suspended in RPMI1640 medium after 24 h culture. After adjusting the cell density, they were inoculated into the Transwell chamber at 1×10^5 cells/well. Then, serum free solution was added to the upper chamber to a final volume of 200 μ L, while 600 μ L complete medium + 10%FBS was put into the basolateral chamber. The cells remaining in the apical chamber following 24 h of conventional culture were wiped off with Q-tips, rinsed with phosphate buffer saline (PBS) twice, fastened with paraformaldehyde solution (4%, 20 min), dyed with crystal violet (0.1%, 20 min), and photographed with a microscope for cell counting.

Wound-healing assay

The cells transfected in the logarithmic growth phase of each group (1×10^6 /well) were seeded into 6-well plates and grown to 90% confluence. Subsequently, the tip of a 10 μ L sterile pipette was used to create scratches at the bottom of the cell culture plate through the center of each well to make a straight-line scratch from top to bottom, after which the wells were subjected to 3 gentle PBS rinses. Thereafter,

fresh culture medium was added for another 24 h of cultivation. Wound area imaging and quantification at 0 and 24 h were performed by inverted microscope (Olympus) and Image Pro Plus v.6.0 respectively. The cell healing rate was calculated as $(0 \text{ h scratch wound width} - 24 \text{ h scratch wound width}) / (0 \text{ h scratch wound width}) \times 100\%$. The experiment was repeated 3 times to obtain the average value.

Nuclear and cytoplasmic extraction and fluorescence in situ hybridization (FISH)

The nuclear and cytoplasmic fractions of RNA were extracted with PARIS™ kit (Invitrogen, USA). Then, the subcellular localization of the MALAT1 in cells was identified through a fluorescent in situ hybridization kit (RiboBio, China). MALAT1 probes were designed and synthesized by GenePharma (Shanghai, China). Briefly, Hep-2 cells were fixed in 4% formalin for 15 minutes. After PBS pre hybridization, we hybridized in 37°C hybridization solution for 30 minutes. Then the nuclei were counterstained by DAPI (4',6-diamidino-2-phenylindole) staining (Beyotime Biotechnology, China). Images were taken using a fluorescence microscope (Olympus, Japan).

RNA pull-down assay

After enzyme digestion and gel recovery, the pGEM-T-MALAT1 vector constructed by Beijing Rui Biotech was added into the *in vitro* transcription mixture and incubated at 37°C for 2-5 h. After the colorless liquid became turbid, DNase I was added to digest DNA template for 15 min of incubation at 37°C. Then, RNA was extracted using the kit (Guangzhou Genesee Biotech Co., Ltd.) and biotin-labeled RNA transcripts were obtained. After that, 3 µg RNA was heated at 90°C for 2 min to denature RNA. An equal volume of RNA binding buffer was added for 30 min of indoor incubation, so that RNA was folded to form advanced structures. The folded RNA was then mixed with 1 mg of total cell protein solution and incubated for 1 h in room temperature vortex, while part of protein lysate was retained as input. Thereafter, magnetic beads (Beads) were added at 40 µL/sample to culture (4°C) for 3 h to overnight. The magnetic beads were rinsed 8 times, after which proteinase K buffer was added into the molecular hybridization furnace for vortex incubation (55°C, 30 min). The RNA of the supernatant

transferred to the new centrifugal tube was isolated using the phenol-chloroform extraction. Finally, the adsorbed miRNA was detected by qRT-PCR after reverse transcription.

RNA binding protein immunoprecipitation (RIP) assay

RIP was performed using Magna RIP™ (Millipore, Billerica, USA) following the manufacturer's protocol. Hep-2 cells were lysed in RNA immunoprecipitation lysis buffer, and magnetic beads were conjugated with human anti-Ago2 antibody (Millipore, Billerica, USA) or control IgG antibody. Then the samples were digested with proteinase K, and RNA was extracted from the beads using TRIzol. Finally, qRT-PCR was performed to detect the expression of MALAT1 and miR-362-5p.

