Iran. J. Biotechnol. July 2024;22(3): e3693 DOI: 10.30498/ijb.2024.394737.3693

Research Article

Inhibition of Chitinase-3-like Protein 1 Reduced Epithelial–Mesenchymal Transition and Vascular Epithelial Cadherin Expression in Oesophageal Squamous Cell Carcinoma

Yanzi Qin[1#], Wenjun Zhao[2#], Qicai Li[3#], Zhaogeng Cai[1], Guowen Wang[3], Nan Wang[4*], Li Ma[1*]

[1]Department of Pathology, The First Affiliated Hospital of Bengbu Medical University, Bengbu 233000, China
[2]Department of Emergency Internal Medicine, The Third the People's Hospital of Bengbu, Bengbu 233000, China
[3]Department of Thoracic and Cardiovascular Surgery, The First Affiliated Hospital of Bengbu Medical University,Bengbu 233000, China
[4]Department of Critical Care Medicine, The Fourth Affiliated Hospital of Anhui Medical University, Hefei 230601, China

[*]*Corresponding author*: Nan Wang, Department of Critical Care Medicine, The Fourth Affiliated Hospital of Anhui Medical University, Hefei 230601, China. Tel: +86-15955228723, Fax: +86-18196608009, E-mail: nw0615@outlook.com

# These authors contributed equally to this study

Received: 2024/07/18 ; **Accepted:** 2024/09/16

Background: Oesophageal cancer (EC) is one of the common malignant tumors, and the prognosis of patients is poor. Further exploration of EC pathogenesis remains warranted.

Objective: The relationship between vascular epithelial cadherin (VE-cadherin) and chitinase-3-like protein 1 (CHI3L1) in EC is currently unknown. To further explore the relationship, immunohistochemical staining was performed to detect the expression level of CHI3L1 and VE-cadherin in oesophageal squamous cell carcinoma (ESCC).

Materials and Methods: Small interfering RNAs (siRNAs) inhibited CHI3L1 expression in KYSE-150 and TE1 cells. Western blot and quantitative fluorescence polymerase chain reaction were used to detect the levels of CHI3L1, VE-cadherin and epithelial–mesenchymal transition (EMT)-related proteins *in vitro* and *in vivo*, and KYSE-150 cells were used to establish an *in-vivo* model and observe tumour growth.

Results: High levels of CHI3L1 and VE-cadherin expression were closely associated with the progression of ESCC; the pathologic tumour-node-metastasis stage was also closely related with the progression of ESCC ($p < 0.05$). High levels of CHI3L1 and VE-cadherin expression led to poor prognosis in patients with EC. In KYSE-150 and TE1 EC cell lines, the invasion, migration and proliferation of cells decreased, and the apoptotic rate increased after CHI3L1 expression was decreased using siRNA. The CHI3L1, VE-cadherin, Snail, Twist1 protein and mRNA expression levels decreased, whereas the E-cadherin levels increased.

Conclusions: Chitinase-3-like protein 1 could promote the EMT of ESCC, and the inhibition of CHI3L1 decreases the expression of VE-cadherin, which inhibits tumour angiogenesis and tumour progression in ESCC.

Keywords: CHI3L1; Oesophageal cancer; Squamous cell carcinoma; VE-cadherin

1. Background

Oesophageal cancer (EC) is a malignant tumour and the sixth leading cause of tumour-related death (1); oesophageal squamous cell carcinoma (ESCC) accounts for >90% of ECs (2). Although significant progress has been made in the early detection,

accurate diagnosis and combined treatment of ESCC, its 5-year survival rate remains extremely low (3). The primary reason for this is that the invasion and metastasis of ESCC fatally affect the patients' prognosis. Therefore, it is necessary to explore the pathogenesis of ESCC further and provide new therapeutic targets for it.

Chitinase-3-like protein 1 (CHI3L1) is a protein similar to mammalian chitinase. It belongs to the glycoside hydrolase family 18 and is located on the human chromosome 1q32. Its amino acid sequence has extremely high homology (4,5). It is a secreted glycoprotein first discovered in the osteosarcoma MG-63 cell line cultured *in vitro* in 1992 (6). Chitinase-3-like protein 1 participated in the growth, proliferation, differentiation and remodelling of tissue. It played an important role in chronic inflammatory diseases, such as severe bacterial infections (7) and liver fibrosis (8, 9). The expression of CHI3L1 was elevated in the serum and tumour cells of patients with various malignancies, and CHI3L1 indicated poor survival and prognosis in patients with tumours (10-12).

Chitinase-3-like protein 1 could promote tumour angiogenesis and stromal remodelling. In the colon cancer SW480 cell line, overexpression of CHI3L1 could promote tumour angiogenesis by activating the ERK1/2 and JNK pathways of tumour cells (13). In a co-culture system of osteosarcoma cell line MG-63 and fibroblasts, inhibiting the expression of CHI3L1 in tumour cells could lead to a decrease in the expression of MMP-1 and vascular endothelial growth factors (VEGF) in fibroblasts (14). In gliomas, overexpression of CHI3L1 could promote tumour cells to express VEGF, thereby promoting the formation of new blood vessels (15). In EC, CHI3L1 expression indicated that CHI3L1 was highly correlated with the increased macrophage signature genes in oesophageal tumour tissue (16).

