Cancer Letters 460 (2019) 18–28

Contents lists available at ScienceDirect

Cancer Letters

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

Original Articles

CCL18-induced HOTAIR upregulation promotes malignant progression in esophageal squamous cell carcinoma through the miR-130a-5p-ZEB1 axis

Wenjian Wang[a,b,1], Duoguang Wu[a,b,1], Xiaotian He[a,b,1], Xueting Hu[a,b], Chuwen Hu[a,c], Zhiwen Shen[a,c], Jiatong Lin[a,b], Zihao Pan[a,b], Zhanghai He[a,d], Huayue Lin[a,e,**], Minghui Wang[a,b,*]

[a] Guangdong Provincial Key Laboratory of Malignant Tumor Epigenetics and Gene Regulation, Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University, Guangzhou, 510120, China
[b] Department of Thoracic Surgery, Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University, Guangzhou, 510120, China
[c] Department of Anesthesiology, Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University, Guangzhou, 510120, China
[d] Department of Pathology, Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University, Guangzhou, 510120, China
[e] Breast Tumor Center, Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University, Guangzhou, 510120, China

ARTICLE INFO

Keywords:
Long non-coding RNA
MicroRNA
ceRNA

ABSTRACT

Accumulating evidence indicates that CCL18 and the long non-coding RNA, HOTAIR, have critical roles in cancer progression and metastasis, but the correlation between CCL18 and HOTAIR in esophageal squamous cell carcinoma (ESCC) and their downstream molecular mechanisms remain unclear. Overexpression of CCL18 in ESCC tissues was associated with a worse survival in patients with ESCC. CCL18 enhanced the invasiveness of ESCC cells in a dose-dependent manner, whereas CCL18 knockdown inhibited their invasiveness. In particular, CCL18 expression was positively associated with HOTAIR expression in ESCC tissues. Furthermore, CCL18 upregulated the expression of HOTAIR, and knockdown of HOTAIR alleviated the CCL18-induced invasiveness of ESCC cells. HOTAIR may act as a competing endogenous RNA and could effectively becoming a sponge for miR-130a-5p, thereby modulating the derepression of ZEB1 and promoting epithelial–mesenchymal transition in ESCC. Our study suggests that CCL18 contributes to the malignant progression of esophageal cancer by upregulating HOTAIR expression. HOTAIR overexpression may promote tumor invasiveness and progression in ESCC, given that HOTAIR functions as a miR-130a-5p sponge, positively regulating ZEB1. This provides new therapeutic targets for early diagnosis and treatment of ESCC.

1. Introduction

Esophageal squamous cell carcinoma (ESCC) is the main histopathological subtype of esophageal cancer [1,2]. ESCC ranks eighth and sixth in terms of incidence and mortality worldwide, respectively, because of its aggressiveness and therapeutic difficulties [3]. Every year, approximately 300,000 people die because of esophageal cancer worldwide [4]. Although advances in the diagnosis and treatment of ESCC have increased the possibility of its treatment, ESCC is still largely incurable due to its poor prognosis and recurrence, with a five-year survival rate of 15–25% [5]. Therefore, the development of innovative, targeted therapies is imperative and of great clinical significance.

It has been well established that the tumor microenvironment plays an important part in cancer development and metastasis. Furthermore, mechanistic studies have revealed that a variety of cytokines, including chemokines, inflammatory factors, and growth factors, promote tumor progression and metastasis in the tumor microenvironment [6,7]. Chemokine (C–C motif) ligand 18 (CCL18) is a major chemokine produced mainly by M2 macrophages and cancer cells; it plays a pivotal role in the progression and metastasis of tumors [8–12]. Currently, the role of CCL18 in cancer progression is controversial. CCL18 has been reported to have an immunosuppressive role in ovarian cancer [13,14], but it has been reported to be associated with prolonged survival in patients with gastric cancer [11]. The role of CCL18 in the progression of ESCC and its downstream signaling pathways remain elusive.

Long non-coding RNAs (lncRNAs), which are extensively

* Corresponding author. Guangdong Provincial Key Laboratory of Malignant Tumor Epigenetics and Gene Regulation, Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University, Guangzhou, 510120, China.
** Corresponding author. Guangdong Provincial Key Laboratory of Malignant Tumor Epigenetics and Gene Regulation, Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University, Guangzhou, 510120, China.
E-mail addresses: linhy29@mail.sysu.edu.cn (H. Lin), wmingh@mail.sysu.edu.cn (M. Wang).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.canlet.2019.06.009
Received 23 December 2018; Received in revised form 8 June 2019; Accepted 12 June 2019

transcribed from the mammalian genome, have gained widespread attention in recent years. They are important and powerful regulators of various biological activities and play a critical role in the progression of a variety of diseases, including cancer [15–17]. Recently, a new regulatory mechanism has been identified in which lncRNAs and mRNAs interact by competition for microRNA (miRNA) response elements. In this case, lncRNAs may function as competing endogenous RNAs (ceRNAs) and sponge miRNAs to regulate the derepression of miRNA targets and impose an additional level of post-transcriptional regulation [18]. The disequilibrium of ceRNAs and miRNAs can be critical for tumorigenesis [19]. The lncRNA, Malat1, is overexpressed in gallbladder cancer; it promotes the development of gallbladder cancer via its competitive binding with miR-206 to upregulate ANXA2 and KRAS [20]. Similarly, lncRNA Unigene56159 promotes epithelial-mesenchymal transition (EMT) by acting as a ceRNA of miR-140–5p in hepatocellular carcinoma cells [21]. Therefore, we believe that some lncRNAs may also play role of ceRNAs, linking miRNAs and the post-transcriptional network in ESCC. Hox transcript antisense intergenic RNA (HOTAIR) is a 685-nt lncRNA; it is located in the Hoxc gene cluster but represses the transcription of the Hoxd locus in foreskin fibroblasts [22]. Being a novel regulator of tumorigenesis, HOTAIR was initially found to promote invasiveness and metastasis in breast cancer [22]. HOTAIR is also associated with chromatin modifications and exhibits pro-oncogenic activity in pancreatic cancer [23]. Moreover, its upregulation was positively correlated with poor prognosis, tumor progression, and recurrence of gastrointestinal cancers such as colorectal cancer, liver cancer, and gastrointestinal stromal tumors [24–28]. We previously identified that HOTAIR is a novel diagnostic biomarker and a prognostic marker for progression and survival in ESCC [29,30]. However, the mechanism underlying the HOTAIR-mediated regulation of ESCC progression has not largely characterized. In the present study, for the first time, we demonstrate that HOTAIR is upregulated by CCL18 and promotes tumor progression in ESCC via its involvement in the CCL18 signaling pathway. Moreover, mechanistic analyses reveal that HOTAIR may play a carcinogenic role in ESCC by acting as a ceRNA that competes with ZEB1 for miR-130a-5p, thereby regulating ZEB1 expression. The present work provides the first evidence of the positive correlation between CCL18/HOTAIR and the crosstalk between miR-130a-5p, HOTAIR, and ZEB1, which promotes ESCC progression of ESCC; it also provides new insights relevant to the treatment of ESCC.

2. Materials and methods

2.1. Tissue sample specimens

A total of 25 fresh primary ESCC samples and paired adjacent non-tumorous tissues were obtained from the Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University, between January 2010 and December 2012. All cases were reviewed by a pathologist and histologically confirmed as ESCC based on the histopathological evaluation. None of the patients had undergone any local or systemic treatment before operation. All samples were collected with informed consent, and this study was approved by the Internal Review and the Ethics Boards of the Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University.

2.2. Cell culture, treatment, and transfection

Five ESCC cell lines (ECA109, KYSE30, KYSE140 KYSE510, and TE-1) and a normal esophageal squamous cell line (HEEC) were used in this study. All cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Invitrogen) supplemented with 10% fetal bovine serum and 1% penicillin–streptomycin. For chemokine treatment, the ESCC cells were exposed to CCL18 (Peprotech, Princeton, USA) for 1 h.

Table 1
qRT-PCR primers.

Gene	Primer sequence
ccl18	Forward: 5′-TGGCAGATTCCACAAAAGTTCA-3′
	Reverse: 5′- GGATGACACCTGGCTTGGG-3′
HOTAIR	Forward: 5′- GGTAGAAAAAGCAACCACGAAGC-3′
	Reverse: 5′- ACATAAACCTCTGTCTGTGAGTGCC-3′
miR-130a-5p	Forward: 5′-CCAGGGCTTTTCAAAAATGA-3′
	Reverse: 5′-CCGATCCAATCTGTTCTGGT-3′
ZEB1	Forward: 5′-CCAGGGCTTTTCAAAAATGA-3′
	Reverse: 5′-CCGATCCAATCTGTTCTGGT-3′
E-Cadherin	Forward: 5′-TGAAGGTGACAGAGCCTCTGGA-3′
	Reverse: 5′-TGGGTGAATTCGGGCTTGTT-3′
ZO-1	Forward: 5′-TGAACGCTCTCATAAGCTTCGTAA-3′
	Reverse: 5′-ACCGTACCAACCATCATTCATTG-3′
N-Cadherin	Forward: 5′-GCGCGTGAAGGTTTGCCAGTG-3′
	Reverse: 5′-CCGGCGTTTCATCCATACCACAA-3′
Vimentin	Forward: 5′-TGGCCGACGCCATCAACACC-3′
	Reverse: 5′-CACCTCGACGCGGGCTTTGT-3′
Snail	Forward: 5′- AAGATGCACATCCGAAGCCA-3′
	Reverse: 5′-CAAAAACCCACGCAGACAGG-3′
Gapdh	Forward: 5′-GAGTCAACGGATTTGGTCGT-3′
	Reverse: 5′-GACAAGCTTCCCGTTCTCAG-3′

2.3. Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted using TRIzol (Invitrogen), according to the manufacturer's instructions. Quantitative real-time polymerase chain reaction (qRT-PCR) was performed with a Light Cycler 480 system (Roche Diagnostics, Switzerland) using an SYBR Premix ExTaq kit (Takara, Dalian, China). The oligonucleotide sequences of the qRT-PCR primers are listed in Table 1.

2.4. Immunohistochemistry

Immunohistochemistry (IHC) assays were performed and quantified as described in a previous report [27]. As previously described, two independent observers assessed and scored the degree of immunostaining of the indicated proteins; the proportion of positively stained tumor cells and the staining intensities were also scored. The staining intensities and scores representing the proportion of positively stained tumor cells were graded as: low (< 25%), medium (25–75%), or high (> 75%).

