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Original Article

HOTAIR promotes proliferation, migration and invasion of esophageal squamous cell carcinoma by regulating MAPK1

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Abstract: Hox transcript antisense intergenic RNA (HOTAIR), a lncRNA expresses from the HOXC locus but represses transcription in more distal HOXD locus and genes on other chromosomes, is overexpressed in esophageal squamous cell carcinoma (ESCC) cell lines and patient samples. HOTAIR could promote ESCC cell proliferation and metastasis, but the underlying mechanism was unclear. The objective of this study was to evaluate the interaction between HOTAIR and mitogen-activated protein kinase 1 (MAPK1) and potential mechanism of HOTAIR in the tumorigenesis and progression of ESCC. In our study, the results of qRT-PCR and western blot showed that expression of HOTAIR and MAPK1 were increased in ESCC patient samples while microRNA (miR)-217 decreased compared with normal tissues, and the expression levels of HOTAIR was correlated with the disease stage. Silencing HOAIR or MAPK1 by shRNA could down-regulate the expression level of each other, but co-silenced with dsRNA endoribonuclease Dicer abolished these effects. These results indicate that HOTAIR might be a ceRNA of MAPK1. Bioinformatics analysis predicts that miR-217 is shared by HOTAIR and MAPK1. MiR-217 mimics could suppress the expression of HOTAIR and MAPK1 in TE1 and KYSE520 while silencing HOTAIR or MAPK1 could increase the expression level of miR-217, indicating the interaction of HOTAIR and MAPK1 was mediated by miR-217. Luciferase reporter assay confirmed this. The cell proliferation, migration and invasion *in vitro* and tumor formation *in vivo* of TE1 or KYSE520 were inhibited or decreased after HOTAIR silencing with decreased expression of MAPK1 and activation of its downstream p90RSK. In conclusion, HOTAIR and MAPK1 were up-regulated in ESCC patient samples and HOTAIR could regulate cell proliferation, migration, invasion and tumor formation of ESCC cell line by regulating MAPK1.

Keywords: Hox transcript antisense intergenic RNA (HOTAIR), esophageal squamous cell carcinoma (ESCC), long non-coding RNAs (LncRNAs), miR-217, mitogen-activated protein kinase 1 (MAPK1)

Introduction

Human esophageal cancer (EC) is the eighth most common and sixth leading causes of cancer-related mortality worldwide [1]. According to the cancer statistics data, there would be 16,940 new cases of EC in USA in 2017 [2] and 477,900 new cases in China in 2015 [3], ranking the 21th in USA and 3rd in China among all cancers. These data indicated a different incidence of EC in Western countries and East Asia. There are two major types of EC: esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC), with different risk factors and clinicopathological features. ESCC is the most frequent subtype of EC, and more than 90% EC were ESCC in China [4]. ESCC is one of the most aggressive and lethal malignancies that have a five-year survival rate of only 15-34% [5], mainly due to the advanced stage at initial diagnosis and lacking for efficacious therapies. The risk factors known for ESCC are chronic smoking and alcohol consumption. In recent years, the identification and characterization of molecular alternations in ESCC by tumor sample sequencing had detected a variety of somatic mutations such as TP53, CDKN2A, FAT1, NOTCH1, PIK3CA and KMT2D, and elucidation of molecular changes could improve disease diagnosis and therapy.

Long non-coding RNAs (lncRNAs), non-protein coding transcripts longer than 200 bases, have been intensively studied in recent years and more than 5000 human lncRNAs founded. Accumulated evidences showed that lncRNAs

were involved in many biological process and many of them act as scaffolds to regulate the interaction between molecular such as DNA and protein [6]. The expression level of lncRNAs was deregulated in many cancers, indicating a potential role in tumorigenesis [7]. Hox transcript antisense intergenic RNA (HOTAIR), a lncRNA expresses from the HOXC locus but represses transcription in more distal HOXD locus and genes on other chromosomes, is overexpressed in many cancers such as breast cancer, gastric cancer, liver cancer and sarcoma [8-11]. The two binding domains of HOTAIR are known to interact with the polycomb repressive complex 2 (PRC2), a histone H3 lysine 27 (H3K27) methylase which involves in the transcriptional regulation of thousands of genes, and lysine-specific demethylase 1 (LSD1) complexes, respectively. The HOTAIR-PRC2-LSD1 complex could target silencing the genes on chromosome 2 and *in vitro* analysis revealed that elevated HOTAIR expression could promote cancer cells proliferation, invasion and metastasis, while inhibit apoptosis [12]. Recent studies also revealed that HOTAIR was overexpressed in ESCC cell lines and patient samples and could promote ESCC cell proliferation and metastasis, but underlying mechanism needed to be elucidated [13].

The MAP kinase signaling cascade Ras/Raf/MEK/ERK takes parts in multiple cellular processes such as cell proliferation, differentiation, migration and survival. Deregulation of this pathway is commonly occurred in cancers. Mitogen-activated protein kinase (MAPK) 1 (also known as extracellular regulated protein kinases 2, ERK2), the effector kinase of this signaling cascade, was showed to elevate expression in ESCC and participate in ESCC progression [14]. MAPK1 is a target gene of miR-217 and overexpressed miR-217 could suppress the expression of MAPK1 in cancer cells [15]. In 2011, a "competitive endogenous RNA" (ceRNA) hypothesis was brought up by Leonardo Salmena et al [16] and this hypothesis elucidated a crosstalk between lncRNAs, mRNAs and microRNAs by microRNA response elements (MREs). In this study, we investigated interaction between HOTAIR and MAPK1 in ESCC and elucidated the potential mechanism of HOTAIR in the tumorigenesis and progression of ESCC.

Materials and methods

Patient samples

The ESCC patient samples and matched adjacent normal tissues were obtained from The First Affiliated Hospital of Henan University of Science and Technology from October 2013 to December 2015. Informed consent was written by every enrolled subject. All our methods were according guidelines approved by the Human Research Ethics Committee of Henan University of Science. ALL specimens were immediately frozen in liquid nitrogen and stored at -80°C until RNA extraction. No previous treatment was received before surgical operation and tumor-node-metastasis (TNM) grading system was used to classify the tumor stage of ESCC patients.