Vascular epithelial cadherin (VE-cadherin), also called cadherin-5, is a specific structural protein that is indispensable for maintaining the polarity and integrity of vascular endothelial cells. It can be used as an intercellular adhesion medium of the same type and is closely related to the stable distribution of cell membranes and the adhesion function of vascular endothelial cells (17). Studies have shown that VE-cadherin was overexpressed in aggressive human-skin cells and highly aggressive melanoma cells but not in non-invasive or low-aggressive melanoma cells (18). Recently, VE-cadherin has been demonstrated to be associated with vasculogenic mimicry (VM) (19, 20). After VE-cadherin inhibition, aggressive melanoma cells could not form an angiogenesis-like network, which indicated that tumour-related VE-cadherin promoted angiogenesis in the process of mimicking cell formation (21). Thus, VE-cadherin could enhance the microvascular formation of malignant tumours and promote their growth and progression (22,23). According to previous studies, CHI3L1 and VE-cadherin were both associated with angiogenesis, but there was no relevant report on the link between CHI3L1 and VE-cadherin in ESCC.

2. Objective

In this study, we aimed to investigate the association between CHI3L1 and VE-cadherin, explore the possible mechanism of oesophageal squamous epithelium carcinogenesis and provide a theoretical basis for targeted gene therapy for patients with ESCC. We hypothesised that CHI3L1 promoted epithelial–mesenchymal transition (EMT) in ESCC and that the inhibition of CHI3L1 decreased VE-cadherin expression, thereby inhibiting tumour angiogenesis and tumour progression.

3. Materials and Methods

3.1. Clinical Data

A total of 120 samples of paraffin specimens of ESCC and 60 samples of normal oesophageal mucosa tissue were collected from our hospital between January 2015 and December 2015. The selected patients were followed up until their death or until December 2015; the follow-up time was 9–78 months. All patients gave their informed consent, and the study was approved by the Ethics Committee of Bengbu Medical University. The clinical information of the patients is shown in **Table 1**. There were 97 men and 23 women; 48 patients were <65 years old, and 72 were >65 years old. Gross tumour morphology included the ulcer type in 44 patients, medullary type in 56 patients, mushroom type in 14 patients and constrictive type in 6 patients. The tumour location was in the upper segment in 10 patients, the middle segment in 51 patients and the lower segment in 59 patients. In terms of histological differentiation, the tumour was well differentiated in 12

patients, moderately differentiated in 78 patients and poorly differentiated in 30. The tumour size was <3.5 cm in 62 patients and >3.5 cm in 58. In 76 patients, the tumour had invaded the serosal layer, and in 44 patients, the tumour had not reached the serosal layer. There were 47 cases of lymph node metastasis and 73 cases without lymph node metastasis. The pathologic tumour-node-metastasis (pTNM) stage was I + II in 82 patients and III + IV in 38 patients. The patients agreed that all data and specimens could be obtained, and no radiotherapy, chemotherapy or other treatments were performed. The results of the pathological diagnosis were interpreted by two senior pathologists.

3.2. Immunochemistry

The paraffin-embedded specimens were sliced to a thickness of 4 μm. The slides were deparaffinised and followed by a 10-min treatment with 3% hydrogen peroxide. Antigen retrieval was performed using sodium citrate. Blocking was performed using goat serum for 1 h at 25 °C. Primary antibodies were subsequently incubated overnight at 4 °C with CHI3L1 (dilution ratio 1:1,000, ab77528, Abcam, USA) and VE-cadherin (dilution ratio 1:1,000, ab232880, Abcam, USA). The DAB reagent was dyed for 3–5 min and then re-stained with haematoxylin and sealed with neutral gum (20).

The appearance of brown–yellow or brown–black particles in the tumour cytoplasm indicated a positive expression of CHI3L1 protein. The formation of brown–yellow or tan particles in the tumour cell membrane and/or cytoplasm indicated a positive expression of the VE-cadherin protein (**Fig. 1A-1D**). According to the proportion of positive cells, <10% = 0 points, 10%–25% = 1 point, 26%–50% = 2 points, 5%–75% = 3 points and >75% = 4 points. Based on the intensity of positive cell staining, 0 = uncoloured, 1 = light yellow or yellow, 2 = brown and 3 = brown–black. The scores of the two items were multiplied; <3 was considered negative, and >3 was considered positive.

3.3. Cell Culture

Oesophageal cancer cell lines, KYSE-150 and TE1, were purchased from NCACC, China and cultured in Dulbecco's modified Eagle medium (DMEM) (Gibco, USA) containing 10% foetal bovine serum (FBS) (Gibco, USA) and 100 $U.mL^{-1}$ of penicillin/streptomycin at 37 °C with 5% CO_2. These two cell lines were the most commonly used ESCC cell lines (20).

3.4. Cell Transfection

A silencing CHI3L1 small interfering RNA (siRNA) fragment was designed (GenePharma, China), and siRNA constructs were performed using the psiRNAH1-neo vector (Testobio, China). The siRNA-control was as follows: (forward), 5'-UUCUCCGAACGUGUCACGUTT-3', Antisense 5'-ACUUGACACGUUCGGAGAATT-3' (reverse), siRNA-1: 5'-CCCACCCUAAUCAAGGAAAUTT-3' (forward), 5'-AUUUCCUUGAUUAGGGUGGTT-3' (reverse), siRNA-2: 5'-GAGCCACAGUCCAUAGAA UTT-3' (forward) and 5'-AUUCUAUGGACUGUGGC UCTT-3' (reverse); see **Table 2** for details. The KYSE-150 and TE1 cell lines were split into three groups: the NC group (siRNA control group), the siRNA-1 group and the siRNA-2 group. The cells in the exponential growth phase were seeded into a six-well plate at a density of 2×10^5/well. After 24 h, siRNA-1 and siRNA-2 were interfered with according to Lipofectamine™ 2000 reagent (Invitrogen, USA) transfection of RNA. After 6 h, the fresh medium was replaced for subsequent experiments. The siRNAs started to show effects 48 h after transfection, and the following assays were consistent with the timing of CHI3L1 knockdown.

