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HOXA1 Promotes Migration, Invasion and Cell Cycle, and Suppresses Cisplatin Sensitivity of Laryngeal Cancer Cells By Mediating AKT/mTOR Pathway

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Homeobox A1 (*HOXA1*) is implicated in the progression of various cancers, but its biological function in laryngeal cancer (LC) remains undefined, which is the foothold of our study. Bioinformatics analysis and survival analysis were performed to predict *HOXA1* expression in LC tissues, and the prognostic relationship between high *HOXA1* expression and LC. Whether high *HOXA1* expression correlated with the clinical characteristics and prognosis of LC patients was analyzed. LC cell viability and sensitivity to cisplatin were determined by Methyl thiazolyl tetrazolium assay. The cell migration, invasion, and cell cycle after transfection were examined by Wound healing, Transwell, and flow cytometry assays, respectively. The corresponding mRNA and protein expressions were measured by quantitative real-time PCR or Western blot. A higher expression of *HOXA1* was detected in LC tissues, which was found to be relevant to poor prognosis of LC patients. The association of high expression of *HOXA1* with lymph node and clinical stage was also confirmed. Silencing of *HOXA1* in LC cells enhanced the cell sensitivity to cisplatin, inhibited viability, migration, invasion and cell cycle, and reduced N-Cadherin, Vimentin, PCNA, p-AKT and p-mTOR expressions, while overexpression of *HOXA1* had the opposite effects. Collectively, *HOXA1* boosts migration, invasion and cell cycle, while suppressing cisplatin sensitivity of LC cells by mediating AKT/mTOR pathway, hinting that *HOXA1* is a promising biomarker for diagnosis and prognosis of LC in clinical practice.

Keywords: AKT/mTOR pathway; homeobox A1; invasion; laryngeal cancer; migration
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Introduction

Laryngeal cancer (LC) is one of the most prevailing malignant tumors in head and neck cancer, of which laryngeal squamous cell carcinoma (LSCC) occupies a high proportion, with a high recurrence rate as well as a low survival rate (Thompson 2017; Mai and Ma 2019; Xiong et al. 2019). The development of modern medical technology contributes to the clinical diagnosis and treatment of LC (Obid et al. 2019). However, owing to the complex biological behaviors of LC, problems, such as low early diagnosis rate and the poor prognosis of advanced LSCC, still exist (Chai et al. 2014; Cossu et al. 2019; Sheng et al. 2019). Therefore, the search for new therapeutic target biomarkers and prognostic molecular markers has become one of the main directions of current LC research.

The postoperative recurrence and 5-year survival rate of LC are related to the primary site of tumor, the pathological type and differentiation of tumor, the lymph node metastasis, etc., and the diversity of LC clinical manifestations interferes in prognostic assessment (Gross et al. 2013; Zhao et al. 2016). At present, more new accurate prognostic indicators of LC have been found at the cellular and molecular levels by cell molecular biology techniques (Kara et al. 2017). For instance, the red blood cell distribution width and estrogen receptor α (ERα) have been identified to

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serve as reliable predictors of prognosis in LC; tight junction proteins (claudin-1, claudin-3, claudin-7 and claudin-8) and Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2 (PLOD2) may be useful molecular markers for the diagnosis and prognosis of LC; and various microRNAs (miRs), long non-coding RNAs (lncRNAs) and mRNAs, as the molecular technology progresses, have been confirmed to function as effective diagnostic and prognostic biomarkers for LC (Bradford et al. 2014; Zhang et al. 2017; Gao et al. 2018; Cossu et al. 2019; Fan et al. 2019; Fang et al. 2019; Sheng et al. 2019; Verma et al. 2019; Zhou et al. 2019).

Homeobox (HOX) genes can regulate biological processes, and participate in migration, apoptosis and proliferation of LC cells as well as drug resistance of tumor cells (de Barros et al. 2016; Bhatlekar et al. 2018; Casaca et al. 2018). The homeobox A cluster (HOXA) gene family belongs to HOX genes, several members of which are overexpressed in LC tissues including HOXA10, HOXA11 and HOXA13 (Li et al. 2020). Over the past few years, increasing discoveries have highlighted the role of *HOXA1* in different cancers. *HOXA1* expression is markedly up-regulated and is implicated in lymphatic metastasis in bladder cancer (Chen et al. 2022). In breast cancer, the expression of *HOXA1* is associated with poor prognosis and tumor progression (Zhang et al. 2006, 2019b; Liu et al. 2019). It has been found in breast cancer and ovarian cancer cells that miR-99a restrains cell invasion by targeting *HOXA1* based on modulation of the protein kinase B (AKT)/mTOR signaling pathway (Wang et al. 2015b; Zhang et al. 2019a). However, there are no available reports on the role of *HOXA1* in LC.

Collectively, the purpose of our study is mainly to fathom out the role of *HOXA1* in LC and preliminarily shed light on the corresponding potential mechanism.

Methods and Materials

Ethics statement

This study was approved by the Ethics Committee of Inner Mongolia Medical University (Approval No. YKD202202002). Prior to the collection of tumor tissues and corresponding normal tissue samples, all patients and their families had signed the written informed consent.

Tissue specimens and reagent preparation

Seventy pairs of LC tissues and corresponding normal tissue samples were collected from LC patients in the Inner Mongolia Medical University from March 2015 to April 2015. Clinical characteristics of these patients were recorded in Table 1.

Cisplatin ($Cl_2H_4N_2Pt$, CAS No. 15663-27-1, HY-17394) was acquired from MedChemExpress (Monmouth Junction, NJ, USA). N, N-Dimethylformamide (DMF, HY-Y0345, MedChemExpress, USA) was employed to prepare cisplatin stock solution.