Cell culture

The ESCC cell lines TE1, TE3, TE8, KYSE180, KYSE150, KYSE140, KYSE450, KYSE520 and HEK293T were grown in RPMI-1640 (Gibco, Life Technologies, USA) supplemented with 10% feta bovine serum (Gibco, Rockville, MD) and 1% penicillin/streptomycin (Gibco, Rockville, MD) at 37°C, 5% CO_2 in a humidified atmosphere.

Luciferase report assay

Full length 3'UTR of HOTAIR or MAPK1 were cloned into the pCDH-CMV-MCS-EF1-PURO (System biosciences, Palo Alto, CA, American) vector. MiR-217 mimics were constructed by cloning the full length of has-miR-217 (MI0000293) into the pCDH-CMV-MCS-EF1-PURO (System biosciences, Palo Alto, CA, American) vector. TE1 and KYSE520 were transfected with HOTAIR or MAPK alone or in combination with miR-217 mimics. Luciferase report assay were done 3 d after transfection following the manufactory's protocols from Promega company (#E1910, Madison, WI, USA). Luminescence signal of luciferin was detected by BioTek Synergy 2 machine (BioTec Inc., Beijing, China).

Lentivirus generation and RNA interference using shRNA

HEK293T cells were seeded at a density of 3×10^6 cells in 10 cm culture dishes. After 48 h

when the cell confluence reached 80-95%, HEK293T cells were transfected with HOTAIR shRNA, MAPK1 shRNA, miR-217 mimics or a negative control shRNA plasmids plus lentivirus packaging vectors δ8.9 and VSVG using lipofectamine reagents as protocols. The medium with virus particles were collected at 24 h, 48 h and 72 h after transfection and stored at -80°C for use. RNA interference was conducted by infecting TE1 or KYSE520 with corresponding shRNA virus particles. In brief, TE1 or KYSE520 cells were seeding in 6-well plates at 5×10^5 cells and transfected with HOTAIR or MAPK1 shRNA lentiviral particles and negative control shRNA lentiviral particles 24 h later. Successful infected cells were selected by 1 ug/ml puromycin for 4 days post infection. Sequences of HOTAIR shRNA vectors: shHOTAIR-1, 5'-GAT CCC GAA CGG GAG TAC AGA GAG ATT CAA GAG ATC TCT CTG TAC TCC CGT TCT TTT TTG GAA A-3'; and shHOTAIR-2, 5'-GAT CCC CCA CAT GAA CGC CCA GAG ATT CAA GAG ATC TCT GGG CGT TCA TGT GGT TTT TTG GAA A-3'. MAPK1 shRNA sequence: shMAPK1-1, 5'-CCG GTG GAA TTG GAT GAC TTG CCT ACT CGA GTA GGC AAG TCA TCC AAT TCC ATT TTT-3', shMAPK1-2, 5'-CCG GCA AAG TTC GAG TAG CTA TCA ACT CGA GTT GAT GCT ACT CGA ACT TTG TTT TT-3'.

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

Total RNA was extracted from ESCC patient samples or cell lines using TRIzol reagent (Invitrogen, CA, USA) according to the manufacturer's protocol. The quality and quantity of extracted RNA was measured by Nano Drop ND-2000 spectrophotometer. Complementary DNA was amplified using ReverTra Ace kit (Toyobo, Japan) and quantified using a standard SYBR-Green PCR kit protocol (Takara, Japan) in ABI 7900 Real Time PCR system. All samples were done in triplicate and normalized to GAPDH, the relative expression levels were calculated by the equation $2^{-\Delta\Delta CT}$. The primers for qRT-PCR were: HOTAIR, forward: 5'-ATA GGC AAA TGT CAG AGG GTT-3' and reverse: 5'-ATT CTT AAA TTG GGC TGG GTC-3'; MAPK1, forward: 5'-ATG ACC TCC TAT GGC ATC GA-3', and reverse: 5'-TGA TGT TCT GTG CGT GGA GC-3'.

Cell proliferation, migration and invasion assay

Cell proliferation assays were performed using Cell Counting Kit-8 (Dojindo, Japan) according to the manufacturer's protocol. About 5000 TE1 or KYSE520 cells per well were seeded in 96-well plates and infected with HOTAIR shRNA or negative control shRNA viral particles overnight. The cell proliferation was evaluated by the 450 nm absorbance of WST-8 at indicated time points.

The cell migration was evaluated by wound healing assay. A total of 1×10^6 cells were seeded in 6-well plates and when the cells reached 85% confluence, the cell monolayer was scratched with a sterile plastic tip right in the middle and washed with culture medium. Cells were cultured for 48 h with medium containing 1% FBS. Take images of the plates at different time points under a microscope (IX71, Olympus, Tokyo, Japan). Digimizer software system (MedCalc software, Ostend, Belgium) was used to measure the distance between two edges of the scratch.

A Matrigel cell invasion assay was performed and 8 um Matrigel Invasion Chamber in 24-well plates (BD Bioscience, San Jose, CA, USA) was used. 1×10^5 cells were plated in upper chamber without serum and the lower chamber were filled with RPMI-1640 with 10% FBS. After incubation for 24 h, the cells invading through the membrane were stained with Giemsa dye and imaged.

Three duplicates were done for each assay.

Tumor formation assay

Six weeks old male BALB/c nude mice were purchased from Lingchang biotechnology company (Shanghai, China). Tumorigenicity of KYSE520 stable expressing HOTAIR shRNA was determined. 2×10^6 KYSE520 cells stable expressing HOTAIR shRNA or a negative control shRNA were suspended in 150 ul saline and subcutaneously injected into the armpits of mice. Tumor growth was measured by tumor diameter with a digital Vernier caliper every 4 days from 8^{th} day until 32^{th} day after cell injection. Tumor volume (mm^3) was calculated from the following equation: Tumor volume = $W^2 \times L/2$, W and L represented the shortest and the longest diameter respectively. At the end of the experiment all mice were sacrificed and the tumor samples were weighed and collected for western blot analysis and qRT-PCR.