Dual-luciferase reporter (DLR) assay

The possible binding sites of miR-362-3p and MALAT1 were predicted. The miR-362-3p binding site mutation vector of pGL3-MALAT1-3'UTR was completed by Wuhan GeneCreate Biological Engineering Co., Ltd. The wild-type plasmid containing the target sequence was named MALAT1-WT 3'-UTR, while the mutant plasmid through site-directed mutagenesis was MALAT1-MUT 3'-UTR. In the DLR assay, the constructed luciferase reporter vector (100 ng) was co-transfected with reninase vector (20 ng, Promega) and miR-122-5p (100 nM) and miR-NC (100 nM) into HCCC9810 and RBE with use of Lipofectamine 2000 (Invitrogen, USA). The cells collected 48 h after transfection were determined by the DLR system (Promega) for renin and firefly luciferase activities, with renin activity as the standardized index.

Statistical processing

In this study, SPSS 22.0 and Graphpad Prism 8.0 were used for data processing and image rendering, respectively. Differences in the expression of miR-362-3p and MALAT1 in carcinoma tissues and normal counterparts were tested by rank sum test. Measured data were expressed as mean ± standard deviation (Mean ± SD). Each test was repeatedly determined ≥3 times. The measured data conforming to normal distribution were compared between groups using independent samples T test. One-

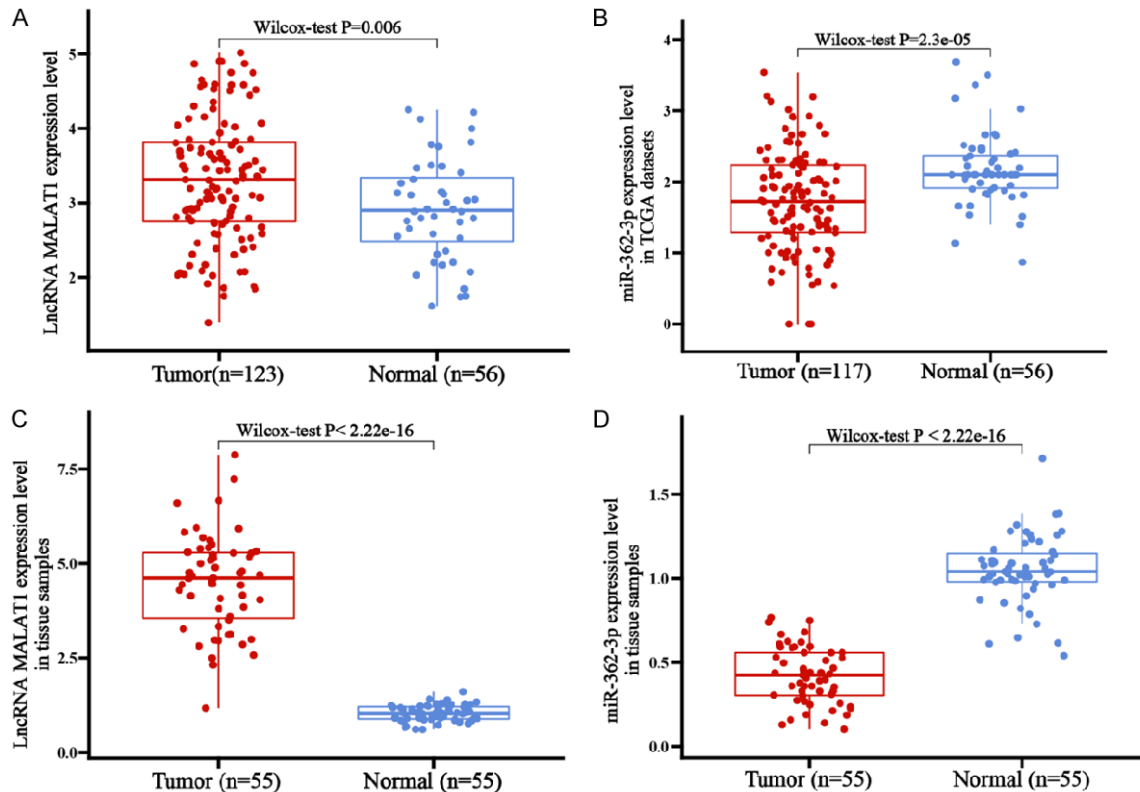


Figure 1. Expression of lncRNA MALAT1 and miR-362-3p in laryngeal squamous cell carcinoma patients. A: lncRNA MALAT1 expression in TCGA database patients; B: miR-362-3p expression in patients in TCGA database; C: lncRNA MALAT1 expression in tissue samples from clinical patients; D: miR-362-3p expression in tissue samples from clinical patients.

way ANOVA followed by Tukey post hoc test were used for multi-group comparisons. Double tailed *P* values <0.05 implied that the differences were of significance.