Bioinformatics analysis

Firstly, *HOXA1* expression in tumor (n = 519) and normal (n = 44) samples was analyzed using TCGA and GTEx databases in the Gene Expression Profiling Interactive Analysis (GEPIA2) website (http://gepia.cancer-pku.cn/). Secondly, the correlation between high (n = 208) or low (n = 208) *HOXA1* expression and overall survival of HNSC patients was dissected by GEPIA2 website.

Cell culture

One normal human laryngeal cell line HuLa-PC (CRL-3342, American Type Culture Collection, Manassas, VA, USA) and four human laryngeal squamous cell lines including AMC-HN-8 (CL0666, Fenghui Biotechnology, Changsha, China), SNU-46 (CTCC-001-0175, Amyjet Scientific, Wuhan, China), SNU-899 (CTCC-001-0176, Amyjet Scientific, China) and SNU-1076 (CTCC-001-0174, Amyjet Scientific, China) were selected for this experiment. All cell lines were rest in Dulbecco's modified Eagle's medium (DMEM; PM150210, Procell, Wuhan, China) containing 10% fetal bovine serum (FBS; 10091, Gibco, Grand Island, NY, USA) and 1% Penicillin-Streptomycin (V900929, Sigma-Aldrich, Missouri, MO, USA). The culture dish was placed in a cell incubator (Forma 370, Thermo fisher, Massachusetts, CA, USA), and the culture medium was changed every 2 days.

Cell transfection

The *HOXA1* overexpression vector (primer that amplifies overexpression sequences: Forward (F): 5′-GTGGGCTCGCCTCAATACAT-3′, Reverse (R): 5′-TTTTGGCTTTTGAAGGGAGTTC-3′), and short hairpin RNA (shRNA) targeting *HOXA1* (shHOXA1; 5′-CTGGCCCTGGCTACGTATAAT-3′) were purchased from GenePharma Company (Shanghai, China). The empty vector (Vector) was used as negative control (pcDNA3.1; V79520, Invitrogen, Carlsbad, CA, USA). The synthesized *HOXA1*, shHOXA1 and Vector were separately transfected into AMC-HN-8 and SNU-46 cells according to the instructions of Lipofectamine™ 2000 Transfection Reagent (11668027, Thermo Fisher, USA).

Cell treatment

To elucidate the effects of *HOXA1* on the sensitivity of AMC-HN-8 and SNU-46 cells to cisplatin, these cells were treated with 5, 10, 30, and 50 μM cisplatin for 48 h at 37°C (Jia et al. 2020).

Wound healing assay

The transfected cells were collected, adjusted to a concentration of approximately 2×10^3/ml, and incubated with 5% CO_2 at 37°C. When the cell confluence reached 95%, a line was drawn vertically on the back of the 6-well plate, and cells were scratched with a disinfected 10 μL white tip perpendicular to the line, followed by washing with phosphate buffered saline (PBS; P4417, Sigma-Aldrich, USA).

Table 1. Association between HOXA1 and clinicopathological characteristics in patients with laryngeal cancer.

Features	No. of cases	*HOXA1* expression		P-value
		Low (n=35)	High (n=35)	
Age				0.454
<60	25	14	11	
≥60	45	21	24	
Sex				0.788
male	51	25	26	
female	19	10	9	
Smoking				0.143
Nonsmokers	42	18	24	
Current smokers	28	17	11	
Drinking				0.086
drinker	43	18	25	
nondrinker	27	17	10	
Differentiation				0.385
well	27	13	14	
Moderate	33	15	18	
Poor	10	7	3	
Lymph node				<0.001
Negative	42	28	14	
Positive	28	7	21	
Tumor depth (pT)				0.504
T1	16	9	7	
T2	21	12	9	
T3	18	9	9	
T4	15	5	10	
Clinical stage				0.034
I	19	7	12	
II	15	4	11	
III	31	21	10	
IV	5	3	2	

Table 2. Sequence of Primers.

Gene	Sequence (5'-3')
HOXA1 forward	AAGCAGACGTAAGTGGTGGG
reverse	AGTCAAAAGTCTGCGCTGGA
PCNA forward	TGTTGGAGGCACTCAAGGAC
reverse	TAGGTGTCGAAGCCCTCAGA
N-Cadherin forward	GGGAAATGGAAACTTGATGGCA
reverse	TGGAAAGCTTCTCACGGCAT
Vimentin forward	CAGGACTCGGTGGACTTCTC
reverse	TAGTTGGCGAAGCGGTCATT
GAPDH forward	ATTGACCTCAACTACATGGT
reverse	CATACTTCTCATGGTTCACA

The above-treated cells were incubated with serum-deprived DMEM in 6-well plates, followed by observation and photographing at 0 h and 24 h under an inverted microscope (DMi8, Leica, Weztlar, Germany) at × 100 magnification. The relative distance of cell migration was measured by Imagej-1.38x software (National Institutes of

Health, Bethesda, MD, USA).

Transwell assay

The transfected cells were washed with serum-free medium, and transferred into the upper Transwell chambers (ECM550, Sigma-Aldrich, USA), together with 200 μL serum-free DMEM. 700 μL DMEM enriched with 10% FBS was added to the lower Transwell chambers. After 24-h incubation, the culture medium in the Transwell chamber was discarded, and cells failing to pass through the membrane were wiped off by a cotton swab. Cells invading into the lower chambers were fixed using 4% paraformaldehyde solution (P395744, Aladdin, Shanghai, China) for 15 min, and dyed by 0.1% crystal violet (C196471, Aladdin, China) for 10 min in the dark at room temperature, followed by observation under an inverted microscope (magnification × 250), and cell counting with Imagej-1.38x software.