3.5. Transwell Assay

Differently transfected ESCC cells were seeded in a 24-well plate. In the invasion assay, the upper chambers were coated with Matrigel (BD, USA) before the cells were seeded, whereas the migration was not. The upper chamber was inoculated with 2×10^5 KYSE-150 and TE1 cells suspended in a serum-free medium, and the lower chamber had DMEM containing 10% FBS added. After 48 h, the filter membrane was removed, and the upper chamber cells were wiped with a cotton swab to fix them before being stained with crystal violet for 1 h (20).

3.6. Wound Healing Assays

Horizontal lines were appropriately drawn on the back of the six-well plate. The cells were inoculated in the six-well plate at a density of 5×10^5 cells/well, and the transfection was carried out for 24 h. The pipette tip was used to draw three lines perpendicular to the previously drawn horizontal lines, making parallel vertical lines. The cells were rinsed with phosphate buffered saline three times to remove the exfoliated cells, and then the cells were returned to the incubator to continue

incubating. The specimens were taken for analysis (20).

3.7. MTT Assay

Experiments were performed using 96-well plates. A total of 2,000 cells were seeded per 96-well plate. After culturing the cells for 3 days, cell growth was detected using MTT (thiazolyl blue tetrazolium, from Sigma). Cells were incubated for 4 h using MTT and then measured at OD490 using a Biotek Synergy H1 microplate reader (20).

3.8. Cell Apoptosis Assay

Cells were treated via transfections, as noted above. The cells were then collected and subjected to V-fluorescein isothiocyanate (FITC) and propidium iodide (PI) staining, following the reagent manufacturer's instructions. The cells' apoptosis was detected via flow cytometry (20).

3.9. In-vivo Animal Experiments

The conduct of the animal study was approved by the ethics committee. Experiments were performed using 5-week-old male nude mice. Each mouse was injected subcutaneously with 1×10^7 KYSE-150 cells. The tumour growth rate and size were measured (20). Five mice were used in each experimental group.

3.10. Western Blot

The proteins of the KYSE-150 and TE1 cell lines were lysed and extracted using a strong radioimmunoprecipitation assay lysis buffer. The protein bands were separated by SDS page gel. Then, the protein was transferred to a PVDF membrane (Millipore Company, USA), antibody VE-cadherin (1:1,000, ab232880, Abcam, USA), CHI3L1 (1:1,000, ab77528, Abcam, USA), E-cadherin (1:500, AF0131, Affinity Biosciences, USA) were incubated after blocking, Snail (1:500, Affinity Biosciences, USA) and Twist1 (1:500, AF4009, Affinity Biosciences, USA). Glyceraldehyde 3-phosphate dehydrogenase (ab128915, Abcam, USA) was used as the internal reference protein.

3.11. Fluorescence Quantitative Real-Time Polymerase Chain Reaction

The cells were collected, and the total RNA was extracted using the TRIzol™ (Invitrogen, USA) lysis method; an ultraviolet spectrophotometer was used to measure the concentration and purity. The design primers and synthesise primers (Sangon Biotech, Shanghai, China) were as follows: CHI3L1 F:5'-GTGGAATGATGTGACGCTCTACGG-3',R:5'-TCTGGGTGTAGGCTATCTTGG-3', VE-cadherin F:5'-CCTCTGTGGGCTCTCTGTTTGTTG-3',R:5'-TGTCTCAATGGTGAAAGCGTCCTG-3',E-cadherin F:5'-GCATACTCACTGTCGAAGCCTACG-3',R:5'-GCAAGCAGCCACTGTTGTCAATG-3',Snail F:5'-CGCTCCACCTTCAAGTATGCCTTC-3',R:5'-AGGACGACGGAAAAGACGGAAATC-3', Twist1 F:5'-GACTTCCTCTACCAGGTCCTCCAG-3' and R:5'-TCCAGACCGAGAAGGCGTAGC-3' (**Table 2**). Quantitative real-time polymerase chain reaction (PCR) was performed with cDNA as a template. Three replicates were set for each sample to obtain the cycle threshold of each sample. The reaction conditions were 95 °C for 30 s, 95 °C for 5 s and 60 °C for 30 s (35 cycles). The $2^{-\Delta\Delta CT}$ method was used to perform a relative quantitative analysis of the differential expression of sample genes (20).

3.12. Statistical Analysis

All data were collected using the SPSS 26.0 software (IBM, USA). Data were shown as mean ± SD. The Kaplan–Meier method was used to evaluate the survival characteristics of CHI3L1 and VE-cadherin positive and negative protein expressions. The log-rank test was used for comparison. Multivariate analysis was conducted using the Cox regression model, and Pearson correlation analysis was used for CHI3L1 and VE-cadherin protein expression. A p value of <0.05 was considered statistically significant.