Results

Expression of MALAT1 and miR-362-3p in LSCC patients

Excluding samples that did not express MALAT1 or miR-362-3p from the downloaded data from TCGA database, a total of 123 samples expressing MALAT1 and 117 samples expressing miR-362-3p were obtained, in addition to 56 normal samples. The analysis identified up-regulated MALAT1 and down-regulated miR-362-3p in TCGA database samples (**Figure 1A, 1B**). The qPCR results of 55 cases of clinical cancer tissues and adjacent normal counterparts collected in our hospital showed that compared to normal, MALAT1 was significantly up-regulated and miR-362-3p was down-regulated in cancerous tissues (*P*<0.05, **Figure 1C, 1D**).

Correlation analysis showed that MALAT1 was significantly negatively correlated with miR-362-3p (*r*=-0.3075, *P*=0.0232, **Figure 2A**). The prognostic follow-up results showed that high expression of MALAT1 was significantly correlated with poor one-year prognosis of patients (*P*<0.05, **Figure 2B**), and there was no significant difference in one-year prognosis between patients with high and low miR-362-3p expression (*P*>0.05, **Figure 2C**).

Expression of MALAT1 and miR-362-3p in Hep-2 cells

Compared to NP69 cells, MALAT1 was up-regulated while miR-362-3p was downregulated in Hep-2 cells (**Figure 3A**). Compared to the si-NC group, MALAT1 decreased and miR-362-3p increased in cells in si-MALAT1 group after si-MALAT1 transfection (*P*<0.05). However, no significant difference was observed in the expression of MALAT1 and miR-362-3p between the control group and si-NC group (*P*>0.05) **Figure 3**.

lncRNA MALAT1 in laryngeal squamous cell carcinoma

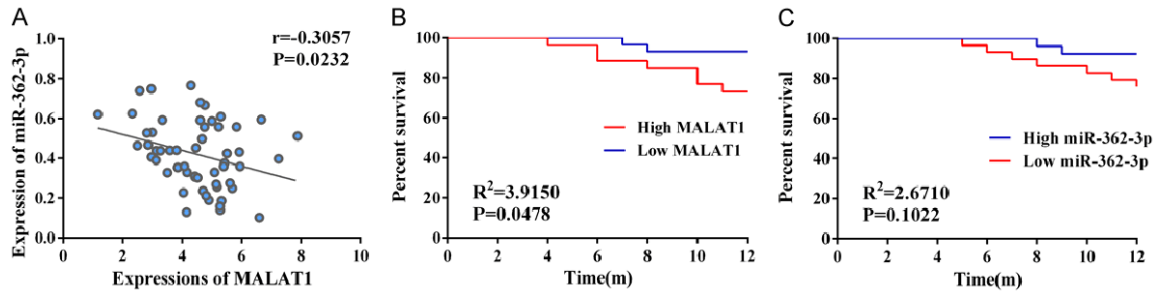


Figure 2. Correlation of the expression of lncRNA MALAT1 and miR-362-3p with prognosis in laryngeal squamous cell carcinoma patients. A: Correlation of the expression of lncRNA MALAT1 and miR-362-3p in clinical patients; B: Relationship between lncRNA MALAT1 expression level and one-year survival in clinical patients; C: Relationship between miR-362-3p expression level and one-year survival in clinical patients.

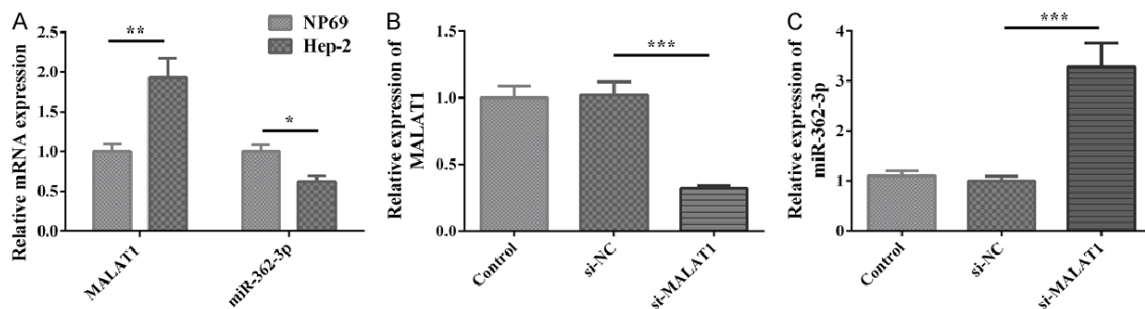


Figure 3. Expression of lncRNA MALAT1 and miR-362-3p in Hep-2 cells. A: Comparison of lncRNA MALAT1 and miR-362-3p expression in cell lines; B: Comparison of lncRNA MALAT1 expression; C: Comparison of miR-362-3p expression; ***P<0.001.