Western blot analysis

Cancer cells or tumors were lysed in RIPA buffer containing protease inhibitors (Sigma-Aldrich, Carlsbad, CA, USA). Protein concentra-

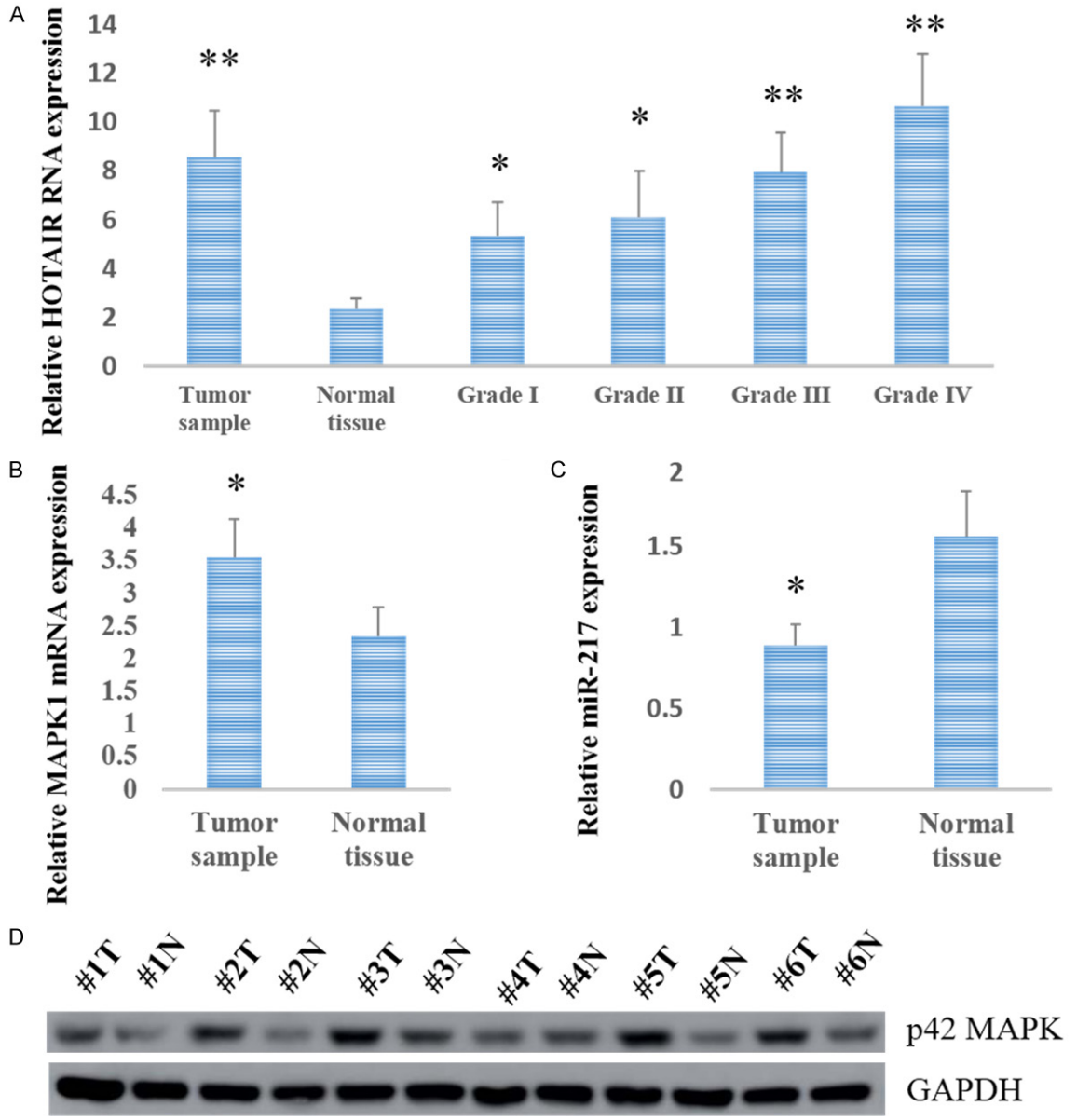

Figure 1. Expression of HOTAIR, MAPK1 and miR-217 in human ESCC patient samples. A. HOTAIR expression in human ESCC tumor samples and normal tissues was measured by qRT-PCR. B. Relative mRNA expression of MAPK1 in human ESCC tumor samples and normal tissues was measured by qRT-PCR. C. Relative miR-217 expression in human ESCC tumor samples and normal tissues was measured by qRT-PCR. D. Protein expression of MAPK1 in human ESCC tumor samples and normal tissues was measured by Western blot. T represents tumor sample and N represents Normal tissue. $^{**}P < 0.01$ and $^{*}P < 0.05$ versus Normal tissue group.

tion was quantified using the BCA protein assay kit (Thermo Scientific, Grand Island, NY, USA). The same amounts of protein were electrophoresed by 10% SDS-PAGE and transferred onto nitrocellulose membranes and incubated with specific first antibodies and corresponding second antibodies. The specific first antibodies were list as follows: p42 MAP Kinase antibody (#9108, CST, Danvers, MA, USA), RSK1/RSK2/RSK3 (32D7) Rabbit mAb (#9355, CST, Danvers, MA, USA), Phospho-p90RSK (Ser380) (D5D8) Rabbit mAb (#12032, CST, Danvers, MA, USA), GAPDH (#G8795, Sigma, Carlsbad, CA, USA).