2.5. ELISA measurements

To detect the secretion of CCL18, the cell supernatants were collected and analyzed using a CCL18 ELISA kit (R&D Systems, Minneapolis, Minnesota, USA), following the manufacturer's instructions.

2.6. SiRNA transfection

For short interfering RNA (siRNA) transfection, 5×10^5 cells/mL were plated in serum-free medium and transfected with specific siRNA duplexes using Lipofectamine 2000 (Invitrogen, USA) according to the manufacturer's instructions. All siRNAs were purchased from GenePharma (Shanghai, China). The oligonucleotide sequences of the siRNAs are listed in Table 2.

2.7. Invasion assay

The invasiveness of ESCC cells was examined using 24-well Boyden chambers (Corning, USA) with 8 μm inserts coated with fibronectin (Roche) and Matrigel (BD Biosciences). Cells (10^5 cells/well) were plated on the upper cell culture inserts with 0.2% bovine serum albumin in serum-free DMEM; the same medium was added to the lower

Table 2
siRNA sequences.

Gene	siRNA sequence
GFP siRNA	5′-GGCTACGTCCAGGAGCGCACCdTdT-3′
si CCL18-1	5′-ACAAGTTGGTACCAACAAATTdTdT-3′
si CCL18-2	5′-CCAGCATTCTCACTGTGAATTdTdT-3′
si ZEB1-1	5′-GGCAAGTGTTGGAGAATAAdTdT-3′
si ZEB1-2	5′-CCAGAAATACACAGGGTTAdTdT-3′

chambers in the presence of phosphate-buffered saline (PBS) or CCL18. After 12 h of culture at 37 °C, the invading cells were captured and counted by crystal violet staining.

2.8. In situ hybridization

In situ hybridization (ISH) of the lncRNA *HOTAIR* was performed as described in a previous report [30]. Digoxigenin-labeled locked nucleic acid probes for *HOTAIR* were purchased from Exiqon (USA).

2.9. Cell proliferation

Cell proliferation was analyzed using the CCK-8 assay, following the manufacturer's instructions. ESCC cells were cultured in 96-well plates and subjected to different treatments. After that, 10 μL of CCK-8 solution was added to each well, followed by further incubation for 2 h. Absorbance at 450 nm was measured using a microplate reader (Multiskan MK3, Thermo Fisher Scientific Inc., USA). The experiments were performed in triplicate.

2.10. Western blot analysis

Protein extracts were first resolved by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis; the protein bands were then transferred onto polyvinylidene fluoride membranes (Millipore, Billerica, MA, USA), and probed with antibodies against E-cadherin (Cat. No. sc-7870, Santa Cruz), vimentin (Cat. No. sc-66002, Santa Cruz), ZO-1 (8193, CST), ZEB1 (3396, CST), or GAPDH (Cat. No. sc-137179, Santa Cruz). Peroxidase-conjugated anti-mouse or rabbit antibody (CST) was used as the secondary antibody, and the antigen-antibody reaction was visualized by enhanced chemiluminescence assay (Thermo).

2.11. Luciferase reporter assay

Human wild-type and mutant ZEB1 3′-untranslated region (UTR) sequences containing the putative binding sites of miR-130a-5p were synthesized and inserted into the firefly luciferase reporter vector pmiR-RB-Reporter™ (RiboBio Co. Ltd, China), and then validated by sequencing. Wild-type and mutant HOTAIR fragment containing the putative miR-130a-5p binding sites were synthesized and inserted into the psicheck2 vector to generate luciferase reporter. The TE-1 cells were transfected either with miR-mimics (RiboBio Co. Ltd) and pWT or with miR-mimcs and pMut and either with miR-negative control (nc) and pWT or with miR-nc and pMut. The cells were harvested after 24 h, and the luciferase activity was assayed according to the dual luciferase reporter assay system (Promega, USA). The Renilla luciferase signal was normalized to the firefly luciferase signal for each individual analysis.

2.12. Tumor mice xenograft

Tumor formation was studied by establishing a xenograft model. BALB/c female nude mice (4–6 weeks old) were purchased from the Animal Experiment Center of the Sun-Yat-Sen University. The usage and treatment of the mice were approved by the Animal Care and Use Committee of the Sun-Yat-Sen University. ECA109 cells infected with Lv-miR-130a-5p inhibitor, Lv-Sc, Lv-ShHOTAIR, Lv-ShNC, or Lv-miR-130a-5p + Lv-ShHOTAIR were injected subcutaneously into the dorsal flank of the nude mice. Tumor volumes were measured and calculated according to formula $V = 0.5 \times L^2$ (length) × W (width). The mice were sacrificed 6 weeks after cell inoculation.

2.13. Statistical analysis

All statistical analyses were performed with the SPSS 19.0 software. All data were expressed as the mean ± SD of three independent experiments, in which each assay was performed in triplicate. Statistical analysis was performed using ANOVA followed by student's *t* -test. Kaplan–Meier survival curves were plotted, and log rank tests were performed. Pearson's correlation and Spearman's correlation were used to analyze the relationship among the expression levels of CCL18, HOTAIR, and miR-130a-5p in the tissues. Significance was defined at $P < 0.05$. Cutoff values for the high and low expression of the protein of interest were chosen based on a measurement of heterogeneity using the log-rank test with respect to overall survival.

3. Results

3.1. CCL18 expression in ESCC correlates with tumor invasiveness

To evaluate the expression status of CCL18 in ESCC tissues, we detected CCL18 mRNA in 25 pairs of ESCC tissues and adjacent benign esophageal tissues. The statistical analysis showed that the expression levels of CCL18 mRNA in ESCC tissues were significantly higher than those in non-cancerous esophageal tissues ($P < 0.001$, Fig. 1A). Likewise, the expression of CCL18 in patients with metastasis was significantly higher than that in patients without metastasis ($P < 0.01$, Fig. 1B). IHC analysis of CCL18 expression in ESCC tissues revealed that CCL18-positive cells were scattered in the cytoplasm of cancer cells and the tumor stroma of esophageal carcinoma tissues. Furthermore, the expression of CCL18 was associated with the degree of differentiation of the cancer cells; the higher the expression of CCL18, the lesser was the degree of differentiated of the cancer cell (Fig. 1C). Kaplan–Meier survival analysis also demonstrated that patients with low CCL18 expression had a significantly longer survival time than those with high CCL18 expression (Fig. 1D, $P = 0.022$), which was associated with poor survival prognosis in patients with esophageal cancer.

To further confirm the increased expression of CCL18 in ESCC, we detected the expression of CCL18 in the five ESCC cell lines (ECA109, KYSE30, KYSE140 KYSE510, and TE-1) and HEECs by qRT-PCR and ELISA. Compared with HEECs, the expression of CCL18 mRNA (Fig. 1E) and protein (Fig. 1F) in all the types of ESCC cells increased.

Transwell chambers were used to examine the invasiveness of esophageal cancer cells treated with recombinant CCL18. Compared to the case for the PBS-treated cancer cells, treatment with recombinant CCL18 (rCCL18, 5–20 ng/mL) enhanced the invasiveness of ECA109 cells in a dose-dependent manner (Fig. 1G and H). To further determine whether CCL18 contributes to the invasiveness of the cancer cells, two CCL18-siRNAs were transfected into TE-1 cells to interfere with the function of CCL18 (Supplementary Fig. S1, $P < 0.01$). Transfection of TE-1 cells with either of the two CCL18-siRNAs also reduced the number of invasive cancer cells (Fig. 1I). Collectively, these data suggest that CCL18 promotes the process of malignant progression in ESCC.

3.2. HOTAIR mediates CCL18-induced invasion of ESCC

Our previous work indicated that HOTAIR could be used as a prognostic marker for ESCC progression and survival [29,30]. However, whether HOTAIR is involved in CCL18 regulation and the subsequent progression of ESCC remains unclear. To investigate the effect of HOTAIR in ESCC, we performed qRT-PCR to determine the levels of