MALAT1 knockdown significantly inhibited Hep-2 cell proliferation

The findings of cell proliferation activity determined by CCK-8 assay showed that MALAT1 knockdown inhibited the activity of Hep-2 cells (**Figure 4A**). The results of plate clone assay demonstrated that knocking down MALAT1 significantly reduced the number of cell plate clones (P<0.05, **Figure 4B**).

MALAT1 knockdown significantly reduced Hep-2 cell invasiveness

Transwell assay results determined that knocking down MALAT1 could inhibit the invasive potential of Hep-2 cells and reduce the number of invasive cells (P<0.05) **Figure 5**.

MALAT1 knockdown significantly inhibited Hep-2 cell migration

The results of wound-healing assay revealed that after knocking down MALAT1, the percentage of 24-h cell scratch wound width to 0-h scratch wound width was higher than that of

the si-NC group; that is, the cell healing rate was significantly lower versus the si-NC group (P<0.05). This indicates that MALAT1 knockdown can inhibit the migration and mobility of Hep-2 cells **Figure 6**.

Inhibition of miR-362-3p expression reversed the effect of MALAT1 knockdown on Hep-2 cell proliferation and invasiveness

si-MALAT1 + miR-362-3p inhibitor co-transfection into Hep-2 cells led to markedly improved cell proliferation capacity of the si-MALAT1 + miR-362-3p inhibitor group compared to the si-MALAT1 + inhibitor NC group, increased number of plate clones, and enhanced invasiveness and migration ability. This suggests that inhibiting miR-362-3p expression can reverse the effect of MALAT1 knockdown on the proliferation and invasiveness of Hep-2 cells (P<0.05) **Figure 7**.

MALAT1 could sponge miR-362-3p

FISH assay showed that most of MALAT1 (Red) was located in the cytoplasm in Hep-2 cells

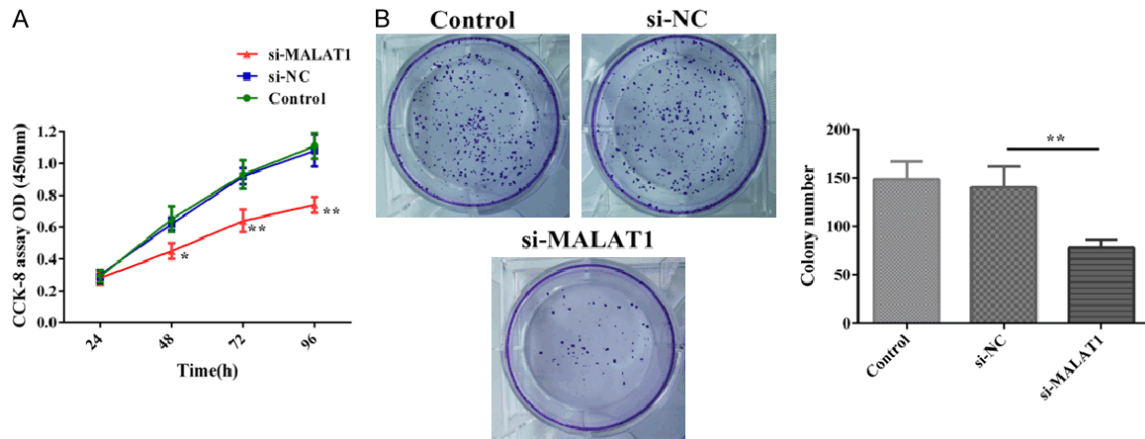


Figure 4. Effect of IncRNA MALAT1 on Hep-2 cell proliferation. A: Cell proliferation detected by CCK-8 assay; B: Number of plated cell clones formation; Compared to si-NC group, * $P < 0.05$; ** $P < 0.01$.