Cell cycle detection

The transfected AMC-HN-8 and SNU-46 cells were transferred to centrifuge tubes, washed 1-2 times with PBS, and fixed by precooled 75% ethanol solution that was prepared with 100% ethanol (E7023, Sigma-Aldrich, USA) for 12 h at 4°C. The cells were later centrifuged at 1,500 rpm/min in a centrifuge (75006590, Thermo Fisher, USA) for 5 min, and underwent 30-min incubation with 100 μL Rnase A (R6148, Sigma-Aldrich, USA) at 37°C. Next, the appropriate amount of propidium iodide (PI; ST511, Beyotime, China) was added into centrifuge tube for staining at 4°C for 30 min in the dark. Finally, a flow cytometer (CytoFLEX, Beckman Coulter, Fullerton, CA, USA) was applied for cell cycle detection.

Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from the homogenized tissues and the transfected AMC-HN-8 and SNU-46 cells by the Beyozol reagent (R0011, Beyotime, China). Subsequently, the purity and concentration of the dissolved RNA were detected by UV spectrophotometer (NanoDrop One, Thermo Fisher, USA) and Agarose gel (ST004Q, Beyotime, China), after which the RNA was stored in a −80°C freezer. RNA was reverse-transcribed into cDNA according to the manual of PrimeScript™ RT Reagent Kit (RR037A, Takara, Tokyo, Japan), followed by PCR amplification. Expression of *HOXA1* was quantified by iQ5 qRT-PCR system (Bio-Rad, Hercules, CA, USA), with SYBR Premix (D7260) purchased from Beyotime (China). GAPDH acted as the reference gene. All primer sequences in this study were displayed in Table 2. The results were analyzed using the 2-$\Delta\Delta$ct method.

Western blot

The transfected AMC-HN-8 and SNU-46 cells were fully lysed with RIPA lysate (89900, Thermo Fisher, USA) to extract total protein. The protein concentration was determined following the specification of the Pierce™ Rapid Gold BCA Protein Assay Kit (A53227, Thermo Fisher, USA). Equal amounts (25 μL) of protein samples were added to the sample wells of the prepared separation gel (P0012AC, Beyotime, China) for electrophoresis. Afterwards, the gel was removed and placed into Tris Buffered Saline with Tween solution (TBST; T9039, Sigma-Aldrich, USA) for 2 min. Then, the protein was transferred to a polyvinylidene fluoride (PVDF) membrane (PFL00010c, Sigma-Aldrich, USA) that had been soaked in methanol solution (34860, Sigma-Aldrich, USA) in advance. The PVDF membrane was washed by TBST solution, and treated with blocking solution prepared from skimmed milk powder (LP0033B, Thermo Fisher, USA) for 1 h at room temperature. After being cultivated with diluted primary antibody overnight at 4°C, and washed again with TBST, the membrane was cultured with secondary antibody at 37°C for 1 h. All antibody information and sources were exhibited in Table 3. Finally, the ECL solution (32134, Thermo Fisher, USA) was evenly dropped on the PVDF membrane, followed by photography with an automatic chemiluminescence image analysis system (Bio-Best, SIM, CA, USA), and analysis using image software.

Methyl thiazolyl tetrazolium (MTT) assay

Cells after transfection and cisplatin treatment were collected, and 100 μL of cell suspension was added into each well of 96-well plate for 24/48-h incubation. Next, cells in each well were successively reacted with 10 μL MTT solution (M6494, Thermo Fisher, USA) for 4 h, and 100 μL dimethyl sulfoxide (S11366, Thermo Fisher, USA). The absorbance of each well was measured using a microplate reader (51119180, Thermo Fisher, USA), with the excitation wavelength of 570 nm.

Statistical analysis

Measurement data were described by mean ± standard deviation. The data in Fig. 1C were analyzed by paired samples t-test and those in multiple groups were compared by one-way ANOVA. Enumeration data were analyzed by Chi-square test or Fisher's exact test, and Kaplan-Meier method was used to assess the correlation between *HOXA1* expression and overall survival of HNSC patients. All statistical analyses were implemented using GraphPad 8.0 software, and the statistical significance was defined when $P < 0.05$.

Results

HOXA1 expression was elevated in LC, and high HOXA1 expression was implicated in poor prognosis including lymph node and clinical stage

First, the bioinformatics analysis indicated that the expression of *HOXA1* was pronouncedly higher in LC patient tissues than in normal tissues (Fig. 1A, $P < 0.05$), and the high *HOXA1* expression in LC was implicated in poor prognosis based on the GEPIA2 database (Fig. 1B, P =

Table 3. Information of antibodies.

Antibodies	Catalog number	Host Species	Dilution ratio	Detected molecular mass (kDa)	Manufacturer
anti-HOXA1 antibody	ab230513	rabbit	1:1000	36	Abcam (Cambridge, UK)
anti-N-Cadherin antibody	ab18203	rabbit	1:1000	130	
anti-Vimentin antibody	ab92547	rabbit	1:000	54	
anti-PCNA antibody	ab92552	rabbit	1:1000	29	
anti-p-AKT antibody	ab38449	rabbit	1:1000	56	
anti-AKT antibody	ab8805	rabbit	1:500	55	
anti-p-mTOR antibody	ab109268	rabbit	1:1000	289	
anti-mTOR antibody	ab2732	rabbit	1:2000	289	
anti-GAPDH antibody	ab8245	mouse	1:1000	36	
HRP-conjugated mouse anti-rabbit IgG	ab99697	mouse	1:1000	N/A	
HRP-conjugated rabbit anti-mouse IgG	ab6728	rabbit	1:2000	N/A	