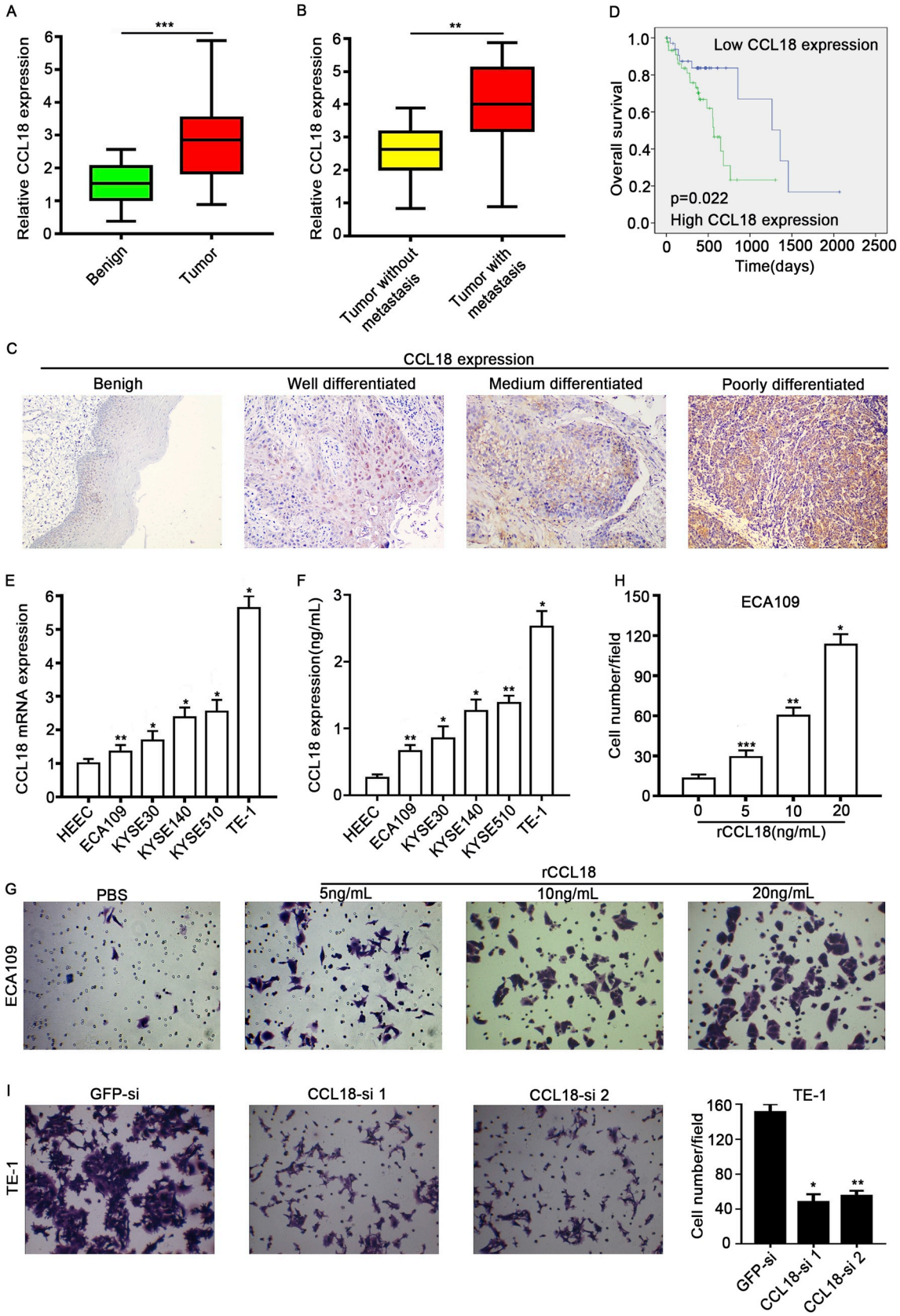

Fig. 1. CCL18 expression in ESCC correlates with tumor invasiveness. (A) Relative expression of CCL18 in ESCC tissues (n = 25) in comparison with adjacent benign esophageal tissues (n = 25). CCL18 expression was examined by qRT-PCR and normalized to GAPDH expression. (B) Relative expression of CCL18 in patients with metastasis (n = 17) compared with those without metastasis (n = 8). (C) Representative images of CCL18 staining in adjacent benign esophageal tissues and ESCC tissues with well, medium, and poor differentiation. (D) Kaplan–Meier survival curve of patients with ESCC with lower and higher CCL18 expression. (E and F) qRT-PCR and ELISA for CCL18 expression in esophageal cancer cells (ECA109, KYSE30, KYSE140 KYSE510, and TE-1) and HEEC. (G and H) Transwell chamber assay for ECA109 with rCCL18 at increasing concentrations (5–20 ng/ml). Bars indicate mean ± SD. (I) Transwell chamber assay for TE-1 transfected with either of the two CCL18-siRNAs or GFP-siRNA. *P < 0.05, **P < 0.01, ***P < 0.001.

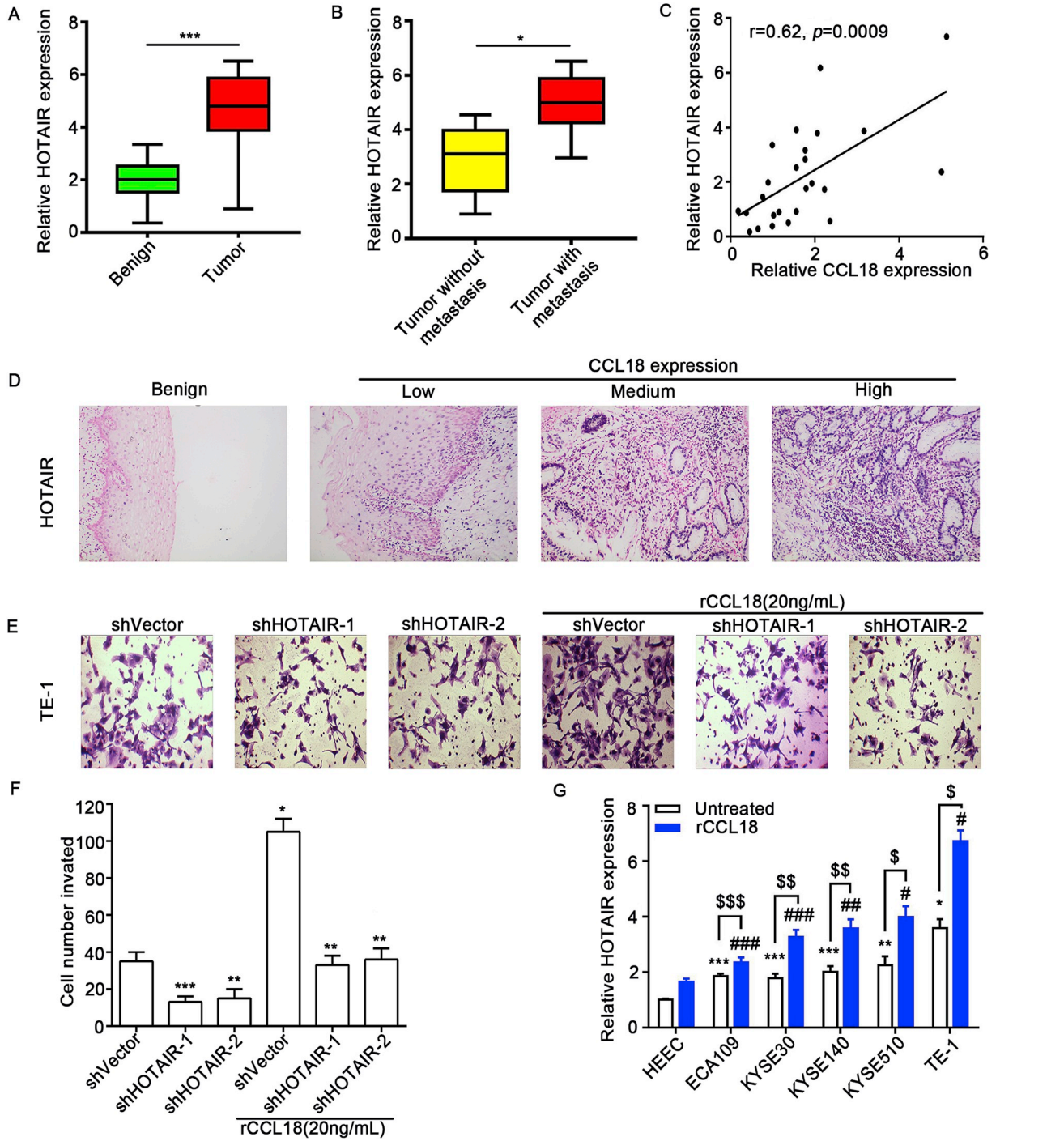

Fig. 2. HOTAIR mediates CCL18-induced invasion of ESCC. (A) Relative expression of HOTAIR in ESCC tissues (n = 25) compared with adjacent benign esophageal tissues (n = 25). HOTAIR expression was examined by qRT-PCR and normalized to GAPDH expression. (B) Relative expression of HOTAIR in patients with metastasis (n = 17) compared with those without metastasis (n = 8). (C) Pearson correlation analysis of HOTAIR expression levels and CCL18 expression in ESCC tissues. (D) HOTAIR expression in benign disease and ESCC tissues with low, medium, and high CCL18 expression, as assayed by in situ hybridization. (E and F) Transwell chamber assay for TE-1 cells transfected with sh-HOTAIR or sh-Vector, and then exposed to CCL18. (G) Alterations in HOTAIR expression in HEEC, ECA109, KYSE30, KYSE140, KYSE510, and TE-1 cells were determined by qRT-PCR after exposure to CCL18. *P < 0.05, **P < 0.01, ***P < 0.001.

HOTAIR in 25 ESCC tissues and 25 adjacent benign esophageal tissues. HOTAIR expression was markedly higher in ESCC tissues than in non-cancerous esophageal tissues ($P < 0.001$, Fig. 2A). Likewise, the expression of HOTAIR was much higher in patients with metastasis than in those without metastasis ($P < 0.05$, Fig. 2B). The HOTAIR and CCL18 expression levels were analyzed to detect the relationship between them. As shown in Fig. 2C, in 25 ESCC tissues, the expression level of HOTAIR was positively associated with that of CCL18 (r = 0.62, $P = 0.0009$). To further examine whether ESCC tissues with high CCL18 expression also showed high HOTAIR expression, we used ISH to detect the expression of HOTAIR in the ESCC tissues. The results showed that HOTAIR was highly expressed in ESCC tissues, but not in adjacent benign esophageal tissues; the HOTAIR expression was related to the expression of CCL18. It is noteworthy that the high expression of CCL18 was significantly associated with HOTAIR positivity (Fig. 2D, Supplementary Figs. S2A–B, r = 0.605, $P < 0.001$). To confirm

whether CCL18 regulates the expression of HOTAIR, we used qRT-PCR to determine the levels of HOTAIR in cancer cells treated with CCL18. The results showed that CCL18 could increase the HOTAIR expression in various cancer cells (Fig. 2G). To investigate whether HOTAIR is involved in the CCL18-mediated promotion of ESCC cell invasiveness, a transwell chamber assay was performed to measure the invasiveness of TE-1 cells. The cells were transfected with short hairpin RNAs (shRNAs) for HOTAIR or mock-transfected (Supplementary Fig. S3, $P < 0.01$). After exposure to CCL18, the invasiveness of mock-transfected TE-1 cells increased, compared to that of the untreated cells. In contrast, the invasiveness of sh-HOTAIR-transfected cells treated with CCL18 reduced significantly (Fig. 2E and F). These data suggest that reducing the expression of HOTAIR in ESCC may alleviate the invasiveness of esophageal cancer cells.

3.3. HOTAIR reduced the miR-130a-5p expression in ESCC

Our results demonstrated that HOTAIR mediates the CCL18-induced invasion of ESCC cells. However, the downstream signaling of HOTAIR in ESCC is still unclear. Recently, accumulating evidence has suggested that non-coding RNAs may participate in ceRNA regulatory networks [18]. To explore whether any miRNAs are involved in the regulation of HOTAIR signaling and the ensuing metastasis of ESCC, we used the Starbase V2.0 online program to search for miRNAs that showed complementary base pairing with HOTAIR. We found 18 miRNAs with such complementary sequences. To further validate whether these miRNAs were regulated by HOTAIR in esophageal cancer cells, we performed qRT-PCR to determine their levels in TE-1 cells transfected with sh-Vector or sh-HOTAIR. Compared with the cancer cells transfected with sh-Vector, the expression levels of all 18 miRNAs in the TE-1 cells transfected with sh-HOTAIR, miR-130a-5p showed the greatest fold change in response to HOTAIR knockdown (Fig. 3A). In addition, a bioinformatics prediction tool indicated that there were complementary sequences with miR-130a-5p seed regions in HOTAIR (Fig. 3B). Therefore, we chose miRNA-130a-5p for the subsequent experiments. In order to verify whether HOTAIR regulated the miR-130a-5p expression through the potential interaction at the putative miR-130a-5p binding sites, the wild type of HOTAIR or mutant HOTAIR fragment containing the putative miR-130a-5p binding sites was cloned into a dual-luciferase reporter. The luciferase activity was analyzed after the co-transfection of TE-1 cells with a miR-130a-5p mimic or scramble and the HOTAIR-Wt or HOTAIR-Mut plasmids. The results showed that the relative luciferase activity of the HOTAIR-Wt plasmid was significantly suppressed after co-transfection with the miR-130a-5p mimic. In contrast, this effect was not detected in the plasmid carrying the HOTAIR-Mut (Fig. 3C). This result demonstrates that the putative binding sites are vital for the reciprocal repression of HOTAIR and mir-130a-5p. To investigate the role of miR-130a-5p in ESCC, the expression of miR-130a-5p in 25 ESCC tissue samples and 25 adjacent benign esophageal tissue samples was detected by qRT-PCR. The results demonstrated that the miR-130a-5p expression in ESCC tissues was significantly lower than that in the adjacent paired tissue samples (Fig. 3D). To assess whether HOTAIR functions as a molecular sponge for miR-130a-5p, we analyzed the expression of HOTAIR and miR-130a-5p in 25 ESCC tissue samples. We observed a negative correlation between the HOTAIR and miR-130a-5p expression (Fig. 3E; $r = 0.52$, $P = 0.008$). These results suggest that HOTAIR may promote CCL18 signaling by acting as a ceRNA for miR-130a-5p.