Immunohistochemistry

Immunohistochemistry was performed as described previously [17]. Briefly, tumor samples were fixed in formalin, embedded in paraffin and sectioned at 5 um thick. Antigen retrieval were done in citrate buffer and washed by

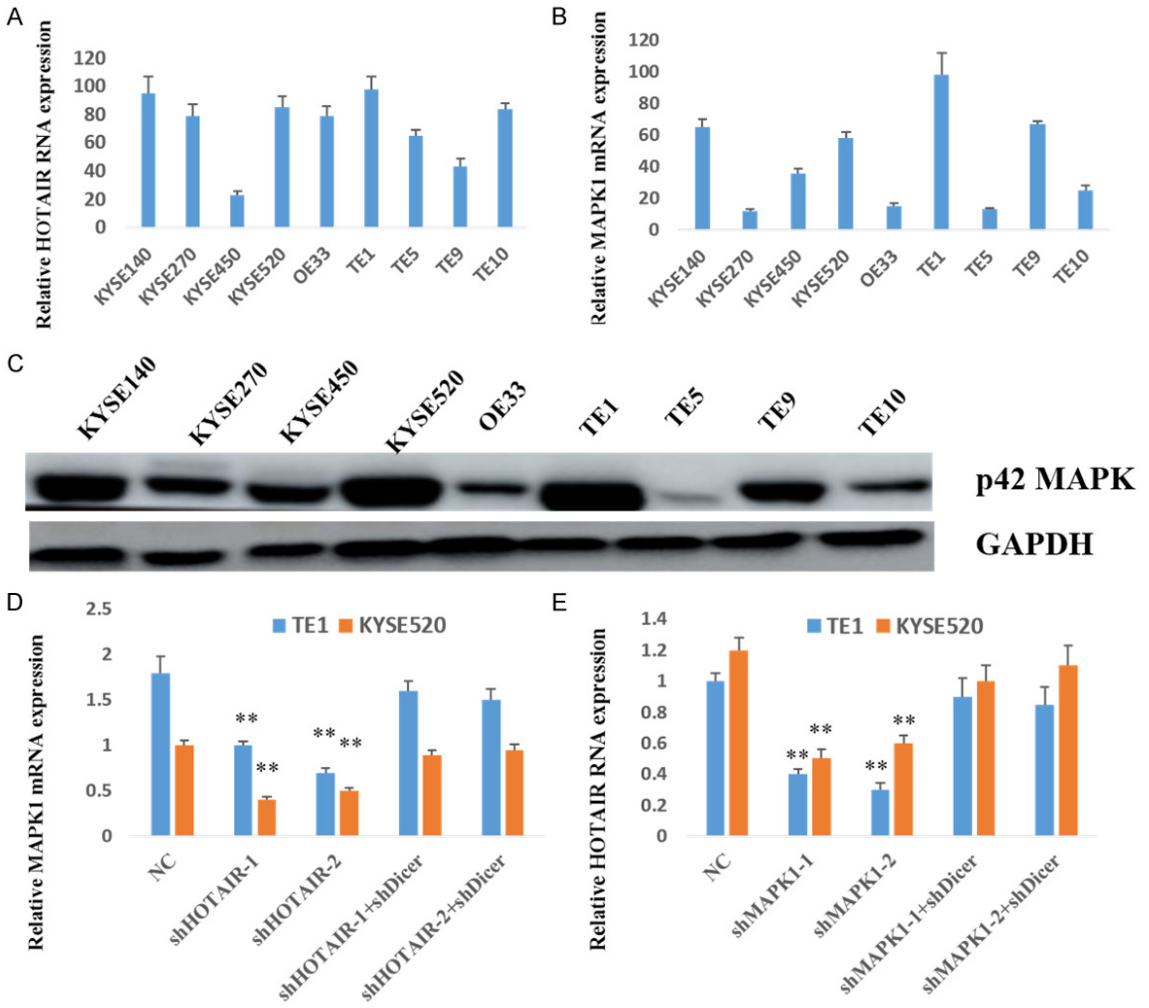

Figure 2. The relative expression of HOTAIR and MAPK1 and the interaction between them. A. Expression of HOTAIR in ESCC cell lines was detected by qRT-PCR. B. mRNA expression of MAPK1 in ESCC cell lines was detected by qRT-PCR. C. Protein expression of MAPK1 in ESCC cell lines was detected by Western blot. D. The mRNA expression of MAPK1 in TE1 and KYSE520 after silencing HOTAIR was detected by qRT-PCR. E. The mRNA expression of HOTAIR in TE1 and KYSE520 after silencing MAPK1 was detected by qRT-PCR. $^{**}P < 0.01$ versus Negative Control (NC).

PBS. Tissue sections were blocked with 10% goat serum (Sigma, St. Louis, MO, USA) for 1 h at room temperature and incubate with primary antibody overnight at 4°C. The anti-rabbit second antibody was diluted (1:300) and incubated at room temperature for 1 h. All slides were incubated in avidin biotin peroxidase complex (Sigma, St. Louis, MO, USA) diluted 1:300 in PBS for 30 min at 37°C. Ki67 (R&D, Minneapolis, MN) was used at 10 ug/ml.

Statistical analysis

All data were shown as mean ± standard (x ± s) deviation. Statistical analyses were performed by the SPSS 20.0 software (IBM Corp, Armonk, New York, USA). The difference between two groups was analyzed by paired sample t-test and one-way analysis of variance for multiple groups. $P < 0.05$ was considered statistically significant.

Results

Expression of HOTAIR, MAPK1 and miR-217 in human ESCC patient samples

To investigate the potential role of HOTAIR, MAPK1 and miR-217 in ESCC, we first evaluated the expression levels of them in human ESCC tissues by qRT-PCR. The expression levels of HOTAIR were significantly higher in human ESCC tumor samples than in normal tissues and strongly correlated with the clinical stage (**Figure 1A**). The mRNA and protein expression level of MAPK1 were also elevated in human