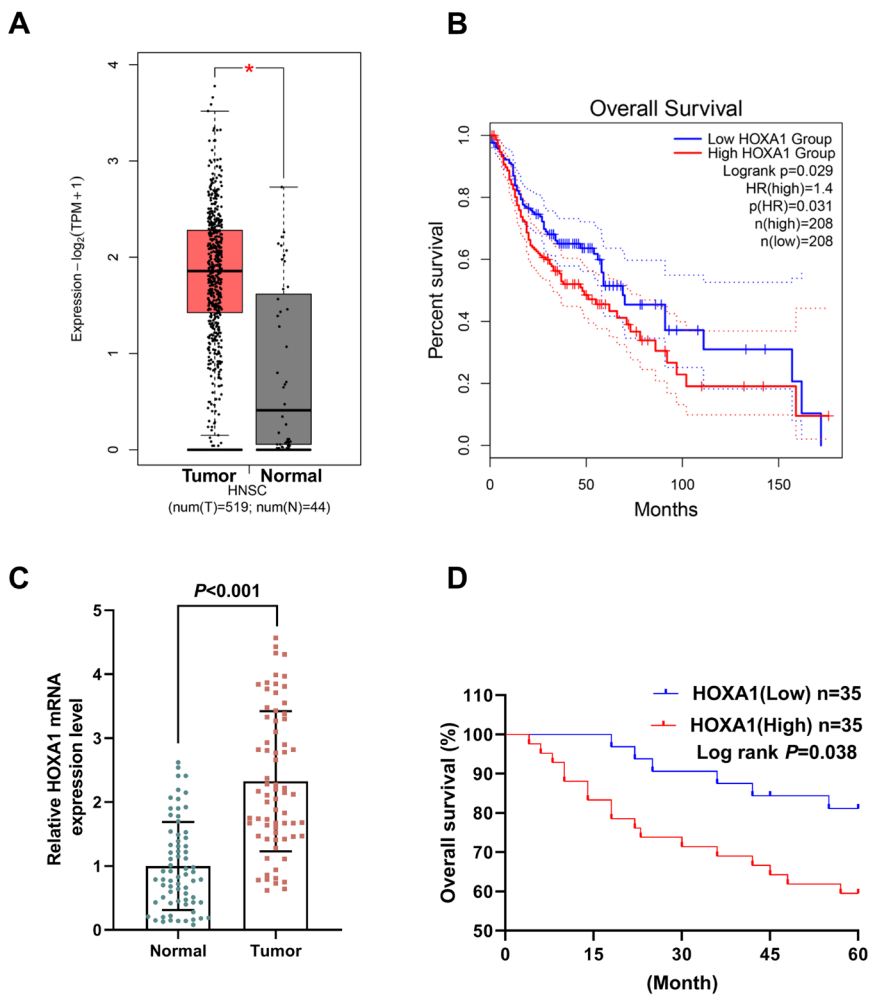

Fig. 1. *HOXA1* expression was up-regulated in LC tissues, and high *HOXA1* expression was associated with poor prognosis. (A) The expression of *HOXA1* in LC tissues and normal tissues was analyzed by bioinformatics. T (tumor samples) = 519, N (normal samples) = 44. (B) After sorting the patient samples according to *HOXA1* expression from high to low, the first 50% samples were high expression, and the last 50% samples were low expression. The prognostic relationship between high *HOXA1* expression and LC was studied by survival analysis, n (high expression samples) = 208, n (low expression samples) = 208. (C) The expression of *HOXA1* in LC tissues and normal tissues was examined by qRT-PCR, and GAPDH was used as a reference gene. (D) High *HOXA1* expression in LC was associated with poor prognosis. *P < 0.05 vs. Normal.
LC: laryngeal cancer; qRT-PCR: Quantitative real-time PCR; *HOXA1*, homeobox A1.

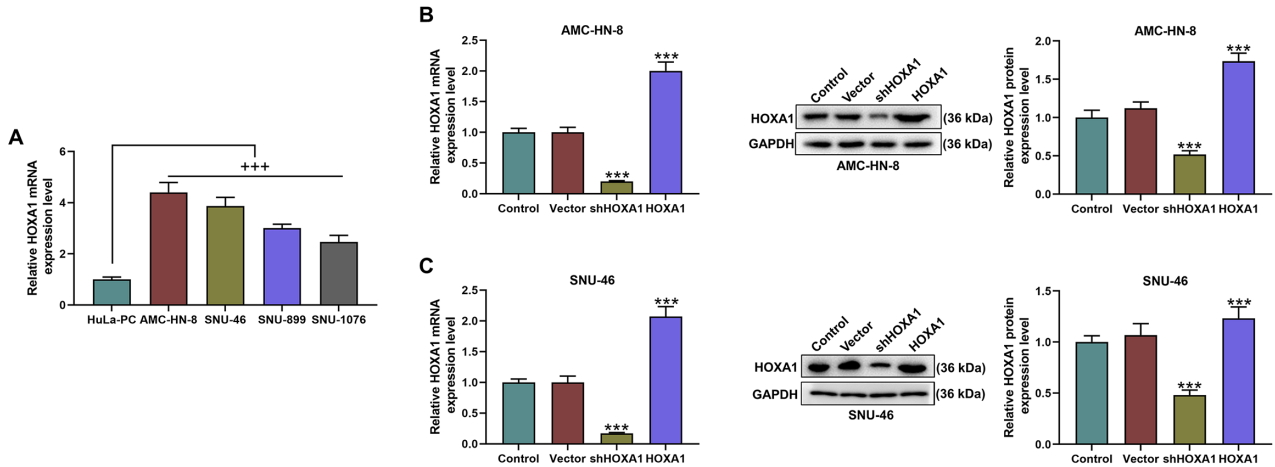

Fig. 2. *HOXA1* expression was up-regulated in LC cell lines.
(A) The expression of *HOXA1* in LC cells and normal cells was examined by qRT-PCR, and GAPDH was used as a reference gene. (B) The expression of *HOXA1* in transfected AMC-HN-8 cells was detected by qRT-PCR and Western blot, where GAPDH was used as a reference gene. (C) The expression of *HOXA1* in transfected SNU-46 cells was measured by qRT-PCR and Western blot, where GAPDH was used as a reference gene. $^{+++}P < 0.001$ vs. HuLa-PC; ***P < 0.001 vs. Vector. ShHOXA1: short hairpin RNA targeting *HOXA1*.