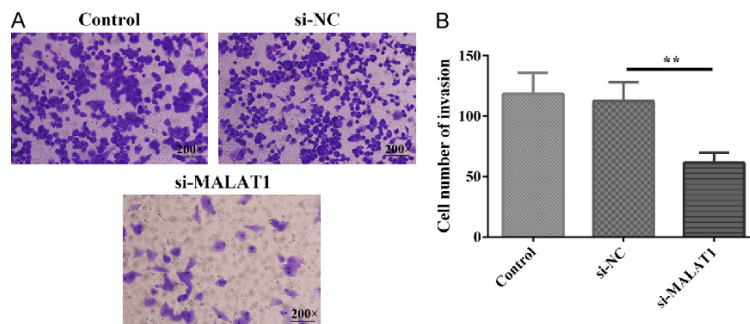


Figure 5. Effect of IncRNA MALAT1 on the invasiveness ability of Hep-2 cells. A: Microscopic invasion of Hep-2 cells; B: Number of invasive cells in each group; ** $P < 0.05$.

(Figure 8A). Starbase2.0 was used to predict the possible binding sites between MALAT1 and miR-362-3p (Figure 8B). RNA pull-down assay was used to verify the binding of endogenous miR-362-3p to *in vitro* transcribed MALAT1 in Hep-2 cells, and qRT-PCR was used to detect MALAT1-bound miR-362-3p. This identified that the binding of miR-362-3p to MALAT1 was significantly higher compared to the control group ($P < 0.05$, Figure 8C). Subsequently, DLR assay was used for validation. The results revealed that the fluorescence activity decreased under the co-transfection of MALAT1-WT miR-362-3p mimic ($P < 0.05$), while the fluorescence activity of other co-transfected combinations had no significant change (Figure 8D). The RIP results showed that both miR-362-3p and MALAT1 could bind to the Ago2 protein in Hep-2 cells (Figure 8E). The results suggest that MALAT1 can modulate

cell biological behavior through sponging miR-362-3p.

Discussion

The occurrence and development of HNSCC is a complicated process involving multi-factor, multi-stage and multi-gene mutations [27], which means that LSCC diagnosis and staging necessitates complex assessments. Today, molecular approaches are receiving increasing attention for their potential in diagnos-

ing and treating LSCC. The expression profiling or expression abnormalities of lncRNAs are closely related to human diseases, including those that seriously endanger human health such as neoplastic and neurodegenerative diseases [28]. Studies have shown that MALAT1 recruits members of the serine/arginine (SR) protein family with high specificity, and participates in epigenetic regulation and cell cycle regulation. It is highly expressed in many tumor cells, with the function of promoting tumor cell proliferation, metastasis, and invasion [18, 29].

In this study, we found that MALAT1 presented upregulated expression in both LC samples from TCGA database and clinical LC samples, which was consistent with previous studies. Many studies have confirmed elevated MALAT1 expression in HNSCC tissues including throat, nasopharynx and oral samples [30-32]. In clini-

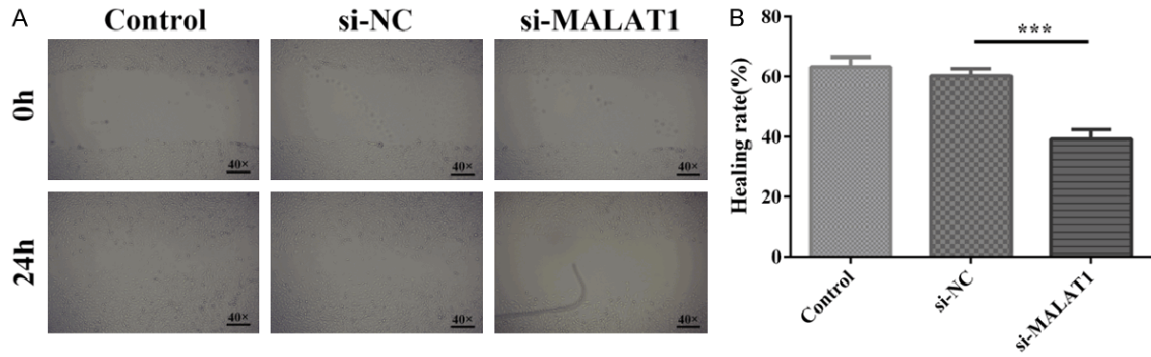


Figure 6. Effect of IncRNA MALAT1 on the migration ability of Hep-2 cells. A: Cell scratches at 0 h and 24 h; B: Cell healing rate; ***P<0.05.