4. Results

The relationship between CHI3L1 and VE-cadherin in ESCC is currently unknown. In this study, CHI3L1 and VE-cadherin were highly expressed in ESCC tissues. Knockdown of CHI3L1 inhibited invasion, migration and proliferation of ESCC cells and increased the apoptosis of ESCC cells. Therefore, it was thought that the simultaneous knockdown of CHI3L1 could inhibit VE-cadherin expression and reduce the EMT of ESCC.

4.1. Comparison of the Expression of Chitinase-3-Like Protein 1 and Vascular Epithelial Cadherin in Oesophageal Squamous Cell Carcinoma

To explore the relationship between VE-cadherin and CHI3L1 in ESCC, 120 patient samples were collected

Table 1. The relationship between CHI3L1, VE-cadherin expression and the clinicopathological characteristics of ESCC

Variable	CHI3L1		χ^2	P	VE-cadherin		χ^2	P
	Positive	Negative			Positive	Negative		
Gender			0.213	0.644			1.294	0.255
Male	64(65.98%)	33(34.02%)			63(64.95%)	34(35.05%)		
Female	14(60.87%)	9(39.13%)			12(52.17%)	11(47.83%)		
Age (years)			0.22	0.639			0.593	0.441
<65	30(62.50%)	18(37.50%)			28(58.33%)	20(41.67%)		
≥65	48(66.67%)	24(33.33%)			47(65.28%)	25(34.72%)		
Gross type			6.012	0.111			3.387	0.336
Ulcerative	30(68.18%)	14(31.82%)			30(68.18%)	14(31.82%)		
Medullary	39(69.64%)	17(30.36%)			36(64.29%)	20(35.71%)		
Mushroom	5(35.71%)	9(64.29%)			6(42.86%)	8(57.14%)		
Constrictive	4(66.67%)	2(33.33%)			3(50.00%)	3(50.00%)		
Location			10.85	0.004			9.208	0.01
Upper	9(90.00%)	1(10.00%)			8(80.00%)	2(20.00%)		
Middle	25(49.02%)	26(50.98%)			24(47.06%)	27(52.94%)		
Lower	44(74.58%)	15(25.42%)			43(72.88%)	16(27.12%)		
Differentiation			1.341	0.511			3.783	0.151
Well	8(66.67%)	4(33.33%)			6(50.00%)	6(50.00%)		
Moderate	48(61.54%)	30(38.46%)			46(58.97%)	32(41.03%)		
Poor	22(73.33%)	8(26.67%)			23(76.67%)	7(23.33%)		
Diameter (cm)			7.817	0.005			10.901	0.001
<3.5	33(53.23%)	29(46.77%)			30(48.39%)	32(51.61%)		
≥3.5	45(77.59%)	13(22.41%)			45(77.59%)	13(22.41%)		
Infiltrate the serosal layer			29.175	0			20.249	0
Yes	63(82.89%)	13(17.11%)			59(77.63%)	17(22.37%)		
No	15(34.09%)	29(65.91%)			16(36.36%)	28(63.64%)		
Lymph node metastasis			23.83	0			27.703	0
Yes	43(91.49%)	4(8.51%)			43(91.49%)	4(8.51%)		
No	35(47.95%)	38(52.05%)			32(43.84%)	41(56.16%)		
pTNM stage			21.615	0			24.657	0
I + II	42(51.21%)	40(48.78%)			39(47.56%)	43(52.44%)		
III+IV	36(94.74%)	2(5.26%)			36(94.74%)	2(5.26%)		

for the study. The positive rate of CHI3L1 in ESCC (65.0%, 78/120) was higher than its positive rate in normal oesophageal epithelial tissue (13.3%, 8/60). The positive rate of VE-cadherin in ESCC (62.5%, 75/120) was higher than its positive rate in normal oesophageal epithelial tissue (5.0%, 3/60). The expression of CHI3L1 and VE-cadherin in tumour tissues and adjacent tissues was detected using immunohistochemical staining, and the results showed that the expression of CHI3L1 (positive rate: 65.0%) and VE-cadherin (positive rate: 62.5%) in tumour tissues was significantly higher than the expression of CHI3L1 (positive rate: 13.3%) and VE-cadherin (positive rate: 5.0%) in adjacent tissues ($p < 0.05$) (**Fig. 1A–1D**).