0.031). In addition, our results showed that *HOXA1* was highly expressed in cancer tissues (Fig. 1C, P < 0.001), and the high expression of *HOXA1* in LC correlated with poor prognosis of patients in our cohort (Fig. 1D, P = 0.038). Clinical characteristics of these patients, including the age, sex, smoking, drinking, differentiation, tumor depth, lymph node and clinical stage, were recorded in Table 1. The results showed that the high expression of *HOXA1* positively correlated with lymph node and clinical stage in LC patients (Table 1). Additionally, *HOXA1* expression was also confirmed to be up-regulated in cancer cells (Fig. 2A, P < 0.001).

ShHOXA1 inhibited migration, invasion and cell cycle, whereas overexpression of HOXA1 had the opposite effect on LC cells

According to Fig. 2B, C, shHOXA1 obviously inhibited, whereas *HOXA1* overexpression plasmid promoted the expression of *HOXA1* in AMC-HN-8 and SNU-46 cells (P < 0.001). MTT and Wound healing assay results corroborated that *HOXA1* silencing significantly dampened, while *HOXA1* overexpression facilitated cell viability and migration (Fig. 3A, B, D, P < 0.001). The data in Fig. 3C, E uncovered that silencing of *HOXA1* brought about weakened cell invasive ability, while overexpression of *HOXA1* led to the opposite trend (P < 0.001). The experimental data showed that silencing of *HOXA1* suppressed the cell cycle and the cell cycle was arrested at the G0/G1 phase. Also, shHOXA1 markedly reduced the S and G2/M phases of the cell cycle, but overexpression of *HOXA1* had the opposite effect on the cell cycle of transfected AMC-HN-8 and SNU-46 cells (Fig. 4A, B, P < 0.05).

ShHOXA1 suppressed N-Cadherin, Vimentin and PCNA expressions, and activation of AKT/mTOR signaling pathway, whereas HOXA1 overexpression did conversely in LC cells

In addition, the expressions of epithelial-mesenchymal transition (EMT)-related factors (N-Cadherin and Vimentin) as well as cell proliferation-related factor (PCNA) were quantified through qRT-PCR and Western blot. The outcomes reflected that shHOXA1 obviously inhibited but *HOXA1* overexpression promoted the expressions of N-Cadherin, Vimentin and PCNA in AMC-HN-8 and SNU-46 cells (Fig. 5A, B, P < 0.05). Similarly, the data demonstrated that shHOXA1 declined p-AKT and p-mTOR expressions and the ratios of p-AKT/AKT and p-mTOR/mTOR, while *HOXA1* overexpression had the opposite effect on the activation of AKT/mTOR signaling pathway (Fig. 5C, D, P < 0.05).

ShHOXA1 enhanced LC cell sensitivity to cisplatin, whereas HOXA1 overexpression did conversely

Moreover, our study also probed into the effects of *HOXA1* on LC cell sensitivity to cisplatin by MTT assay. As displayed in Fig. 6A, B, *HOXA1* overexpression led to the increased viability of 30 μM cisplatin-treated AMC-HN-8 and SNU-46 cells, yet shHOXA1 did oppositely (P < 0.05). In addition, *HOXA1* knockdown lessened the IC50 value of AMC-HN-8 and SNU-46 cells, while its overexpression upregulated the IC50 value of two cell lines (Fig. 6C, D, P < 0.05).

Discussion

It has been confirmed that multiple members of HOXA gene family are involved in the pathogenesis of LC, including the abnormal expressions of HOXA2, HOXA4, HOXA7 and HOXA9, and M stage and gender of LC patients are relevant to the prognosis (Li et al. 2020). There