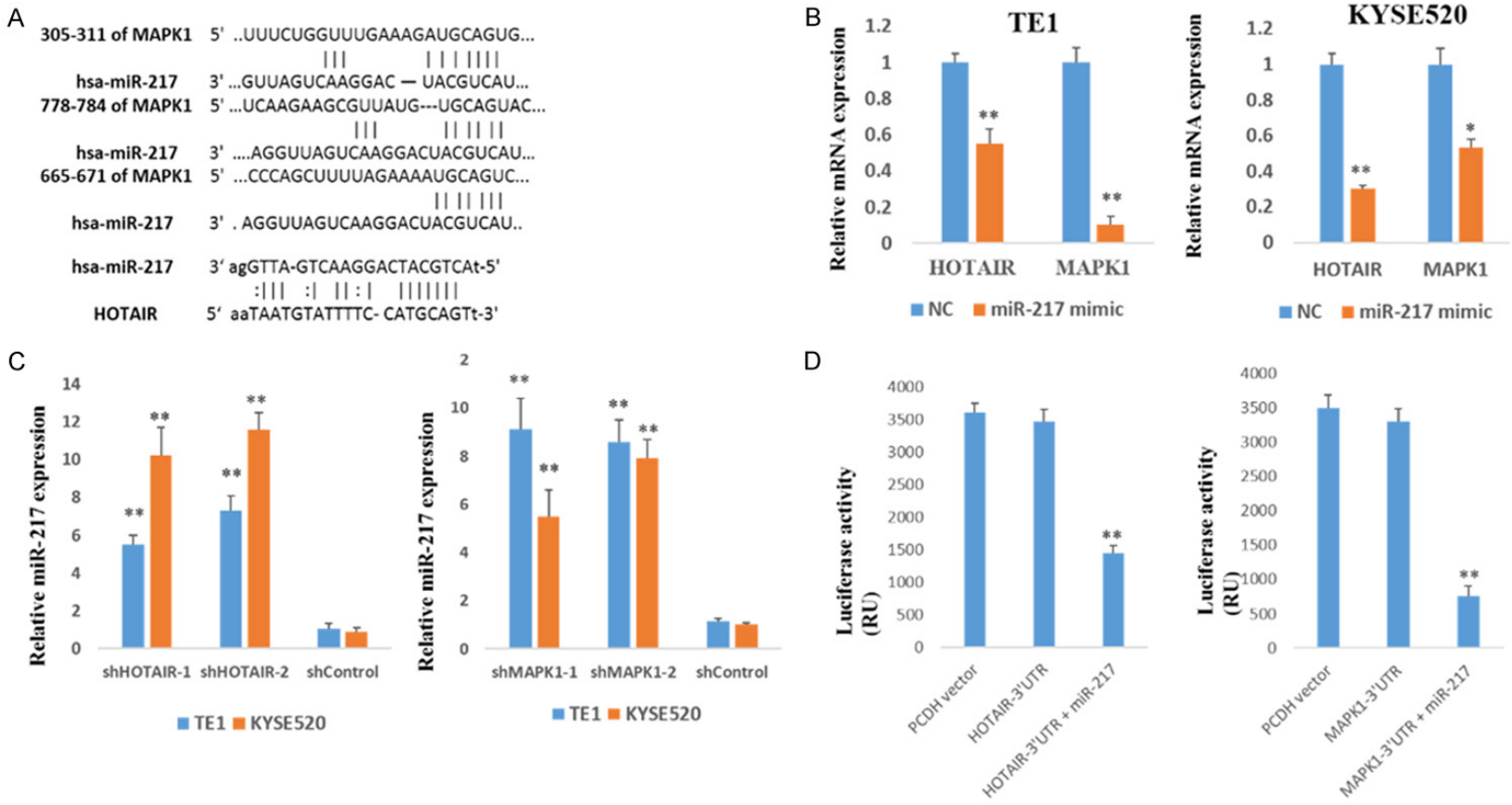

Figure 3. HOTAIR and MAPK1 were targets of miR-217. A. MAPK1 and HOTAIR had seed regions of miR-217 predicted by TargetScan, starBase or PITA. B. The mRNA expression of MAPK1 or HOTAIR in TE1 and KYSE520 after transfected with miR-217 mimics was detected by qRT-PCR. C. The expression of miR-217 after silencing HOTAIR or MAPK1 in TE1 and KYSE520 was detected by qRT-PCR. D. Luminescence signal of vector constructed with HOTAIR mRNA 3'UTR or MAPK1 mRNA 3'UTR in the presence of miR-217 mimic was detected by luciferase reporter assay. $^{**}P < 0.01$ and $^{*}P < 0.05$ versus Negative Control (NC) or shControl group.

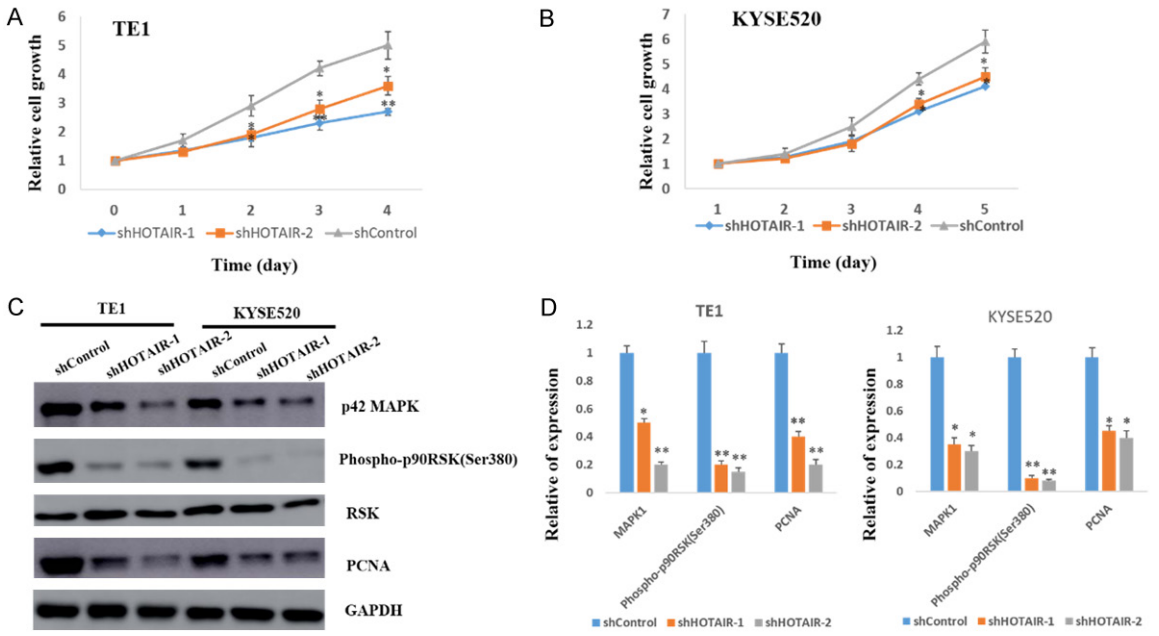

Figure 4. HOTAIR promotes cell proliferation and silencing HOTAIR caused decreasing of MAPK1 and downstream p90RSK. A, B. Cell growth of TE1 and KYSE520 after silencing HOTAIR was measured by Cell Counting Kit-8 assay. C, D. MAPK1 and its activation of downstream p90RSK of TE1 and KYSE520 after silencing HOTAIR was measured by western blot and. $^{**}P < 0.01$ and $^{*}P < 0.05$ versus shControl group.