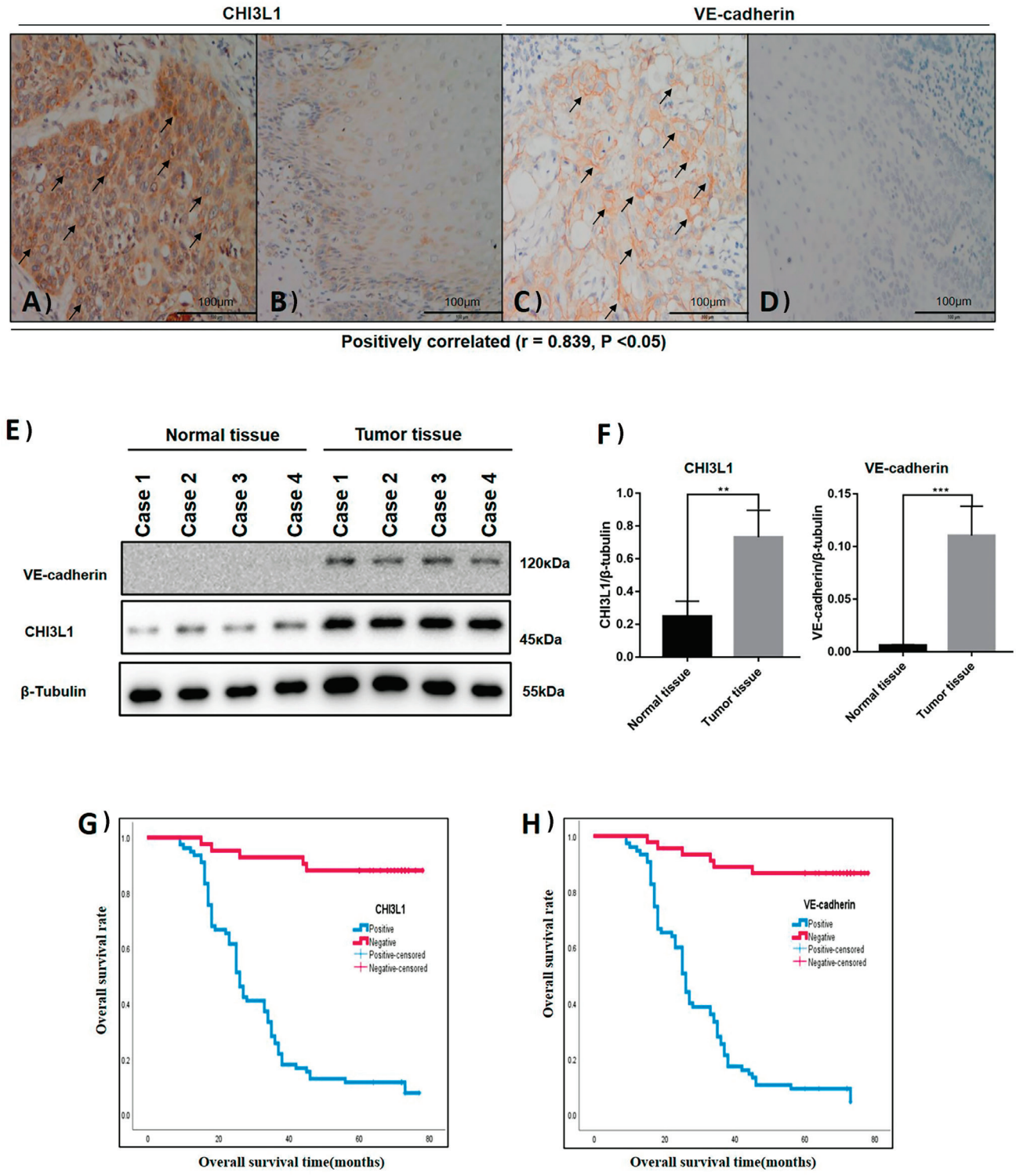

Figure 1. CHI3L1 protein and positive expression of VE-cadherin protein in oesophageal squamous cell carcinoma and normal esophageal epithelium. A,B) Positive staining of CHl3L1 in ESCC and normal esophageal epithelium tissue, respectively (40X). Arrows indicated positive staining. **C,D)** Positive staining of VE-cadherin in ESCC and normal esophageal epithelium tissue, respectively (40X). Arrows indicated positive staining. **E)** Expression of CHl3L1and VE-cadherin in normal and ESCC tissues. **F)** Quantifications of Figure 1E. **G)** The overall survival (OS) rate of CHI3L1 positive patients with ESCC. **H)** The overall survival (OS) rate of VE-cadherin positive patients with ESCC. ***P*<0.01，****P*<0.001.

Table 2. siRNA sequence and primers used for qRT-PCR.

Gene	Forward	Reverse
siRNA-control	5′-UUCUCCGAACGUGUCACGUTT-3′	5′- ACUUGACACGUUCGGAGAATT-3′
siRNA-1	5′-CCCACCCUAAUCAAGGAAAUTT-3′	5′-AUUUCCUUGAUUAGGGUGGTT-3′
siRNA-2	5′ -GAGCCACAGUCCAUAGAAUTT-3′	5′-AUUCUAUGGACUGUGGCUCTT-3′
CHI3L1	5’-GTGGAATGATGTGACGCTCTACGG-3’	5’-TCTGGGTGTAGGCTATCTTGG-3’
VE-cadherin	5’-CCTCTGTGGGCTCTCTGTTTGTTG-3’	5’-TGTCTCAATGGTGAAAGCGTCCTG-3’
E-cadherin	5’-GCATACTCACTGTCGAAGCCTACG-3’	5’-GCAAGCAGCCACTGTTGTCAATG-3’
Snail	5’-CGCTCCACCTTCAAGTATGCCTTC-3’	5’-AGGACGACGGAAAAGACGGAAATC-3’
Twist1	5’-GACTTCCTCTACCAGGTCCTCCAG-3’	5’-TCCAGACCGAGAAGGCGTAGC-3’

The positive rates of CHI3L1 protein and VE-cadherin were closely related to the depth of tumour invasion, pTNM stage, tumour size and tumour location (r = 0.839, $p < 0.05$). An increase in tumour invasion depth was accompanied by an increase in 1: the lymph node metastasis of the tumour and the pTNM stage of the tumour and 2: the protein levels of CHI3L1 and VE-cadherin. However, there was no correlation between CHI3L1 protein expression and patient gender, age, tumour type and degree of differentiation ($p > 0.05$). The results were shown in **Table 1**. The Pearson analysis indicated that CHI3L1 and VE-cadherin were significantly positively correlated ($r = 0.839$, $p < 0.05$), and the western blot results also showed a high expression of CHI3L1 and VE-cadherin in ESCC tissues (**Fig.1E, 1F**). The relative expression of CHI3L1 and VE-cadherin in ESCC tissues was 0.72 ± 0.11 and 0.106 ± 0.031, respectively; the relative expression of CHI3L1 and VE-cadherin in ESCC tissues was 0.22 ± 0.16 and 0.002 ± 0.001, respectively (**Fig. 1F**).