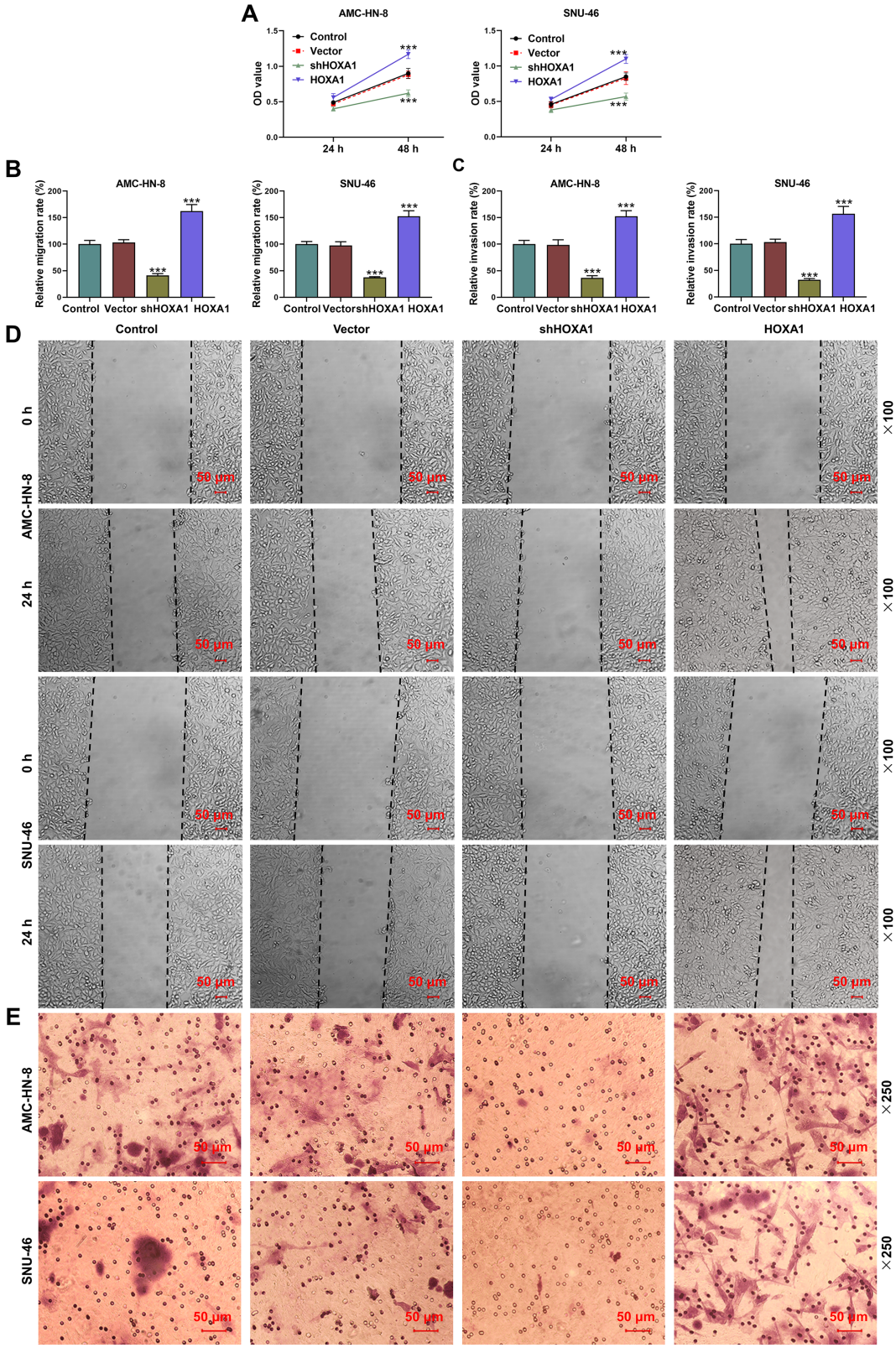

Fig. 3. ShHOXA1 inhibited viability, migration, and invasion of LC cells, whereas overexpression of *HOXA1* had opposite effects on LC cells.

(A) The viability of the transfected cells was measured by MTT assay. (B, D) The migration ability of the transfected cells was examined by Wound healing assay (magnification: ×100; scale: 50 μm). (C, E) The invasion ability of the transfected cells was determined by the Transwell assay (magnification: × 250; scale: 50 μm). ***P < 0.001 vs. Vector. MTT: Methyl thiazolyl tetrazolium.

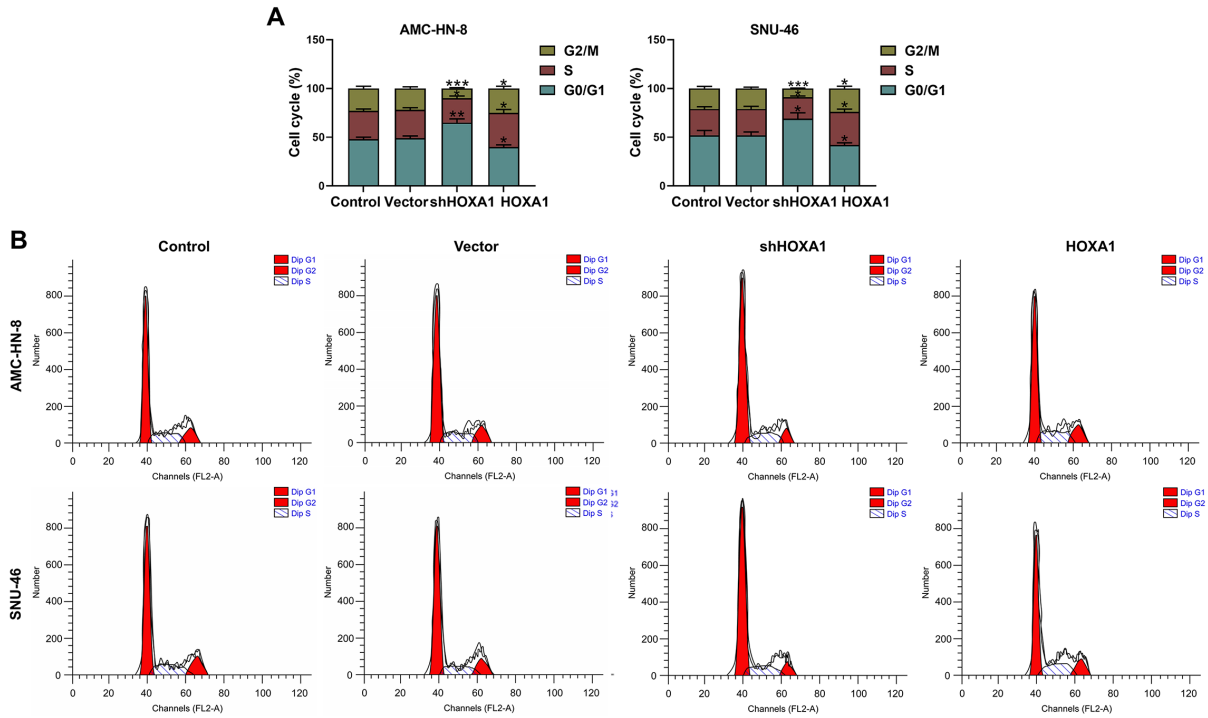

Fig. 4. ShHOXA1 inhibited the cell cycle of LC cells, whereas overexpression of *HOXA1* had opposite effects on LC cells. (A, B) The cell cycle of the transfected cells was tested by flow cytometry. *P < 0.05, **P < 0.01, ***P < 0.001 vs. Vector.