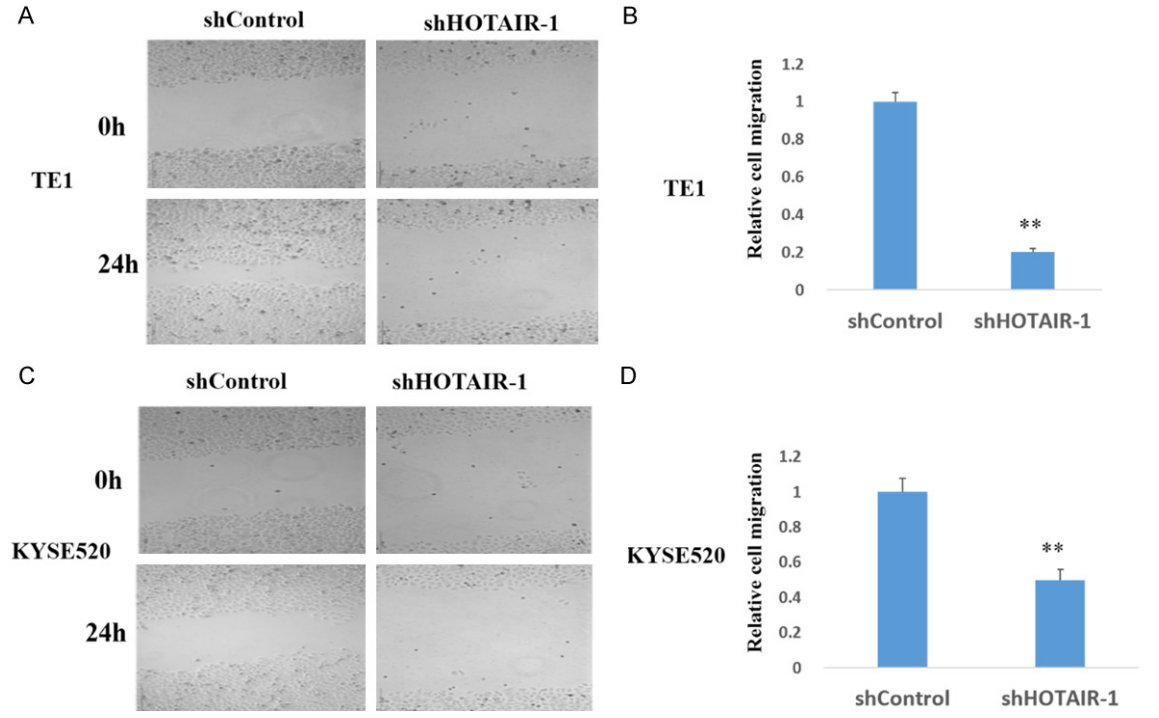

Figure 5. HOTAIR silencing caused a decreasing in cell migration in ESCC. A, B. Cell migration of TE1 cells after silencing HOTAIR was imaged, the migration distance was measured under a microscope. C, D. Cell migration of KYSE520 after silencing HOTAIR was imaged and the migration distance was measured under a microscope. $^{**}P < 0.01$ versus shControl group.

ESCC samples compared to the adjacent normal tissues (**Figure 1B**, **1D**), but the expression levels of miR-217 were downregulated in human ESCC tumor samples (**Figure 1C**).

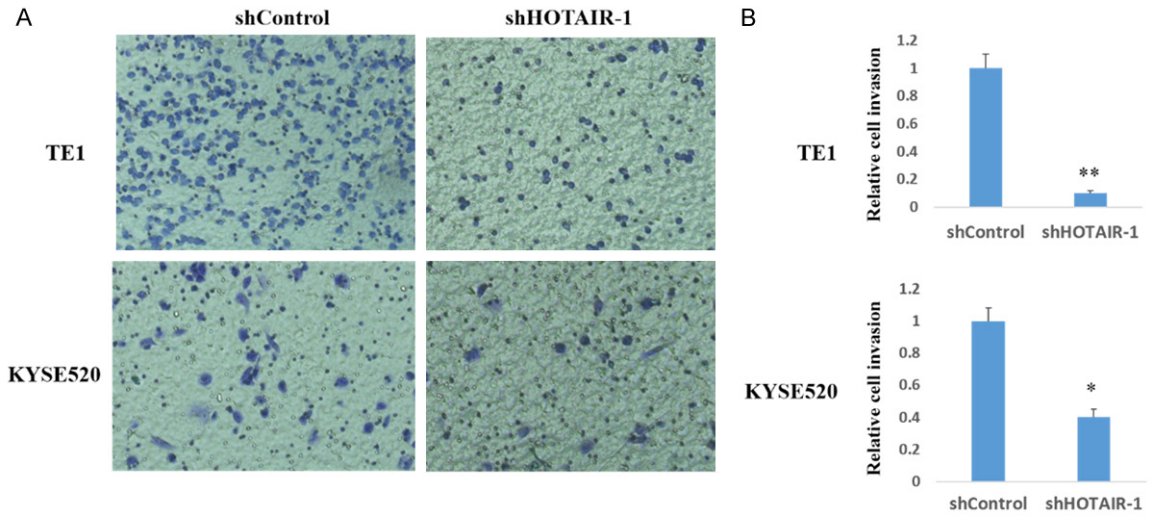

Figure 6. HOTAIR silencing caused a decreasing in cell invasion in ESCC. A. Cell invasion of TE1 and KYSE520 after silencing HOTAIR was evaluated by matrigel cell invasion assay. B. Relative cell number of TE1 and KYSE520 invaded through membrane after silencing HOTAIR was measured by taking photos and counted. $**P < 0.01$ and $*P < 0.05$ versus shControl group.