3.4. Effect of ZEB1 on the malignant progression of ESCC

To further investigate the molecular mechanisms whereby HOTAIR

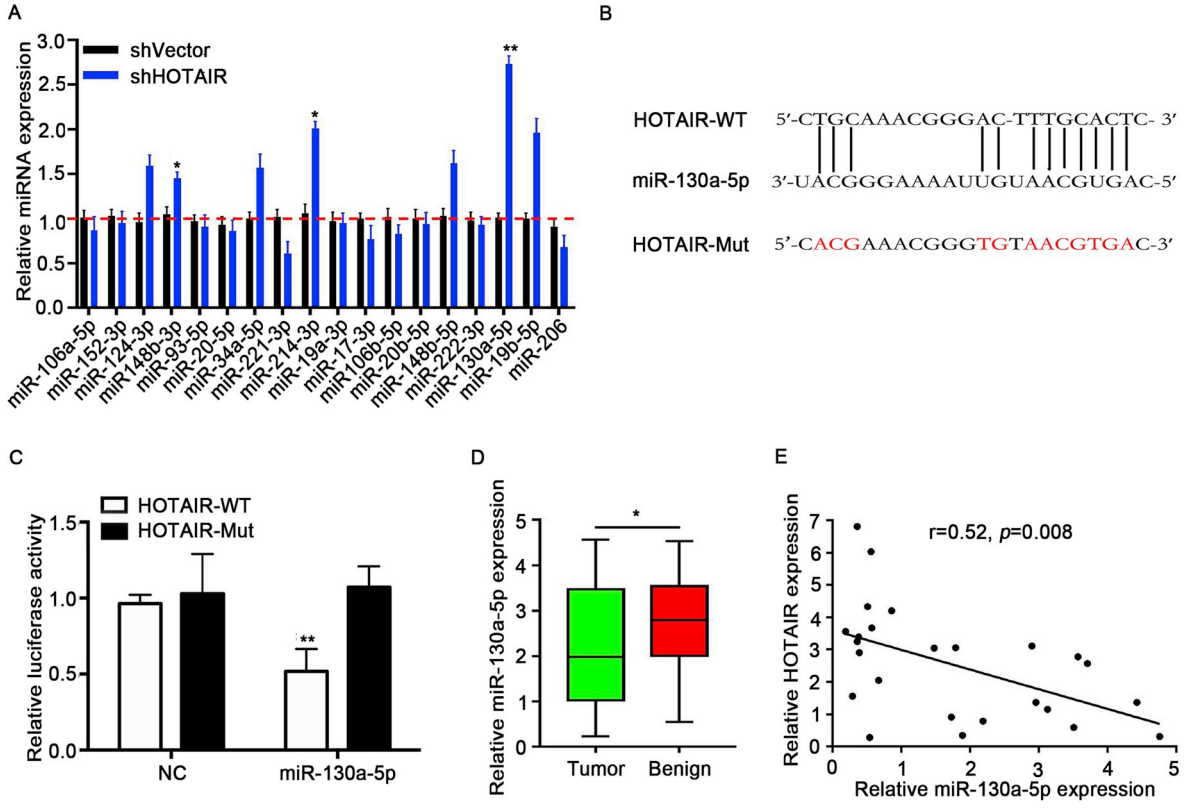

Fig. 3. HOTAIR reduced the miR-130a-5p expression in ESCC. (A) qRT-PCR analysis of the effect of knocking down HOTAIR on miRNAs expression. (B) Bioinformatics analysis revealed that HOTAIR contains binding sequences complementary to the seed region of miR-130a-5p. (C) miR-130a-5p mimics suppressed the luciferase activity of the wild-type but not mutant of HOTAIR reporter in TE-1 cells. (D) qRT-PCR analysis of miR-130a-5p expression in 25 ESCC tissues. (E) Pearson correlation analysis of HOTAIR expression levels and miR-130a-5p expression in ESCC tissues. *P < 0.05, **P < 0.01.

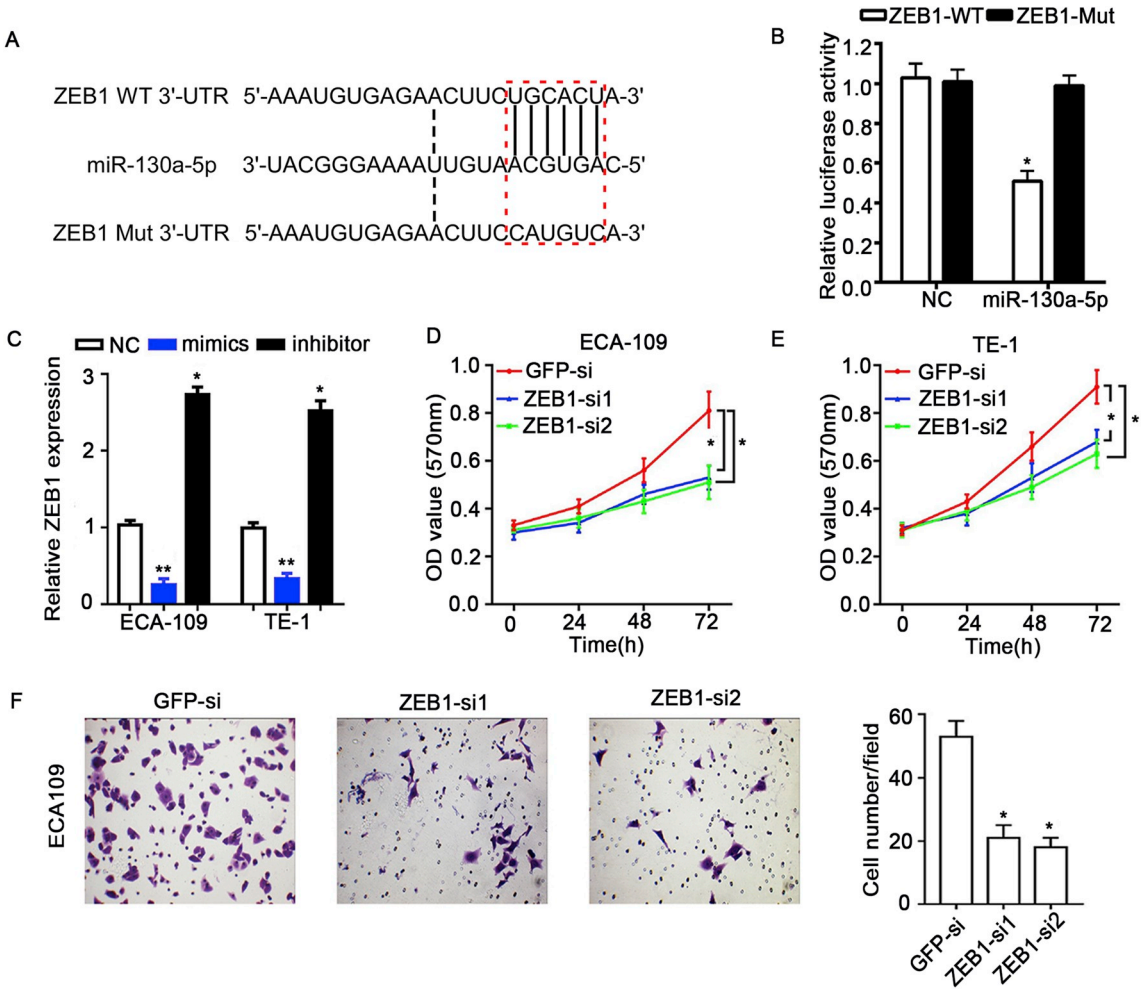

Fig. 4. Effect of ZEB1 on the malignant progression of ESCC. (A) The binding sites of miR-130a-5p within the 3′-UTR of ZEB1 were predicted by TargetScan. (B) Overexpression of miR-130a-5p led to a markedly decreased in luciferase activity of ZEB1-WT, without any change in luciferase activity of ZEB1-Mut in TE-1 cells. (C) ZEB1 expression was assessed by qRT-PCR when transfected with miR-130a-5p mimics or miR-130a-5p inhibitor in ECA109 and TE-1 cells. (D and E) Impact of ZEB1 on ECA-109 and TE-1 cell proliferation. (F) Influence of ZEB1 on ECA-109 cell invasion. *P < 0.05, **P < 0.01.

and miR-130a-5p regulate ESCC cell invasion, targetscan was used to carry out bioinformatics-based targeting prediction analysis, and ZEB1 was found to be a potential target of miR-130a-5p (Fig. 4A). To verify that ZEB1 was a direct target of miR-130a-5p, the 3′-UTR of ZEB1 with wild-type or mutant seed sequence recognition sites was cloned into a luciferase reporter. The luciferase activity was analyzed after the co-transfection of TE-1 cells with a miR-130a-5p mimic or scramble and the ZEB1 3′UTR-Wt or ZEB1 3′UTR-Mut plasmids. The results showed that the overexpression of miR-130a-5p led to a marked decrease in the luciferase activity of the plasmid carrying ZEB1 3′UTR-Wt, while no significant changes in luciferase activity were observed in TE-1 cells transfected with the ZEB1 3′UTR-Mut plasmid (Fig. 4B). To confirm that miR-130a-5p regulates the expression of ZEB1, we examined the expression of ZEB1 mRNA in ECA109 and TE-1 cells transfected with miR-130a-5p mimics or a miR-130a-5p inhibitor (Supplementary Fig. S4). We found that miR-130a-5p overexpression markedly decreased the expression of ZEB1 in ECA109 and TE-1 cells, while the down-regulation of miR-130a-5p increased the expression of ZEB1, compared with the control group (Fig. 4C). Subsequently, we explored the role of ZEB1 in ESCC. Two ZEB1-siRNAs were transfected into TE-1 cells to interfere with the function of ZEB1 (Fig. 4D and E, Supplementary Fig. S5). The effects of ZEB1 on the proliferation and invasion of ECA-109 and TE-1 were examined by the CCK-8 method using transwell chambers. The results showed that compared with the control group, the proliferation rate of ECA109 and TE-1 cells transfected with siRNAs decreased. Transfection of ECA-109 with either of the two ZEB1-siRNAs reduced the number of invasive cancer cells (Fig. 4F). Collectively, these data suggest that ZEB1 may promote the process of malignant progression in ESCC.