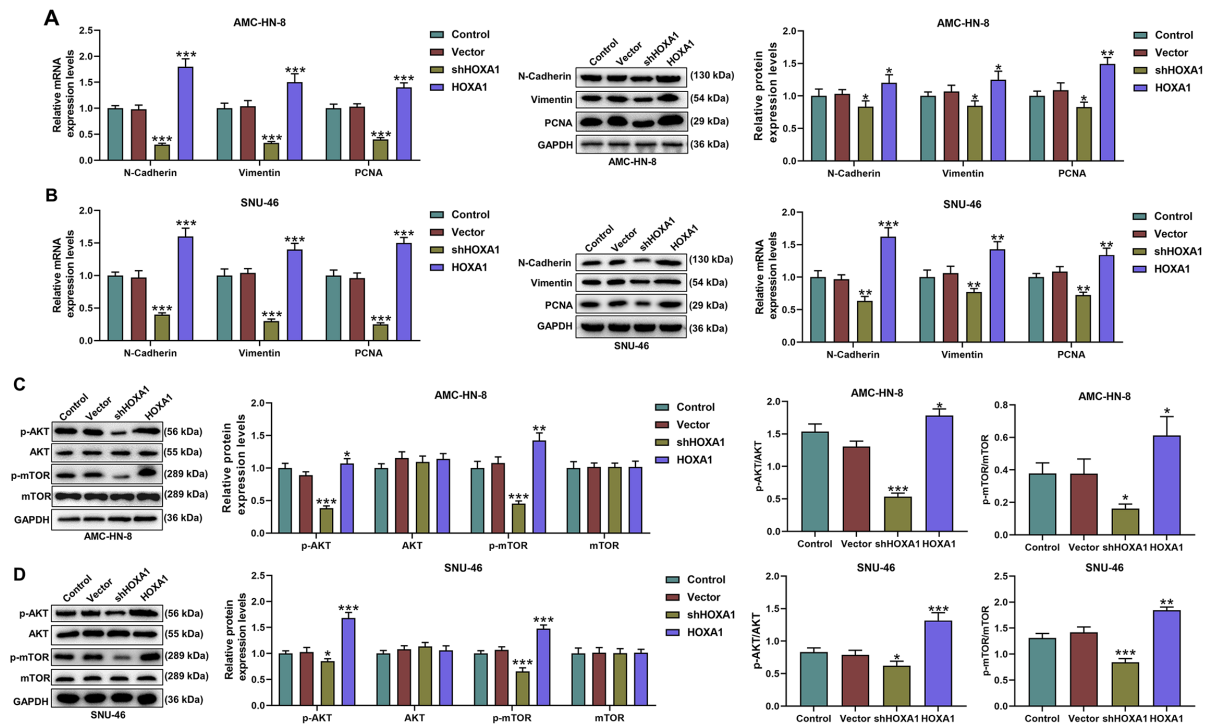

Fig. 5. ShHOXA1 suppressed N-Cadherin, Vimentin and PCNA expressions, and activation of AKT/mTOR signaling pathway, while *HOXA1* overexpression did conversely in LC cells.
(A, B) The expressions of N-Cadherin, Vimentin and PCNA in transfected AMC-HN-8 and SNU-46 cells were examined by qRT-PCR and Western blot, with GAPDH as a reference gene. (C, D) The expressions of p-AKT, AKT, p-mTOR and mTOR in transfected AMC-HN-8 and SNU-46 cells were quantified by Western blot, with GAPDH as an internal loading control. *P < 0.05, **P < 0.01, ***P < 0.001 vs. Vector. EMT: Epithelial-Mesenchymal Transition; AKT: protein kinase B; p-AKT: phosphorylated AKT; mTOR: mammalian target of rapamycin; p-mTOR: phosphorylated mTOR; PCNA, proliferating cell nuclear antigen.