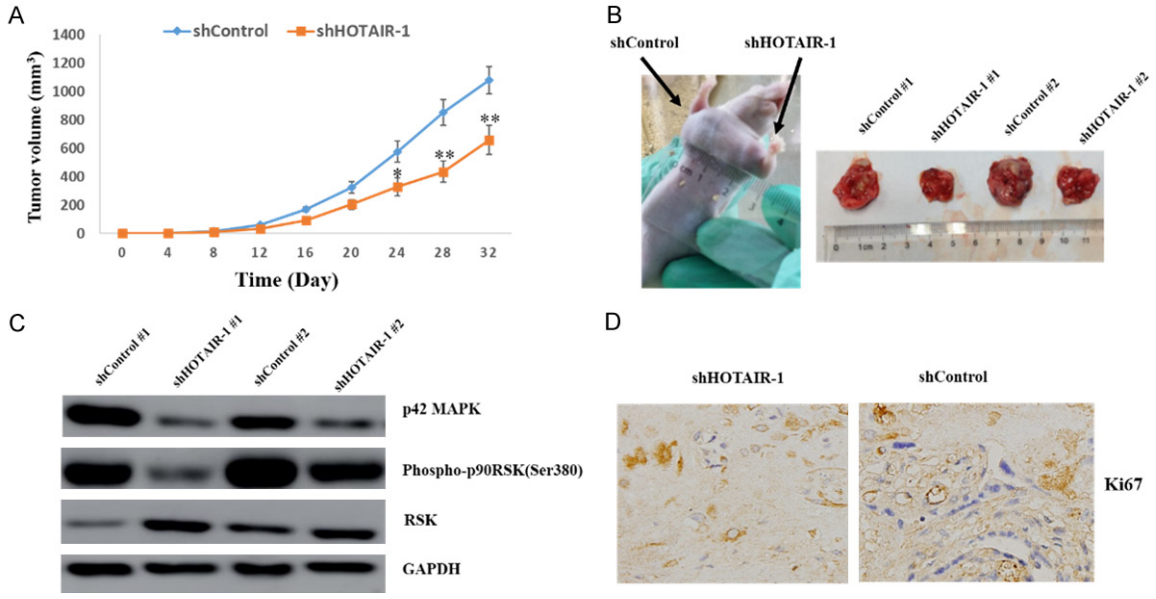

Figure 7. HOTAIR silencing suppressed the tumor growth of KYSE520 in nude mice. A. Tumor volume was measure on indicated days. B. Photos depicted the tumors formed by KYSE520 after silencing HOTAIR or a negative control shRNA. C. MAPK1 (p42 MAPK in image) and its downstream p90RSK in tumors formed by KYSE520 in nude mice after silencing HOTAIR was detected by western blot. D. Ki67 positive cells in tumors formed by KYSE520 after silencing HOTAIR was detected by IHC. $**P < 0.01$ and $*P < 0.05$ versus shControl group.

The interaction between HOTAIR and MAPK1 in ESCC cell lines

We then investigated the expression level of HOTAIR (**Figure 2A**) and MAPK1 (**Figure 2B**, **2C**) in ESCC cell lines. HOTAIR and MAPK1 were both high expressed in ESCC cell line TE1 and KYSE520, so we chose them to study the interaction between HOTAIR and MAPK1 in ESCC. We silenced HOTAIR or MAPK1 in TE1 and KYSE520 respectively. The mRNA level of MAPK1 were downregulated when silencing HOTAIR and the expression level of HOTAIR was decreased when silencing MAPK1 (**Figure 2D**,

2E). When we silenced dicer, these effects abolished (**Figure 2D**, **2E**). These results indicated that HOTAIR might be a ceRNA of MAPK1.

HOTAIR and MAPK1 are targets of miR-217, and silencing HOTAIR or MAPK1 increased expression of miR-217

We used Targetscan (http://www.targetscan.org/vert_71/) to predict the possible interaction between miR-217 and MAPK1. The results showed that MAPK1 had three seed regions of miR-217 and might be a potential target of miR-217 (**Figure 3A**). The interaction between HOTAIR and miR-217 were predicted by starBase (http://starbase.sysu.edu.cn/mirLncRNA.php) and PITA (https://genie.weizmann.ac.il/pubs/mir07/mir07_prediction.html) and there were four seed regions of miR-217 in HOTAIR (**Figure 3A**). The mRNA expression levels of MAPK1 or HOTAIR in TE1 and KYSE520 were decreased when transfected with miR-217 mimics (**Figure 3B**). When we silenced MAPK1 or HOTAIR in TE1 and KYSE520, the expression level of miR-217 increased (**Figure 3C**). In the luciferase reporter assay, results showed that vector constructed with HOTAIR mRNA 3'UTR or MAPK1 mRNA 3'UTR had lower luminescence signal in the presence of miR-217 mimic (**Figure 3D**). These results indicated that MAPK1 and HOTAIR were targets of miR-217.

HOTAIR regulates cell proliferation, migration, invasion and tumor formation of ESCC through regulating MAPK1

To further assess the role of HOTAIR in the pathogenesis of ESCC, we silenced HOTAIR in TE1 and KYSE520. Silencing HOTAIR significantly inhibited the cell proliferation (**Figure 4A**, **4B**) compared to a control shRNA, and the protein expression of MAPK1 and activation of its downstream p90RSK decreased (**Figure 4C**). We also evaluated the migration and invasion of TE1 and KYSE520 after silencing HOTAIR. Stable knockdown of HOTAIR decreased the migration (**Figure 5**) and invasion (**Figure 6**) ability of TE1 and KYSE520 *in vitro*. The tumor growth of KYSE520 in nude mice after silencing HOTAIR was lower than KYSE520 transfected with a control shRNA (**Figure 7A**, **7B**) and the protein expression of MAPK1 and its downstream p90RSK were also decreased (**Figure 7C**). The ki67 staining of tumor tissues formed by KYSE520 after silencing HOTAIR decreased (**Figure 7D**). These results showed that HOTAIR could promote proliferation, migration, invasion and tumor formation of ESCC cell line and this was partially due to regulating of MAPK1.