3.5. HOTAIR-induced reduction of miR-130a-5p expression inhibits invasion and EMT in ESCC via the targeting of ZEB1

In order to further investigate the biological roles of HOTAIR, miR-130a-5p, and ZEB1 in ESCC, a transwell assay was performed. The results showed that compared with the control group, the invasiveness of ECA109 and TE-1 cells transfected with miR-130a-5p mimics decreased, but the invasiveness of cells transfected with miR-130a-5p inhibitor increased. Furthermore, compared with the control group, the invsiveness of ECA109 and TE-1 cells transfected with shHOTAIR decreased. However, co-transfection with shHOTAIR and miR-130a-5p inhibitor rescued the inhibitory effect induced by HOTAIR knockdown. Moreover, compared to the case for transfection with shHOTAIR and miR-130a-5p inhibitor, the invasiveness of cells decreased following transfection with siZEB1, shHOTAIR and miR-130a-5p inhibitor. (Fig. 5A and B). ZEB1 is an important transcriptional regulatory factor that regulates EMT in tumor cells. To explore whether HOTAIR and miR-130a-5p were involved in the regulation of EMT in ESCC cells, we performed qRT-PCR and western blotting to detect the expression of EMT markers. We found that in ESCC cells transfected with shHOTAIR

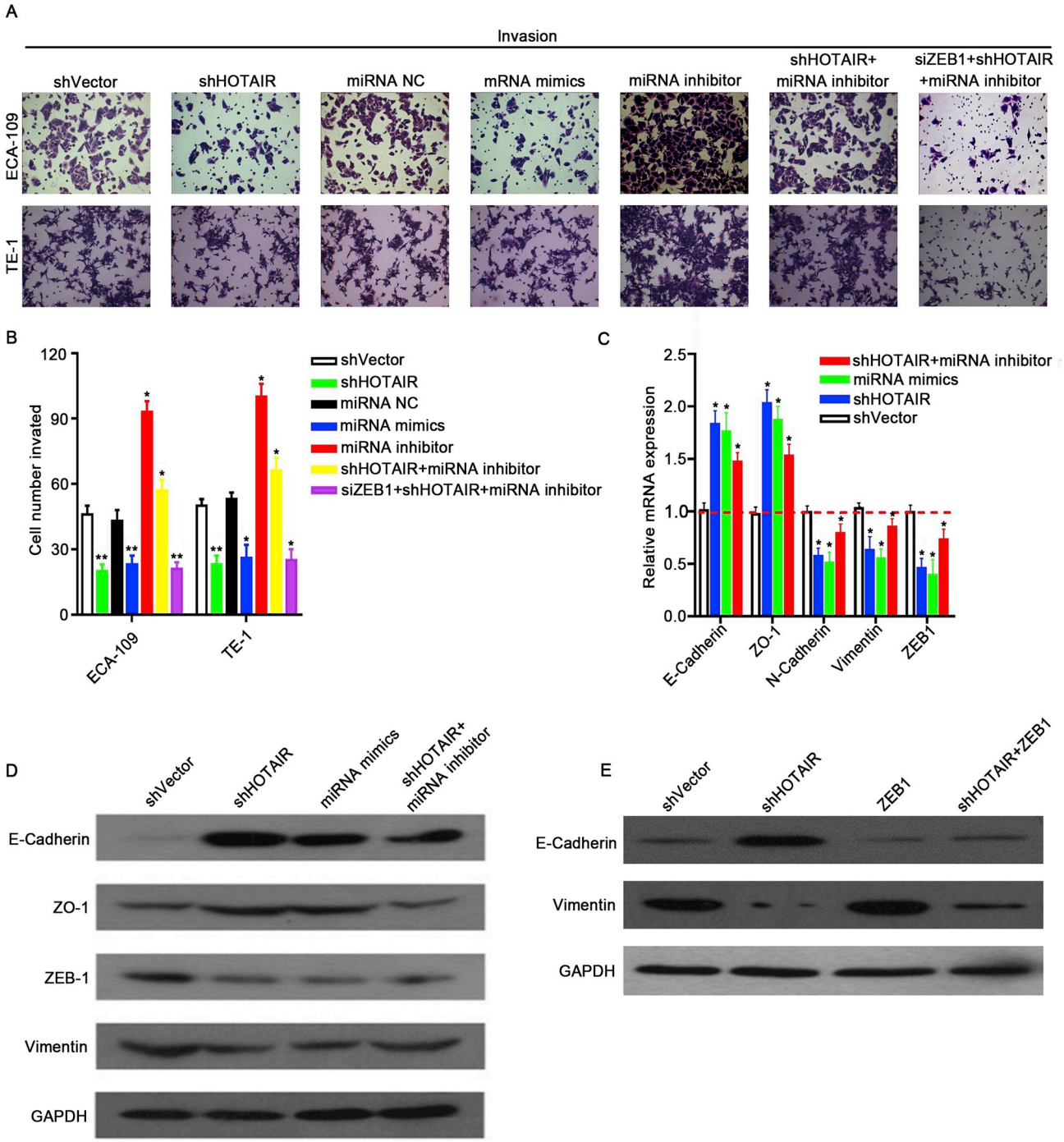

Fig. 5. HOTAIR-induced reduction of miR-130a-5p expression inhibits invasion and EMT in ESCC via the targeting of ZEB1. (A) Transwell assay showed the invasion ability of ECA109 and TE-1 cells transfected with shVector, shHOTAIR, miR-130a-5p mimics, miR-130a-5p inhibitor, shHOTAIR + miRNA inhibitor and siZEB1 + shHOTAIR + miRNA inhibitor. (B) cell invasion analysis. (C) E-Cadherin, ZO-1, N-Cadherin, ZEB1 and Vimentin expression in TE-1 cells transfected with shHOTAIR or miRNA-130a-5p mimics or shHOTAIR + miRNA-130a-5p inhibitor was analyzed by qRT-PCR. (D) E-Cadherin, ZO-1, ZEB1 and Vimentin expression in TE-1 cells transfected with shHOTAIR or miRNA-130a-5p mimics or shHOTAIR + miRNA-130a-5p inhibitor was analyzed by Western blot. (E) E-Cadherin and Vimentin expression in TE-1 cells transfected with shHOTAIR or pcDNA3.1-ZEB1 or shHOTAIR + ZEB1 was analyzed by Western blot. *P < 0.05, **P < 0.01.

or miR-130a-5p mimics, the expression levels of epithelial cell markers (E-cadherin and ZO-1) increased, while those of mesenchymal markers (N-cadherin and Vimentin) and ZEB1 decreased. However, the inhibition of EMT induced by shHOTAIR was reversed by co-transfection with a shHOTAIR + miR-130a-5p inhibitor (Fig. 5C and D). To further explore the effects of HOTAIR on ZEB1 function, we performed western blotting to detect the expression of EMT markers. We found that the E-cadherin expression decreased and Vimentin expression increased in ESCC cells transfected with pcDNA3.1-ZEB1 (Supplementary Fig. S6). Furthermore, HOTAIR knockdown markedly increased the expression of E-cadherin and decreased the expression of Vimentin. However, the inhibition of EMT induced by shHOTAIR was reversed by co-transfection with shHOTAIR + si-ZEB1 (Fig. 5E). These data suggest that HOTAIR may act as an miR-130a-5p sponge to upregulate the expression of its target ZEB1, thereby promoting the progression of EMT in ESCC.

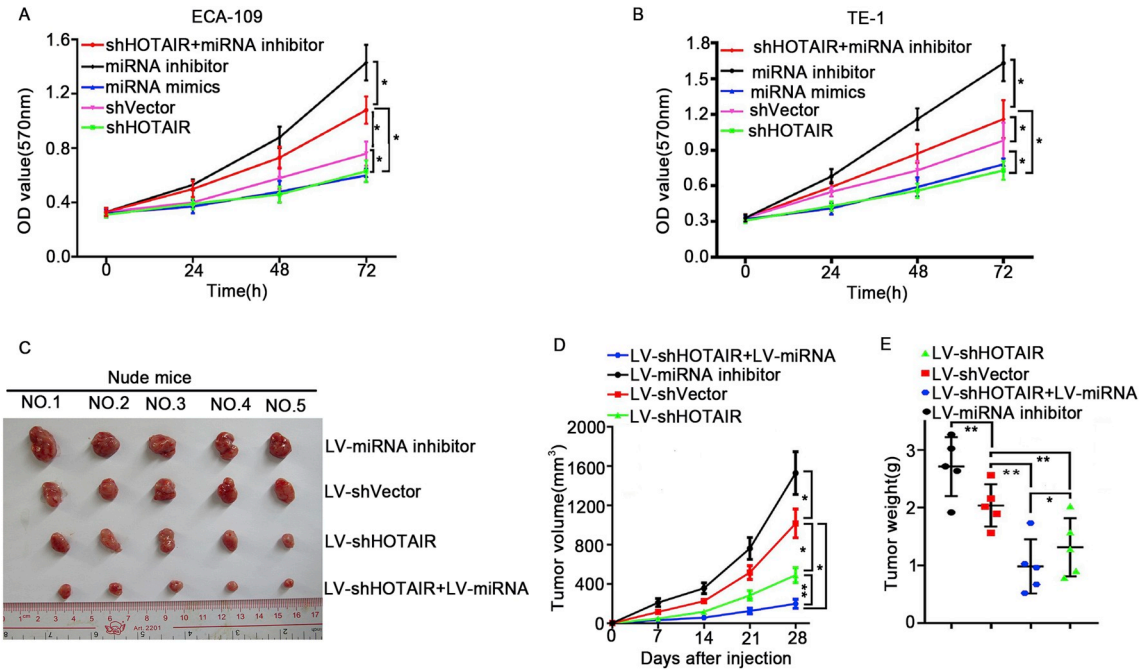

Fig. 6. HOTAIR demonstrates an oncogenic activity in part through negative regulation of miR-130a-5p in vitro and vivo. (A–B) CCK-8 assay showing the proliferation ability of ECA109 and TE-1 cells transfected with shVector, shHOTAIR, miR-130a-5p mimics, miR-130a-5p inhibitor, and shHOTAIR + miRNA inhibitor. (C–E) In vivo tumor xenograft study. (C) Excised TE-1 tumor. (D) Tumor volume was calculated every 7 days after injection. (E) Weight of excised tumor. *P < 0.05, **P < 0.01.