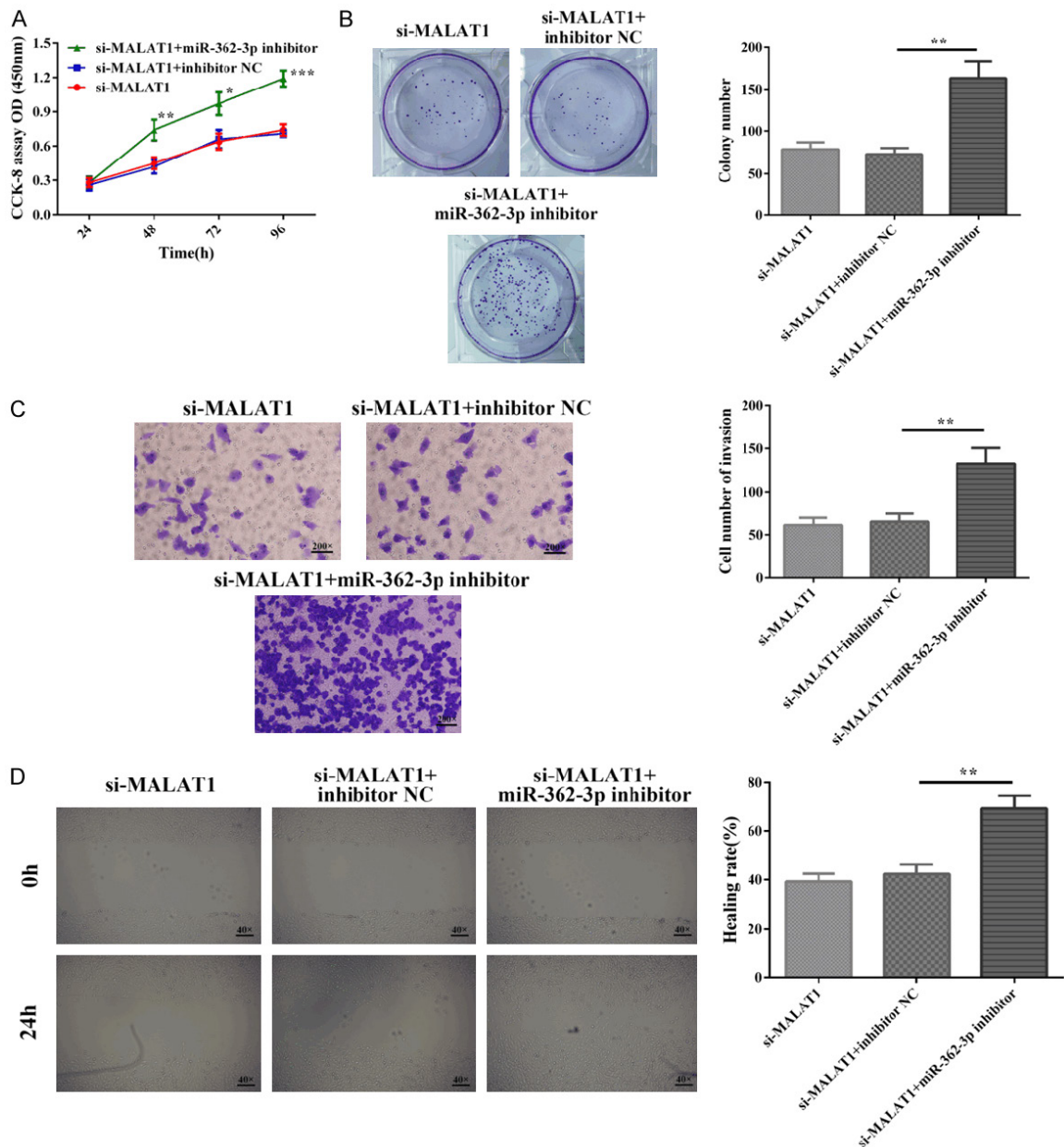


Figure 7. Rescue experiment. A: Cell proliferation detection by CCK-8 assay; B: Number of plate clone cell formation; C: Number of cell invasiveness; D: Number of cell migration; Compared with si-MALAT1 + inhibitor NC group, *P<0.05, **P<0.01, ***P<0.001.