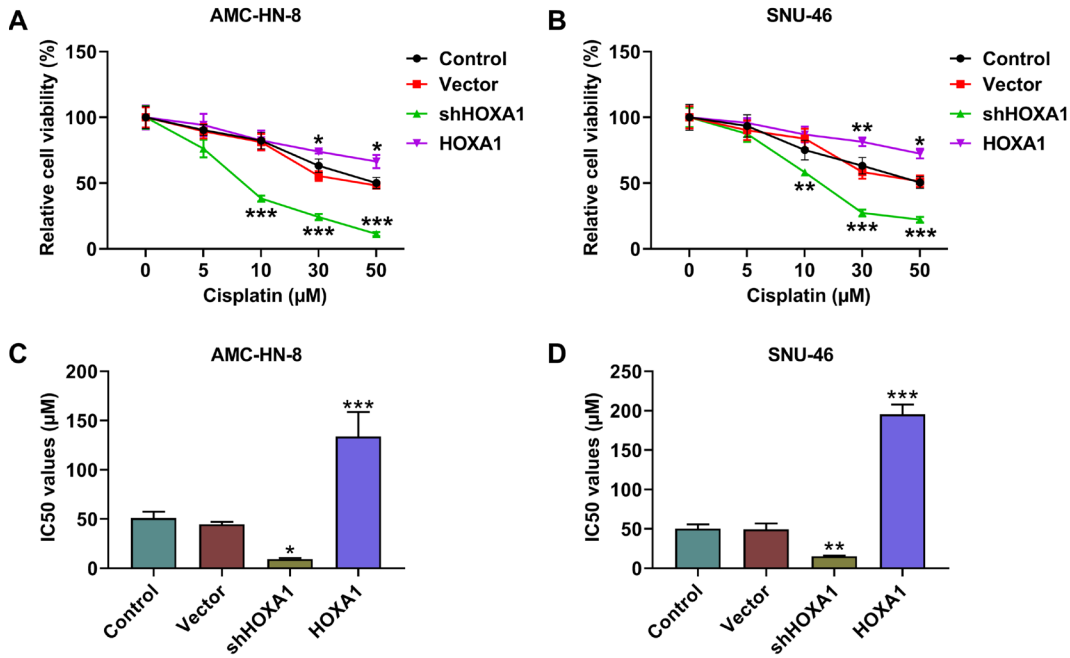

Fig. 6. ShHOXA1 enhanced LC cell sensitivity to cisplatin, whereas HOXA1 overexpression did conversely. (A, B) Viability of transfected AMC-HN-8 and SNU-46 cells following the treatment of 5, 10, 30, and 50 μM cisplatin for 48 h was determined by MTT assay. (C, D) IC50 values of transfected AMC-HN-8 and SNU-46 cells. ***P < 0.001 vs. Vector.

are studies evidencing that in addition to genetic mutations, diabetes and human papillomavirus (HPV) infection, bad lifestyle habits such as smoking and alcohol consumption also contribute to the increased incidence of LC (Menicagli et al. 2017; Beynon et al. 2018). Accordingly, we wondered whether *HOXA1* was abnormally expressed in LC, and can act as a prognostic biomarker for LC. First, through bioinformatics analysis and qRT-PCR experiments, we uncovered highly expressed *HOXA1* in LC tissues, which correlated with lymph node, clinical stage, and poor prognosis in LC patients. Still, further study is needed to explore the specific role of *HOXA1* impacting the progression of LC.

Next, our study was undertaken to explore the possible function of *HOXA1* in LC. Heaps of studies have verified its involvement in other cancers, especially breast cancer (Belpaire et al. 2022), and gastric cancer (Yuan et al. 2016), to name but a few. *HOXA1* blocks breast cancer cell apoptosis, and enhances cell viability and proliferation abilities by activating related signaling pathways (Zhang et al. 2006; Mohankumar et al. 2007). Similarly, in endometrial cancer and prostate cancer, *HOXA1* promotes cell proliferation, migration, and invasion, while in gastric cancer, down-regulation of *HOXA1* inhibits the cell cycle (Wang et al. 2015a; Yuan et al. 2016; Li et al. 2019). In this study, we also found that a low expression of *HOXA1* in LC cells attenuated cell viability, migration, invasion, and cell cycle, but the effect of overexpressed *HOXA1* was opposite, demonstrating that the role of *HOXA1* in LC was similar to that in other cancer cells (Yuan et al. 2016; Li et al. 2019). However, the mechanism of *HOXA1* participating in LC development remains an imperative problem to be explored.

EMT plays a key role in cancer development (Du and Shim 2016). N-Cadherin and Vimentin are both EMT-related factors that are up-regulated in malignant tumors. Of them, N-Cadherin belongs to the Cadherin family that mainly mediates cell adhesion and migration, and Vimentin has a positive effect on maintaining the shape of cells and stabilizing the cytoskeleton (Li et al. 2016; Yamashita et al. 2018). PCNA is instrumental in cell proliferation related to DNA synthesis, which is usually up-regulated in cancer cells (Lv et al. 2016). In addition, *HOXA1* can contribute to the progression of breast cancer through activating PTEN/PI3K/AKT and Wnt/catenin signaling pathways (Zhang et al. 2019b). *HOXA1* inhibits the ovarian cancer cell invasion via regulating AKT/mTOR signaling pathway (Zhang et al. 2019a). Our data revealed that *HOXA1* overexpression in LC cells promoted the expressions of N-Cadherin, Vimentin and PCNA as well as the activation of the AKT/mTOR signaling pathway, whereas shHOXA1 had the opposite effects in LC cells. Hence, these data altogether demonstrated that *HOXA1* promoted the progression of LC by regulating the expressions of related factors through the AKT/mTOR signaling pathway.

As previously mentioned, *HOXA1* has been recognized as an available biomarker predicting the radioresistance in various tumors via different signaling pathways (He et al. 2022). Cisplatin resistance is a vital factor that leads to the poor prognosis of LC (Yi et al. 2021). It has been validated that *HOXA1* correlates to cisplatin resistance in lung adenocarcinoma (Sui et al. 2022). Nevertheless, little was known

about the effects of *HOXA1* on cisplatin resistance in LC. In our study, we uncovered that shHOXA1 enhanced LC cell sensitivity to cisplatin, whereas *HOXA1* overexpression did conversely. In light of this, we rationally inferred that as a biomarker of LC, *HOXA1* accelerated the migration, invasion, and cell cycle of LC cells through the AKT/mTOR pathway.

In conclusion, our study confirms that *HOXA1* is a prognostic biomarker in LC, which facilitates migration, invasion and cell cycle, and attenuates cisplatin sensitivity of LC cells by mediating AKT/mTOR pathway. These findings shed light on the potential clinical application of *HOXA1* in LC therapy. Nevertheless, the present study is limited to assays on the cellular level. Further research on animal models are required to validate the biological role of *HOXA1* in LC, and more studies will be conducted to explore the effect of *HOXA1* on other head and neck cancers. Moreover, in-depth research on mechanisms of *HOXA1* will be supplemented in the future; for example, the mutation status of PIK3CA in LC cell lines will be investigated.

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Conflict of Interest

The authors declare no conflict of interest.

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