3.6. HOTAIR demonstrates an oncogenic activity in part through negative regulation of miR-130a-5p in vitro and vivo

In order to investigate the biological roles of HOTAIR and miR-130a-5p in ESCC, we employed gain-of-function and loss-of-function approaches. Compared with the control group, the proliferation of ECA109 and TE-1 cells transfected with miR-130a-5p mimics decreased, while the cell proliferation increased following the transfection with miR-130a-5p inhibitor. Furthermore, ECA109 and TE-1 cells transfected with shHOTAIR showed reduced cell proliferation compared with the control group. However, co-transfection with shHOTAIR and miR-130a-5p inhibitor rescued the inhibitory effect induced by HOTAIR knockdown. These results imply that the oncogenic activity of HOTAIR may occur partly through the negative regulation of miR-130a-5p (Fig. 6A and B).

We verified these findings in vitro using an in vivo xenograft model. TE-1 cells stably infected with Lv-shHOTAIR, Lv-miR-130a-5p inhibitor, or shHOTAIR + Lv-miR-130a-5p were subcutaneously injected into the dorsal flank of nude mice. After 6 weeks, the nude mice were sacrificed, and the tumor tissues were removed and weighed. Compared with the control group, the volume and size of tumors from mice in the shHOTAIR group and the shHOTAIR + Lv-miR-130a-5p group decreased significantly, while the shHOTAIR + Lv-miR-130a-5p group had a stronger anti-cancer effect. In contrast, the volume and size of tumors from mice in the Lv-miR-130a-5p inhibitor group markedly increased (Fig. 6C and D).

4. Discussion

Chemokines in the tumor microenvironment play important roles in cancer development [31–33]. In particular, CCL18 has a pivotal role in tumor progression and metastasis. However, the role of CCL18 in ESCC remains unclear. In this study, we demonstrated that CCL18 was overexpressed in ESCC tissues and cells and was associated with worse survival in patients with ESCC. Moreover, CCL18 enhanced the invasion of cancer cells in a dose-dependent manner, and knockdown of CCL18 could inhibit cancer cell invasiveness. These findings suggest that CCL18 plays a vital role in the progression and metastasis of ESCC.

CCL18 was primarily expressed in monocytes, macrophages, and dendritic cells [34–36]. Notably, IHC analysis of human pancreatic ductal adenocarcinoma and prostate cancer samples has revealed that CCL18 was expressed in both epithelial tumor cells and macrophages [37,38]. Although the level of CCL18 in body fluids has been shown to be increased in patients with lung cancer, bladder cancer, and ovarian cancer, the origin of CCL18 in these patients was not determined [14,39,40]. The results of the present study showed that the expression of CCL18 was upregulated in ESCC cells compared with that in HEECs. Furthermore, IHC analysis showed that CCL18 was highly expressed in the cytoplasm of ESCC cells. These results suggest that CCL18 at least partially plays an autocrine role in ESCC.

Although the functions of CCL18 have been reported in many cancers, its downstream signaling pathways remain uncertain. To date, three receptors have been proposed for CCL18: PITPNM3, GPR30 (G protein-coupled receptor 30), and CCR8 [12,41,42]. With regard to PITPNM3, it has been reported that PITPNM3-CCL18 binding induces Pyk2-and Src-mediated signaling, thus enhancing the metastasis of breast cancer [12]. Further characterization of downstream signaling pathways will enable people to gain a better understanding of the physiological role of CCL18 in ESCC. Our previous study identified that HOTAIR was a novel diagnostic biomarker, as well as a prognostic marker for progression and survival in ESCC [29,30]. Here, we ascertained whether CCL18 could promote ESCC by regulating the expression of HOTAIR, which has oncogenic functions. Our results confirmed that the expression of HOTAIR in ESCC tissues is significantly higher than that in non-cancerous esophageal tissues, and it is positively associated with the expression of CCL18 in ESCC tissues. In addition, HOTAIR expression was upregulated by CCL18 in ESCC cells, and HOTAIR knockdown alleviated the CCL18-induced invasiveness of ESCC cells. These data suggest that HOTAIR is an important part of the CCL18 regulatory network; it may mediate the CCL18-induced invasiveness of ESCC cells.

In several epidemiological studies, lncRNA, HOTAIR, has been

found to play a role in the epigenetic regulation of gene transcription [23,43]. Our data indicated that HOTAIR could be regulated by CCL18 and function as an oncogene in ESCC. However, the downstream molecular mechanisms underlying the role of HOTAIR in the occurrence and development of ESCC remain unclear. In recent years, significant progress has been made with regard to the research on lncRNA. Multiple studies have demonstrated that some specific endogenous lncRNAs can act as ceRNAs to interfere with miRNA pathways [44,45]. Inspired by the ceRNA regulatory network and emerging evidence that lncRNAs may participate in this regulatory circuitry, we hypothesized that HOTAIR might target miRNAs in ESCC. To test this hypothesis, we searched for potential miRNAs that interact with HOTAIR. Starbase 2.0 was used to predict a total of 18 potential miRNAs that could interact with HOTAIR. The detction of miRNAs silenced by HOTAIR in ESCC cells using qRT-PCR showed that the upregulation rate of miR-130a-5p was highest. Moreover, in ESCC, the expression of miR-130a-5p was inversely correlated with HOTAIR expression. Furthermore, based on a luciferase reporter assay, we found that miR-130a-5p was a direct target of HOTAIR. miR-130a-5p has been shown to be a prognostic marker for inhibiting the proliferation and invasion of cancer cells [46,47]. In this regard, we further explored the role of miR-130a-5p in ESCC. Our results demonstrated that an miR-130a-5p inhibitor enhanced cell proliferation and invasion, whereas HOTAIR knockdown reversed the effects induced by the miR-130a-5p inhibitor. Further in vivo studies revealed that in mice inoculated with TE-1 cells transfected with miR-130a-5p, tumor growth was inhibited. When TE-1 cells were co-transfected with shHOTAIR and miR-130a-5p, the inhibition was stronger. Collectively, these results are consistent with our hypothesis, indicating that HOTAIR may promote ESCC growth and invasiveness by sponging miR-130a-5p. LncRNA-miRNA-mRNA crosstalk forms a network that plays vital roles in human diseases [48]. In this study, we performed bioinformatics analysis and luciferase reporter assays and found that ZEB1 wss a direct target of miR130a-5p. We evaluated the expression of ZEB1 in ESCC cells with miR-130a-5p overexpression or knockdown and found that miR-130a-5p overexpression led to the downregulation of ZEB1. Meanwhile, we found that HOTAIR knockdown inhibited ZEB1 expression, and this inhibitory effect could be reversed by the co-transfection of the cells with the miR-130a-5p inhibitor. ZEB1 is a novel molecule and key transcription factor in the EMT process [49]. Our data showed that HOTAIR could regulate the expression of ZEB1 via the sponging of miR-130a-5p. We hypothesized that HOTAIR and miR-130a-5p were involved in the regulation of EMT in ESCC cells; thus, we detected the expression of EMT markers by western blotting. We found that in ESCC cells transfected with shHOTAIR or miR-130a-5p mimics, the expression levels of epithelial cell markers (E-cadherin and ZO-1) increased, whereas the expression of mesenchymal markers (N-cadherin and vimentin) decreased. However, the shHOTAIR-induced inhibition of EMT was reversed by co-transfection with a shHOTAIR + miR-130a-5p inhibitor. These data suggest that HOTAIR may act as a sponge for miR-130a-5p to upregulate the expression of its target ZEB1, thereby promoting the progression of EMT in ESCC.

In summary, our study suggests that CCL18 may contribute to the malignant progression of esophageal cancer by upregulating HOTAIR expression. Moreover, HOTAIR overexpression may promote tumor invasiveness and progression in ESCC, given that HOTAIR functions as a miR-130a-5p sponge to positively regulate ZEB1 expression. Thus, our findings not only provide new insights into the role of CCL18/HOTAIR and their downstream pathways in the development of ESCC, but also offer new potential therapeutic targets for the early diagnosis and treatment of ESCC. One potential limitation of this study is that the sample size is small. Thus, we will carry out future studies with larger sample sizes to ensure the accuracy of our results.

Declarations of interest

None.

Conflicts of interest

None.

CRediT authorship contribution statement

Wenjian Wang: Writing - original draft, Data curation, Software. **Duoguang Wu:** Formal analysis, Data curation. **Xiaotian He:** Visualization, Resources. **Xueting Hu:** Validation. **Chuwen Hu:** Investigation. **Zhiwen Shen:** Data curation. **Jiatong Lin:** Formal analysis. **Zihao Pan:** Visualization. **Zhanghai He:** Data curation. **Huayue Lin:** Supervision, Methodology, Writing - review & editing. **Minghui Wang:** Conceptualization, Project administration, Funding acquisition.

Acknowledgements

This work was supported by grants from the National Natural Science Foundation of China [grant numbers 81672415, 81871886]; the Natural Science Foundation of Guangdong Province, China [grant number No. 2017A030313474]; and the Guangdong Science and Technology Department [grant number 2017B030314026].

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.canlet.2019.06.009.