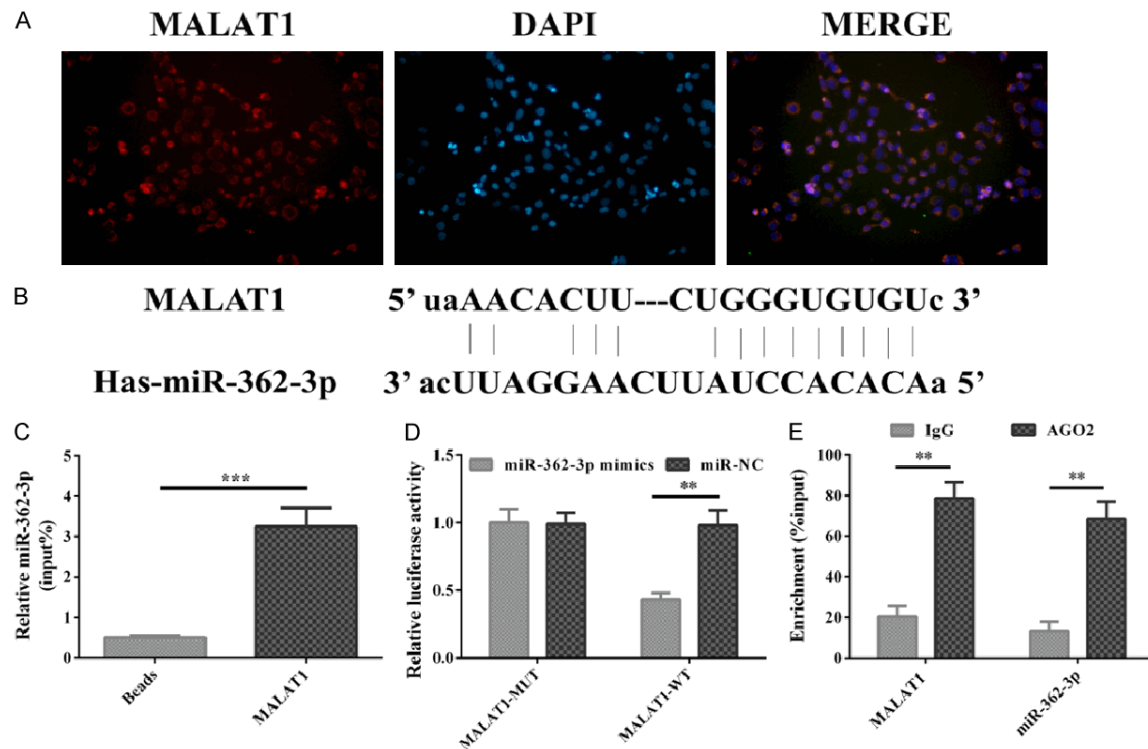


Figure 8. LncRNA MALAT1 can sponge miR-362-3p. A: Subcellular fractionation and FISH staining confirmed the expression of MALAT1 in cytoplasm; B: The predicted binding site of miR-362-3p and MALAT1; C: The binding between MALAT1 and miR-362-3p detected by RNA pull-down assay; D: Fluorescence activity detected by dual luciferase reporter assay; E: RIP assay was performed to confirm the interaction between MALAT1 and AGO2, miR-362-3p, and AGO2 in Hep-2 cells; **P<0.01, ***P<0.001.

cal patients, a high expression level of MALAT1 was correlated with short overall survival time. Subsequently, by knocking down the expression of MALAT1 in Hep-2 cells, we found that the vitality of Hep-2 cell was inhibited and the number of plate clone-forming cells was reduced. Prior studies suggest that MALAT1 is involved in the modulation of cell proliferation, cell cycle, and apoptosis. A previous study by Yamada et al. [33] indicated that MALAT1 expression was up-regulated in both normal endometrial stromal cells and endometrial stromal sarcomas as compared to secretory endometrial stromal cells. These results indicate that MALAT1 may play an important role in cell proliferation. Herein, we found that knocking down MALAT1 reduced the proliferation of Hep-2 cells, suggesting that MALAT1 may be vital in LSCC cell proliferation. Further, we

found that knocking down MALAT1 effectively reduced the number of migrated and invaded Hep-2 cells, as well as their invasiveness and migration ability. LncRNAs function in many ways, including co-transcription regulation, gene expression regulation, nuclear or cytoplasmic complex scaffolding, and pairing with other RNAs [34]. In addition, lncRNAs can play a carcinogenic role by mediating alternative splicing, transcription, and post-transcriptional regulation. There is abundant evidence that MALAT1 participates in the invasiveness and migration of tumor cells. In normal cells, the interplay between MALAT1 and various pre-mRNA splicing factors, such as SR splicing factors, allows it to affect pre-mRNA alternative splicing by modulating the distribution and activity of SR splicing factors [35]. In the setting of oral squamous cell carcinoma, inhibition of