Discussion

ESCC patients have a dismal prognosis, so looking for new diagnostic and prognostic biomarkers for ESCC is very important and badly needed. The development of genome and transcriptome sequencing technology has gave us a profound understanding of somatic mutations, gene amplifications and deletion in tumorigenesis. By these technologies we also found a wide variety of non-protein coding RNAs such as microRNAs and LncRNAs that were deregulated in cancers and functioned as tumor suppressor genes or oncogenes. Aberrant expression of lncRNAs was found to be closely related to the progression of cancers, including ESCC. Recent studies have found that a variety of lncRNAs were overexpressed in ESCC and could be prognostic factors, such as HOTAIR, MALAT1, CCAT2, UCA1, ZEB1-AS1, PCAT-1 and NEAT1 [18-24]. HOTAIR has been intensively studied in recent years and was first found to be correlated with metastasis in primary breast cancer [25]. HOTAIR was overexpressed in many cancers such as breast cancer, lung cancer, gastric cancer, liver cancer, endometrial cancer, prostate cancer, pancreatic cancer, colorectal cancer, melanoma and sarcoma [11, 17, 26-34]. Overexpression of HOTAIR could induce genome-wide retargeting of PRC2 which led to altered H3K27 methylation and metastasis-related gene expression. Recent studies proved that HOTAIR was closely correlated with the development and progression of ESCC [35]. In our study, we found the expression of HOTAIR was up-regulated in ESCC cell lines and patient samples and closely related to the clinical stage, corresponding to the previous studies. We also tested the expression level of MAPK1 and miR-217 in ESCC patient samples and found that MAPK1 was up-regulated while miR-217 down-regulated.

The ceRNA hypothesis assumes that RNAs (protein coding or non-protein coding RNAs) could influence the expression levels of each other by competing for a limit pool of microRNAs through microRNA binding sites (MREs) [16]. According to this hypothesis, LncRNAs can act as "microRNA sponges" to alter the expression levels of given microRNAs so as to influ-

ence other protein coding RNAs. In our study, we tested the interaction of HOTAIR and MAPK1 in ESCC cell lines. HOTAIR and MAPK1 were all high expressed in TE1 and KYSE520, and silenced HOAIR or MAPK1 in TE1 and KYSE520 could down-regulate the expression level of each other, but co-silenced with Dicer abolished these effects. This indicated that HOTAIR might be a ceRNA of MAPK1. Bioinformatics analysis predicts that miR-217 is shared by HOTAIR and MAPK1, so we tested whether the interaction of HOTAIR and MAPK1 was regulated by miR-217. As we expected, miR-217 mimics could suppress the expression of HOTAIR and MAPK1 in TE1 and KYSE520. When we silenced HOTAIR or MAPK1, the expression level of miR-217 increased. We also conducted luciferase reporter assay to evaluate the interaction between miR-217 with HOTAIR and MAPK1, as we expected, vector constructed with HOTAIR mRNA 3'UTR or MAPK1 mRNA 3'UTR had lower luminescence signal in the presence of miR-217 mimic. These results suggested that the interaction of HOTAIR and MAPK1 was mediated by miR-217.

HOTAIR was enrolled in several biological processes including cooperated with chromatin modifying enzymes to regulate epigenetic activation or gene silencing, and acted as microRNA sponges or inducer of ubiquitin-mediated proteolysis. HOTAIR was involved in tumorigenesis, tumor migration and invasion. HOTAIR was found to promote phosphatase and tensin homolog (PTEN) methylation in human laryngeal squamous cell cancer [36]. PTEN methylation could lead to activate of PI3K/AKT/mTOR pathway and repression of p53-mediated apoptosis, so as to promote tumorigenesis. In breast cancer, HOTAIR was found to be an independent biomarker for predicting the metastasis and mortality and overexpression of it could promote metastasis in vitro and in vivo [25]. In lung cancer, HOTAIR was a prognostic marker and was associated with cell migration and invasion of non-small cell lung cancer (NSCLC) cell lines [27]. HOTAIR was overexpressed in pancreatic cancer and depleting HOTAIR in PANC1 and L3.6pL pancreatic cancer cell line could inhibit cell proliferation and induce apoptosis [37]. In our study, we first silenced HOTAIR in ESCC cell line TE1 and KYSE520, thus cell proliferation, migration and invasion in vitro and tumor formation in vivo were all inhibited or decreased after HOTAIR silencing, corresponding with previous studies [13, 17].

The MAP kinase signaling cascade Ras/Raf/MEK/ERK was commonly deregulated in cancers and closely correlated with tumorigenesis and progression. MAPK1 is activated in human liver cancer, colon cancer and NSCLC [38, 39]. MAPK1 was likely to be activated in ESCC and expression of MAPK1 was found to be upregulated in Kazakh ESCC tissues compared with adjacent normal tissue [40, 41]. In our study, we evaluated the protein expression of MAPK1 and activation of its downstream p90RSK after silencing HOTAIR in TE1 and KYSE520. As we expected, the protein expression of MAPK1 were decreased and so was its activation of downstream p90RSK in TE1 and KYSE520. We also tested this in tumor formed by KYSE520 in nude mice, and had detected a decreasing MAPK1 expression as well as its downstream phospho-p90RSK. These consequences indicated that HOTAIR could regulate cell proliferation, migration, invasion and tumor formation of ESCC cell line and this was partially due to the downregulation of MAPK1 and its downstream phospho-p90RSK.

In summary, our study has found that HOTAIR and MAPK1 were up-regulated while miR-217 down-regulated in ESCC patient samples. HOTAIR could interact with MAPK1 and might be a ceRNA of MAPK1 mediated by miR-217. The cell proliferation, migration, invasion and tumor formation of ESCC cell line TE1 or KYSE520 were inhibited when silencing HOTAIR, and these might partially due to the decreasing of MAPK1 and its downstream phospho-p90RSK. Our study provides a new insight into the oncogenic function of HOTAIR and may increase our understanding of lncRNAs in the pathogenesis of ESCC.

Disclosure of conflict of interest

None.

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