References

[1] V. Nagaraja, G.D. Eslick, Forthcoming prognostic markers for esophageal cancer: a systematic review and meta-analysis, J. Gastrointest. Oncol. 5 (2014) 67–76.
[2] K. Matsushima, H. Isomoto, N. Yamaguchi, N. Inoue, H. Machida, T. Nakayama, T. Hayashi, M. Kunizaki, S. Hidaka, T. Nagayasu, M. Nakashima, K. Ujifuku, N. Mitsutake, A. Ohtsuru, S. Yamashita, M. Korpal, Y. Kang, P.A. Gregory, G.J. Goodall, S. Kohno, K. Nakao, MiRNA-205 modulates cellular invasion and migration via regulating zinc finger E-box binding homeobox 2 expression in esophageal squamous cell carcinoma cells, J. Transl. Med. 9 (2011) 30.
[3] A. Rustgi, H.B. El-Serag, Esophageal carcinoma, N. Engl. J. Med. 372 (2015) 1472–1473.
[4] J. Chai, M.M. Jamal, Esophageal malignancy: a growing concern, World J. Gastroenterol. 18 (2012) 6521–6526.
[5] A. Pennathur, M.K. Gibson, B.A. Jobe, J.D. Luketich, Oesophageal carcinoma, Lancet 381 (2013) 400–412.
[6] J. Condeelis, J.W. Pollard, Macrophages: obligate partners for tumor cell migration, invasion, and metastasis, Cell 124 (2006) 263–266.
[7] P. Allavena, A. Sica, G. Solinas, C. Porta, A. Mantovani, The inflammatory microenvironment in tumor progression: the role of tumor-associated macrophages, Crit. Rev. Oncol. Hematol. 66 (2008) 1–9.
[8] I.U. Schraufstatter, M. Zhao, S.K. Khaldoyanidi, R.G. Discipio, The chemokine CCL18 causes maturation of cultured monocytes to macrophages in the M2 spectrum, Immunology 135 (2012) 287–298.
[9] H. Cai, Y. An, X. Chen, D. Sun, T. Chen, Y. Peng, F. Zhu, Y. Jiang, X. He, Epigenetic inhibition of miR-663b by long non-coding RNA HOTAIR promotes pancreatic cancer cell proliferation via up-regulation of insulin-like growth factor 2, Oncotarget 7 (2016) 86857–86870.
[10] E. Schutyser, S. Struyf, P. Proost, G. Opdenakker, G. Laureys, B. Verhasselt, L. Peperstraete, I. Van de Putte, A. Saccani, P. Allavena, A. Mantovani, J. Van Damme, Identification of biologically active chemokine isoforms from ascitic fluid and elevated levels of CCL18/pulmonary and activation-regulated chemokine in ovarian carcinoma, J. Biol. Chem. 277 (2002) 24584–24593.
[11] S.Y. Leung, S.T. Yuen, K.M. Chu, J.A. Mathy, R. Li, A.S. Chan, S. Law, J. Wong, X. Chen, S. So, Expression profiling identifies chemokine (C-C motif) ligand 18 as an independent prognostic indicator in gastric cancer, Gastroenterology 127 (2004) 457–469.
[12] J. Chen, Y. Yao, C. Gong, F. Yu, S. Su, J. Chen, B. Liu, H. Deng, F. Wang, L. Lin, H. Yao, F. Su, K.S. Anderson, Q. Liu, M.E. Ewen, X. Yao, E. Song, CCL18 from tumor-associated macrophages promotes breast cancer metastasis via PITPNM3, Cancer Cell 19 (2011) 541–555.
[13] D. Duluc, M. Corvaisier, S. Blanchard, L. Catala, P. Descamps, E. Gamelin, S. Ponsoda, Y. Delneste, M. Hebbar, P. Jeannin, Interferon-gamma reverses the immunosuppressive and protumoral properties and prevents the generation of human tumor-associated macrophages, Int. J. Cancer 125 (2009) 367–373.
[14] S.F. Zohny, S.T. Fayed, Clinical utility of circulating matrix metalloproteinase-7 (MMP-7), CC chemokine ligand 18 (CCL18) and CC chemokine ligand 11 (CCL11)