4.2. Survival Analysis

The overall survival rate of patients with ESCC was 37.5% (45/120). The survival rate was significantly lower in the CHI3L1 (survival rate: 10%) and VE-cadherin (survival rate: 7.5%) positive group than in the CHI3L1 (survival rate:90.8%) and VE-cadherin (survival rate: 88.3%) negative group (log-rank = 64.450, 69.586, all $p < 0.05$) (Figures 1G, H). Chitinase-3-like protein 1, VE-cadherin and lymph node metastasis could be used as independent evaluation factors ($p < 0.05$) in the prognosis of patients with ESCC through the Cox multivariate regression model (**Table 1**).

4.3. Inhibition of Invasion and Migration of Oesophageal Squamous Cell Carcinoma Cells by Knockdown of Chitinase-3-Like Protein 1

Wound healing experiments showed that in the KYSE-150 and TE1 cell lines, the post-CHI3L1-knockdown wound healing speed was significantly lower in the siRNA-1 (KYSE-150: 0.66 ± 0.008; TE1: 0.53 ± 0.06) and siRNA-2 (KYSE-150: 0.62 ± 0.04; TE1:0.50 ± 0.04) groups than in the NC group (KYSE-150: 1.0 ± 0.16; TE1:1.0 ± 0.02; $p < 0.05$) (**Fig. 2A**). To further confirm the effects of reducing CHI3L1 on the migration ability of EC cell lines, this experiment used the transwell chamber to detect the changes in cell invasion and migration capabilities (**Fig. 2B**). In KYSE-150 and TE1 cell lines, the invasion ability was significantly lower in the siRNA-1 (KYSE-150: 0.48 ± 0.10; TE1: 0.41 ± 0.11) and siRNA-2 (KYSE-150: 0.46 ± 0.12; TE1: 0.36 ± 0.09) groups than in the NC group (KYSE-150: 1.0 ± 0.02; TE1: 1.0 ± 0.02; $p < 0.05$). Furthermore, the migration ability was significantly lower in the siRNA-1 (KYSE-150: 0.46 ± 0.07; TE1: 0.39 ± 0.01) and siRNA-2 (KYSE-150: 0.35 ± 0.06; TE1:0.40 ± 0.07) groups than in the NC group (KYSE-150: 1.0 ± 0.01; TE1:1.0 ± 0.02; $p < 0.05$). These results indicated that the invasion and migration of EC cells were decreased following knockdown of CHI3L1.

4.4. Inhibition of Proliferation and Promotion of Apoptosis in Oesophageal Squamous Cell Carcinoma by Decrease in Chitinase-3-Like Protein 1 Expression

In this experiment, annexin V-FITC/PI staining was used to detect KYSE-150 and TE1 apoptosis using flow cytometry (**Fig. 3A**).