MALAT1 expression prevents OSCC proliferation, while overexpression of MALAT1 expression promotes OSCC progression. The effect of MALAT1 on OSCC development has been further studied *in vivo*. Reportedly, in the established nude mouse model, down-regulation of MALAT1 significantly inhibited the tumor growth of OSCC [36]. In the present study, the mechanism of MALAT1 in Hep-2 cells was further investigated. We found that transfecting miR-362-3p inhibitor into si-MALAT1 transfected Hep-2 cells reversed the inhibitory effect of si-MALAT1 on the proliferation of Hep-2 cells and promoted cell invasiveness and migration. MALAT1 can sponge miR-362-3p and inhibit its expression. miR-362-3p was found to be under-expressed in LC samples from TCGA database and clinical LC samples. Besides, MALAT1 was significantly negatively correlated with miR-362-3p expression. Also, miR-362-3p has different functions in different tumors with certain tissue specificity. It plays an oncosuppressive role in renal and breast cancers, and an oncogenic role in gastric and liver cancers [24, 37, 38]. However, lncRNA contains a miRNA binding locus, which acts as a ceRNA to inhibit miRNA sponging and antagonize its function. For example, MALAT1 interferes with the growth of hepatocellular carcinoma tumor through sponging miR-200a [39]. Liu, et al. found that inhibition of MALAT1 could elevate the expression of miR-429 to suppress the progression of hypopharyngeal squamous cell carcinoma by reducing ZEB1 [40]. Also, researchers have demonstrated that MALAT1 knockdown suppressed the malignant behaviors of cancer cells *in vitro* by targeting miR-194. Therefore, MALAT1 and miR-194 may serve as novel therapeutic targets for hypopharyngeal squamous cell carcinoma [41].

In this study, FISH assay showed that MALAT1 was mainly located in the cytoplasm in Hep-2 cells. RNA pull-down, RIP, and DLR assays proved that MALAT1 was able to sponge miR-362-3p. Therefore, MALAT1 inhibits LSCC Hep-2 cell proliferation and invasiveness by modulating miR-362-3p. Besides, there would be downstream regulatory mechanisms of miR-362-3p which need to be further investigated. miRNAs usually perform their biological functions by pairing with 3'UTRs of target mRNA [42]. Yang, et al. [43] found that miR-362-3p inhibits migration and invasion by targeting BCAP31 in cervical cancer. Studies have found

that miR-217 inhibits laryngeal cancer metastasis by repressing AEG-1 and PD-L1 expression [44]. On the other hand, the downstream mechanism of miR-362-3p in laryngeal squamous cell carcinoma has not been reported; thus, more research and experiments need to be performed to show the mechanism of miR-362-3p.

In sum, MALAT1 is up-regulated in LC, while miR-362-3p is down-regulated. Knocking down MALAT1 can inhibit the proliferation of Hep-2 cells and reduce cell invasiveness and migration, which may be related to the sponging and regulation of miR-362-3p. This study provides important clues for further exploring MALAT1 as a ceRNA sponging miR-362-3p in the molecular mechanism of LC cell invasiveness and migration. However, there are also some limitations in this study. This study did not verify the cancer-promoting effect of MALAT1 on LSCC *in vivo*, nor did it verify whether MALAT1 plays a cancer promoting role through other ways. In addition, the downstream target genes and pathways of miR-362-3p have not been further verified. In general, the mechanism of MALAT1 regulating Hep-2 cell proliferation and invasion through miR-362-3p is still uncertain. Therefore, our task is to conduct in-depth studies at the animal level to further explore the molecular mechanism of MALAT1 affecting the occurrence and development of LSCC, providing new ideas for prevention and treatment.

Disclosure of conflict of interest

None.

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