as markers for diagnosis of epithelial ovarian cancer, Med. Oncol. 27 (2010) 1246–1253.
[15] T.R. Mercer, M.E. Dinger, J.S. Mattick, Long non-coding RNAs: insights into functions, Nat. Rev. Genet. 10 (2009) 155–159.
[16] E. Birney, J.A. Stamatoyannopoulos, A. Dutta, R. Guigo, T.R. Gingeras, E.H. Margulies, Z. Weng, M. Snyder, E.T. Dermitzakis, R.E. Thurman, M.S. Kuehn, C.M. Taylor, S. Neph, C.M. Koch, S. Asthana, A. Malhotra, I. Adzhubei, J.A. Greenbaum, R.M. Andrews, P. Flicek, P.J. Boyle, H. Cao, N.P. Carter, G.K. Clelland, S. Davis, N. Day, P. Dhami, S.C. Dillon, M.O. Dorschner, H. Fiegler, P.G. Giresi, J. Goldy, M. Hawrylycz, A. Haydock, R. Humbert, K.D. James, B.E. Johnson, E.M. Johnson, T.T. Frum, E.R. Rosenzweig, N. Karnani, K. Lee, G.C. Lefebvre, P.A. Navas, F. Neri, S.C. Parker, P.J. Sabo, R. Sandstrom, A. Shafer, D. Vetrie, M. Weaver, S. Wilcox, M. Yu, F.S. Collins, J. Dekker, J.D. Lieb, T.D. Tullius, G.E. Crawford, S. Sunyaev, W.S. Noble, I. Dunham, F. Denoeud, A. Reymond, P. Kapranov, J. Rozowsky, D. Zheng, R. Castelo, A. Frankish, J. Harrow, S. Ghosh, A. Sandelin, I.L. Hofacker, R. Baertsch, D. Keefe, S. Dike, J. Cheng, H.A. Hirsch, E.A. Sekinger, J. Lagarde, J.F. Abril, A. Shahab, C. Flamm, C. Fried, J. Hackermuller, J. Hertel, M. Lindemeyer, K. Missal, A. Tanzer, S. Washietl, J. Korbel, O. Emanuelsson, J.S. Pedersen, N. Holroyd, R. Taylor, D. Swarbreck, N. Matthews, M.C. Dickson, D.J. Thomas, M.T. Weirauch, J. Gilbert, J. Drenkow, I. Bell, X. Zhao, K.G. Srinivasan, W.K. Sung, H.S. Ooi, K.P. Chiu, S. Foissac, T. Alioto, M. Brent, L. Pachter, M.L. Tress, A. Valencia, S.W. Choo, C.Y. Choo, C. Ucla, C. Manzano, C. Wyss, E. Cheung, T.G. Clark, J.B. Brown, M. Ganesh, S. Patel, H. Tammana, J. Chrast, C.N. Henrichsen, C. Kai, J. Kawai, U. Nagalakshmi, J. Wu, Z. Lian, J. Lian, P. Newburger, X. Zhang, P. Bickel, J.S. Mattick, P. Carninci, Y. Hayashizaki, S. Weissman, T. Hubbard, R.M. Myers, J. Rogers, P.F. Stadler, T.M. Lowe, C.L. Wei, Y. Ruan, K. Struhl, M. Gerstein, S.E. Antonarakis, Y. Fu, E.D. Green, U. Karaoz, A. Siepel, J. Taylor, L.A. Liefer, K.A. Wetterstrand, P.J. Good, E.A. Feingold, M.S. Guyer, G.M. Cooper, G. Asimenos, C.N. Dewey, M. Hou, S. Nikolaev, J.I. Montoya-Burgos, A. Loytynoja, S. Whelan, F. Pardi, T. Massingham, H. Huang, N.R. Zhang, I. Holmes, J.C. Mullikin, A. Ureta-Vidal, B. Paten, M. Seringhaus, D. Church, K. Rosenbloom, W.J. Kent, E.A. Stone, S. Batzoglou, N. Goldman, R.C. Hardison, D. Haussler, W. Miller, A. Sidow, N.D. Trinklein, Z.D. Zhang, L. Barrera, R. Stuart, D.C. King, A. Ameur, S. Enroth, M.C. Bieda, J. Kim, A.A. Bhinge, N. Jiang, J. Liu, F. Yao, V.B. Vega, C.W. Lee, P. Ng, A. Shahab, A. Yang, Z. Moqtaderi, Z. Zhu, X. Xu, S. Squazzo, M.J. Oberley, D. Inman, M.A. Singer, T.A. Richmond, K.J. Munn, A. Rada-Iglesias, O. Wallerman, J. Komorowski, J.C. Fowler, P. Couttet, A.W. Bruce, O.M. Dovey, P.D. Ellis, C.F. Langford, D.A. Nix, G. Euskirchen, S. Hartman, A.E. Urban, P. Kraus, S. Van Calcar, N. Heintzman, T.H. Kim, K. Wang, C. Qu, G. Hon, R. Luna, C.K. Glass, M.G. Rosenfeld, S.F. Aldred, S.J. Cooper, A. Halees, J.M. Lin, H.P. Shulha, X. Zhang, M. Xu, J.N. Haidar, Y. Yu, Y. Ruan, V.R. Iyer, R.D. Green, C. Wadelius, P.J. Farnham, B. Ren, R.A. Harte, A.S. Hinrichs, H. Trumbower, H. Clawson, J. Hillman-Jackson, A.S. Zweig, K. Smith, A. Thakkapallayil, G. Barber, R.M. Kuhn, D. Karolchik, L. Armengol, C.P. Bird, P.I. de Bakker, A.D. Kern, N. Lopez-Bigas, J.D. Martin, B.E. Stranger, A. Woodroffe, E. Davydov, A. Dimas, E. Eyras, I.B. Hallgrimsdottir, J. Huppert, M.C. Zody, G.R. Abecasis, X. Estivill, G.G. Bouffard, X. Guan, N.F. Hansen, J.R. Idol, V.V. Maduro, B. Maskeri, J.C. McDowell, M. Park, P.J. Thomas, A.C. Young, R.W. Blakesley, D.M. Muzny, E. Sodergren, D.A. Wheeler, K.C. Worley, H. Jiang, G.M. Weinstock, R.A. Gibbs, T. Graves, R. Fulton, E.R. Mardis, R.K. Wilson, M. Clamp, J. Cuff, S. Gnerre, D.B. Jaffe, J.L. Chang, K. Lindblad-Toh, E.S. Lander, M. Koriabine, M. Nefedov, K. Osoegawa, Y. Yoshinaga, B. Zhu, P.J. de Jong, Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project, Nature 447 (2007) 799–816.
[17] R.A. Gupta, N. Shah, K.C. Wang, J. Kim, H.M. Horlings, D.J. Wong, M.C. Tsai, T. Hung, P. Argani, J.L. Rinn, Y. Wang, P. Brzoska, B. Kong, R. Li, R.B. West, M.J. van de Vijver, S. Sukumar, H.Y. Chang, Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis, Nature 464 (2010) 1071–1076.
[18] L. Salmena, L. Poliseno, Y. Tay, L. Kats, P.P. Pandolfi, A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language? Cell 146 (2011) 353–358.
[19] P. Johnsson, A. Ackley, L. Vidarsdottir, W.O. Lui, M. Corcoran, D. Grander, K.V. Morris, A pseudogene long-noncoding-RNA network regulates PTEN transcription and translation in human cells, Nat. Struct. Mol. Biol. 20 (2013) 440–446.
[20] S.H. Wang, W.J. Zhang, X.C. Wu, M.D. Zhang, M.Z. Weng, D. Zhou, J.D. Wang, Z.W. Quan, Long non-coding RNA Malat1 promotes gallbladder cancer development by acting as a molecular sponge to regulate miR-206, Oncotarget 7 (2016) 37857–37867.
[21] J. Lv, H.X. Fan, X.P. Zhao, P. Lv, J.Y. Fan, Y. Zhang, M. Liu, H. Tang, Long non-coding RNA Unigene56159 promotes epithelial-mesenchymal transition by acting as a ceRNA of miR-140-5p in hepatocellular carcinoma cells, Cancer Lett. 382 (2016) 166–175.
[22] J.L. Rinn, M. Kertesz, J.K. Wang, S.L. Squazzo, X. Xu, S.A. Brugmann, L.H. Goodnough, J.A. Helms, P.J. Farnham, E. Segal, H.Y. Chang, Functional demarcation of active and silent chromatin domains in human HOX loci by noncoding RNAs, Cell 129 (2007) 1311–1323.
[23] K. Kim, I. Jutooru, G. Chadalapaka, G. Johnson, J. Frank, R. Burghardt, S. Kim, S. Safe, HOTAIR is a negative prognostic factor and exhibits pro-oncogenic activity in pancreatic cancer, Oncogene 32 (2013) 1616–1625.
[24] R. Kogo, T. Shimamura, K. Mimori, K. Kawahara, S. Imoto, T. Sudo, F. Tanaka, K. Shibata, A. Suzuki, S. Komune, S. Miyano, M. Mori, Long noncoding RNA HOTAIR regulates polycomb-dependent chromatin modification and is associated with poor prognosis in colorectal cancers, Cancer Res. 71 (2011) 6320–6326.
[25] H. Endo, T. Shiroki, T. Nakagawa, M. Yokoyama, K. Tamai, H. Yamanami, T. Fujiya, I. Sato, K. Yamaguchi, N. Tanaka, K. Iijima, T. Shimosegawa, K. Sugamura, K. Satoh, Enhanced expression of long non-coding RNA HOTAIR is associated with the development of gastric cancer, PLoS One 8 (2013) e77070.
[26] X.H. Liu, M. Sun, F.Q. Nie, Y.B. Ge, E.B. Zhang, D.D. Yin, R. Kong, R. Xia, K.H. Lu, J.H. Li, W. De, K.M. Wang, Z.X. Wang, Lnc RNA HOTAIR functions as a competing endogenous RNA to regulate HER2 expression by sponging miR-331-3p in gastric cancer, Mol. Cancer 13 (2014) 92.
[27] Z. Yang, L. Zhou, L.M. Wu, M.C. Lai, H.Y. Xie, F. Zhang, S.S. Zheng, Overexpression of long non-coding RNA HOTAIR predicts tumor recurrence in hepatocellular carcinoma patients following liver transplantation, Ann. Surg. Oncol. 18 (2011) 1243–1250.
[28] M. Ishibashi, R. Kogo, K. Shibata, G. Sawada, Y. Takahashi, J. Kurashige, S. Akiyoshi, S. Sasaki, T. Iwaya, T. Sudo, K. Sugimachi, K. Mimori, G. Wakabayashi, M. Mori, Clinical significance of the expression of long non-coding RNA HOTAIR in primary hepatocellular carcinoma, Oncol. Rep. 29 (2013) 946–950.
[29] X. Bao, T. Ren, Y. Huang, K. Sun, S. Wang, K. Liu, B. Zheng, W. Guo, Knockdown of long non-coding RNA HOTAIR increases miR-454-3p by targeting Stat3 and Atg12 to inhibit chondrosarcoma growth, Cell Death Dis. 8 (2017) e2605.
[30] X.B. Lv, G.Y. Lian, H.R. Wang, E. Song, H. Yao, M.H. Wang, Long noncoding RNA HOTAIR is a prognostic marker for esophageal squamous cell carcinoma progression and survival, PLoS One 8 (2013) e63516.
[31] H. Lu, K.R. Clauser, W.L. Tam, J. Frose, X. Ye, E.N. Eaton, F. Reinhardt, V.S. Donnenberg, R. Bhargava, S.A. Carr, R.A. Weinberg, A breast cancer stem cell niche supported by juxtacrine signalling from monocytes and macrophages, Nat. Cell Biol. 16 (2014) 1105–1117.
[32] T. Atsumi, R. Singh, L. Sabharwal, H. Bando, J. Meng, Y. Arima, M. Yamada, M. Harada, J.J. Jiang, D. Kamimura, H. Ogura, T. Hirano, M. Murakami, Inflammation amplifier, a new paradigm in cancer biology, Cancer Res. 74 (2014) 8–14.
[33] P.J. Maxwell, J. Neisen, J. Messenger, D.J. Waugh, Tumor-derived CXCL8 signaling augments stroma-derived CCL2-promoted proliferation and CXCL12-mediated invasion of PTEN-deficient prostate cancer cells, Oncotarget 5 (2014) 4895–4908.
[34] E. Schutyser, A. Richmond, J. Van Damme, Involvement of CC chemokine ligand 18 (CCL18) in normal and pathological processes, J. Leukoc. Biol. 78 (2005) 14–26.
[35] X. Lin, L. Chen, Y. Yao, R. Zhao, X. Cui, J. Chen, K. Hou, M. Zhang, F. Su, J. Chen, E. Song, CCL18-mediated down-regulation of miR98 and miR27b promotes breast cancer metastasis, Oncotarget 6 (2015) 20485–20499.
[36] R. van der Voort, M. Kramer, E. Lindhout, R. Torensma, D. Eleveld, A.W. van Lieshout, M. Looman, T. Ruers, T.R. Radstake, C.G. Figdor, G.J. Adema, Novel monoclonal antibodies detect elevated levels of the chemokine CCL18/DC-CK1 in serum and body fluids in pathological conditions, J. Leukoc. Biol. 77 (2005) 739–747.
[37] G. Chen, Y.X. Liang, J.G. Zhu, X. Fu, Y.F. Chen, R.J. Mo, L. Zhou, H. Fu, X.C. Bi, H.C. He, S.B. Yang, Y.D. Wu, F.N. Jiang, W.D. Zhong, CC chemokine ligand 18 correlates with malignant progression of prostate cancer, BioMed Res. Int. 2014 (2014) 230183.
[38] F. Meng, W. Li, C. Li, Z. Gao, K. Guo, S. Song, CCL18 promotes epithelial-mesenchymal transition, invasion and migration of pancreatic cancer cells in pancreatic ductal adenocarcinoma, Int. J. Oncol. 46 (2015) 1109–1120.
[39] T. Plones, A. Krohn, M. Burger, H. Veelken, B. Passlick, J. Muller-Quernheim, G. Zissel, Serum level of CC-chemokine ligand 18 is increased in patients with non-small-cell lung cancer and correlates with survival time in adenocarcinomas, PLoS One 7 (2012) e41746.
[40] V. Urquidi, J. Kim, M. Chang, Y. Dai, C.J. Rosser, S. Goodison, CCL18 in a multiplex urine-based assay for the detection of bladder cancer, PLoS One 7 (2012) e37797.
[41] J. Catusse, S. Wollner, M. Leick, P. Schrottner, I. Schraufstatter, M. Burger, Attenuation of CXCR4 responses by CCL18 in acute lymphocytic leukemia B cells, J. Cell. Physiol. 225 (2010) 792–800.
[42] S.A. Islam, M.F. Ling, J. Leung, W.G. Shreffler, A.D. Luster, Identification of human CCR8 as a CCL18 receptor, J. Exp. Med. 210 (2013) 1889–1898.
[43] T. Niinuma, H. Suzuki, M. Nojima, K. Nosho, H. Yamamoto, H. Takamaru, E. Yamamoto, R. Maruyama, T. Nobuoka, Y. Miyazaki, T. Nishida, T. Bamba, T. Kanda, Y. Ajioka, T. Taguchi, S. Okahara, H. Takahashi, Y. Nishida, M. Hosokawa, T. Hasegawa, T. Tokino, K. Hirata, K. Imai, M. Toyota, Y. Shinomura, Upregulation of miR-196a and HOTAIR drive malignant character in gastrointestinal stromal tumors, Cancer Res. 72 (2012) 1126–1136.
[44] W. Arancio, G. Pizzolanti, S.I. Genovese, C. Baiamonte, C. Giordano, Competing endogenous RNA and interactome bioinformatic analyses on human telomerase, Rejuvenation Res. 17 (2014) 161–167.
[45] R. Sen, S. Ghosal, S. Das, S. Balti, J. Chakrabarti, Competing endogenous rna: the key to posttranscriptional regulation, Sci.World J. 2014 (2014) 896206.
[46] G. Zhao, J.G. Zhang, Y. Shi, Q. Qin, Y. Liu, B. Wang, K. Tian, S.C. Deng, X. Li, S. Zhu, Q. Gong, Y. Niu, C.Y. Wang, MiR-130a-5p is a prognostic marker and inhibits cell proliferation and invasion in pancreatic cancer through targeting STAT3, PLoS One 8 (2013) e73803.
[47] T. Colangelo, A. Fucci, C. Votino, L. Sabatino, M. Pancione, C. Laudanna, M. Binaschi, M. Bigioni, C.A. Maggi, D. Parente, N. Forte, V. Colantuoni, MicroRNA-130a-5p promotes tumor development and is associated with poor prognosis in colorectal cancer, Neoplasia 15 (2013) 1086–1099.
[48] J. Qu, M. Li, W. Zhong, C. Hu, Competing endogenous RNA in cancer: a new pattern of gene expression regulation, Int. J. Clin. Exp. Med. 8 (2015) 17110–17116.
[49] K. Chandra Mangalhara, S. Manvati, S.K. Saini, K. Ponnusamy, G. Agarwal, S.K. Abraham, R.N.K. Bamezai, ERK2-ZEB1-miR-101-1 axis contributes to epithelial-mesenchymal transition and cell migration in cancer, Cancer Lett. 391 (2017) 59–73.