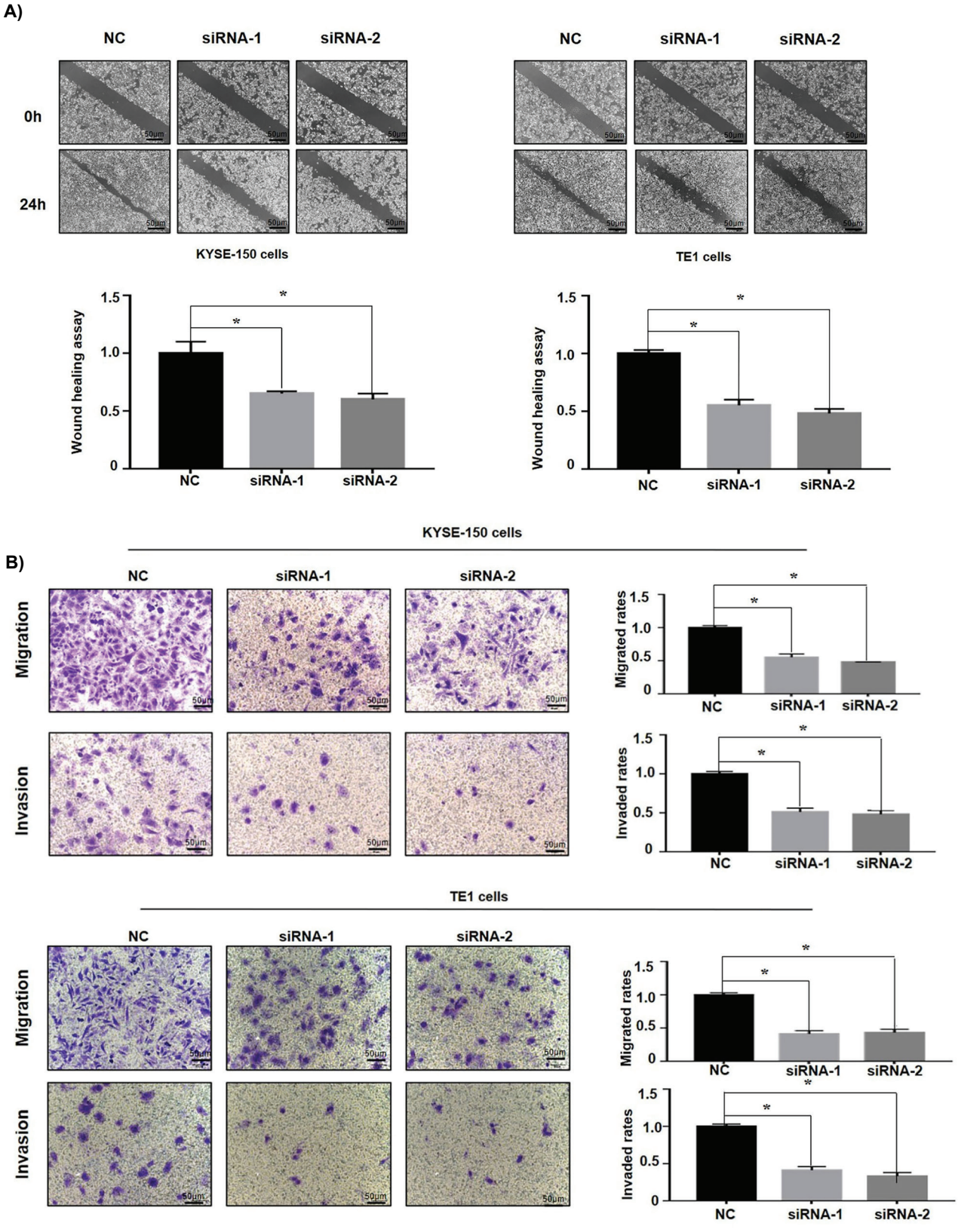

Figure 2. Inhibiting the invasion and migration of EC cells after reducing CHI3L1. A) The scratch healing assay of KYSE-150 and TE1 cells treated with negative control or siRNA. **B)** The migration and invasion assay of KYSE-150 and TE1 cells treated with negative control or siRNA. **P*<0.05, siRNA-1 or siRNA-2 vs control. All the experiments were independently repeated for n=3 times.

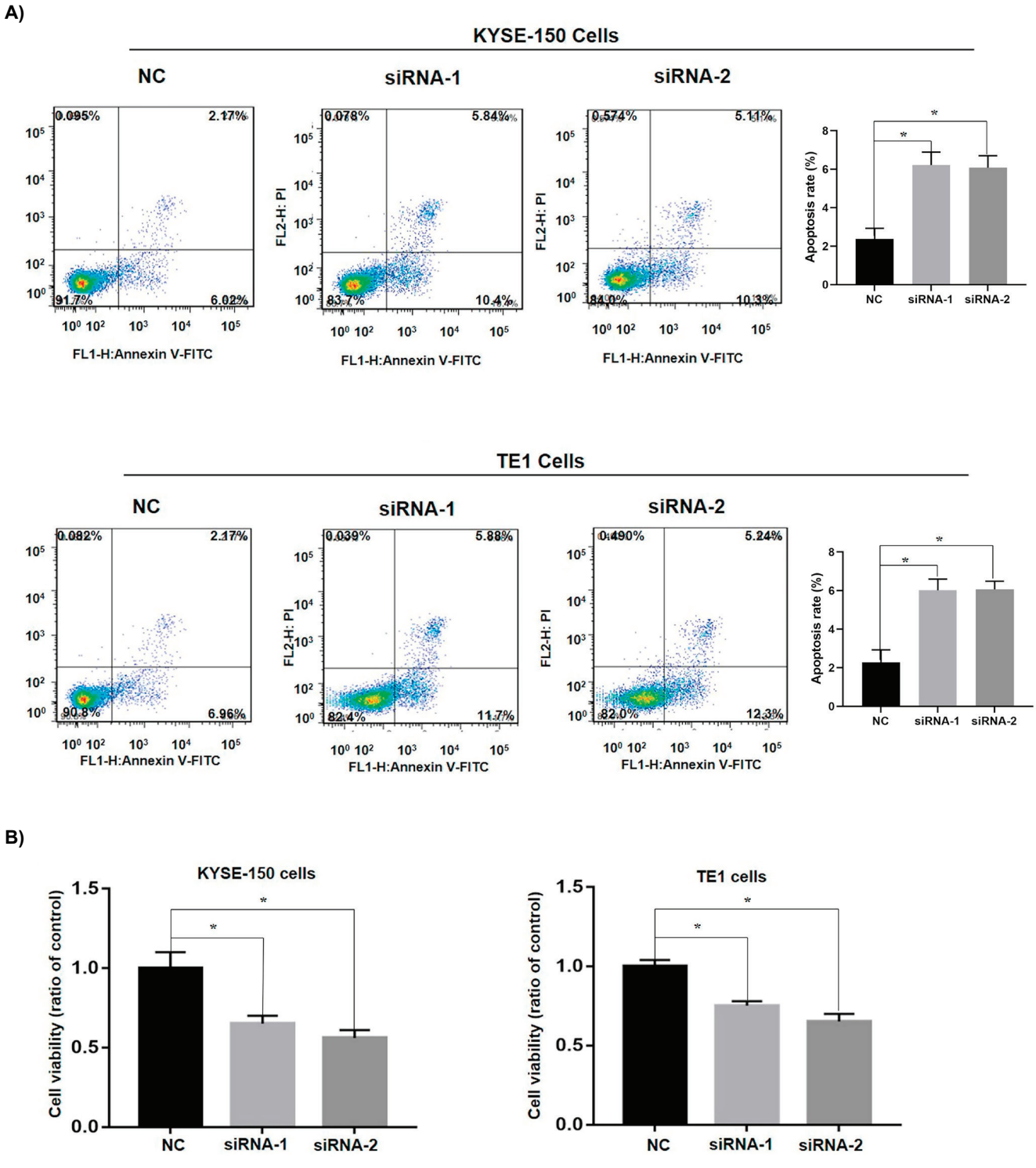

Figure 3. Decreasing CHI3L1 can inhibit the proliferation and promote the apoptosis of EC. A) Flow cytometry analysis of apotosis rate in KYSE-150 and TE1 cells staining with annexin-V treated with negative control or siRNA. **B)** MTT method was used to detect changes in cell proliferation. **P*<0.05, siRNA-1 or siRNA-2 vs control. All the experiments were independently repeated for n=3 times.

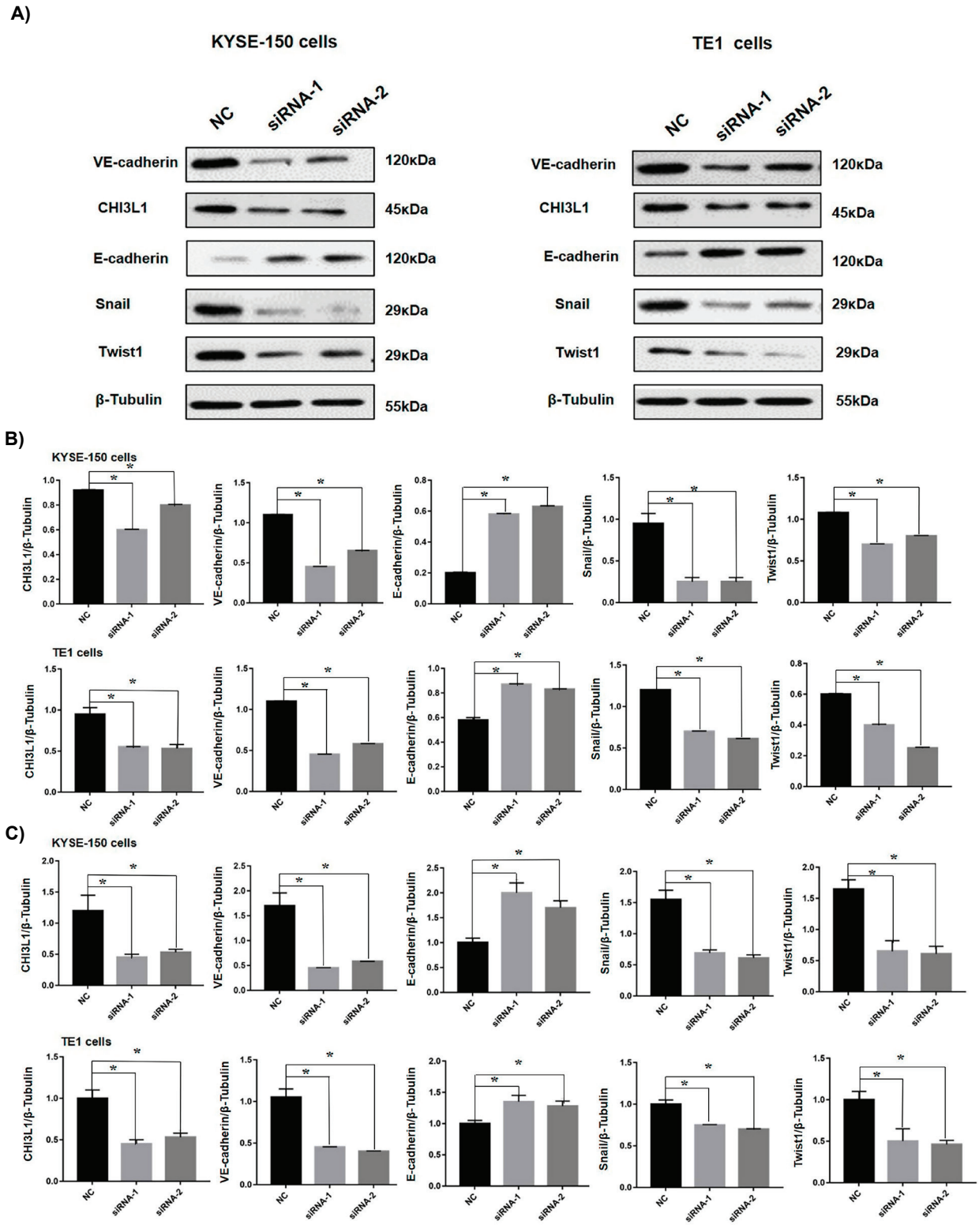

Figure 4. Expression of different molecular proteins and mRNA in EC cells in each group. A) Representative images of indicated proteins expression levels measured by western blot in KYSE-150 and TE1 cells treated with negative control or siRNA. **B)** Statistical graph of indicated protein expression levels in KYSE-150 cells treated with negative control or siRNA. **P*<0.05, siRNA-1 or siRNA-2 vs control. **C)** Statistical graph of indicated protein expression levels in TE1 cells treated with negative control or siRNA. **P*<0.05, siRNA-1 or siRNA-2 vs control. All the experiments were independently repeated for n=3 times.

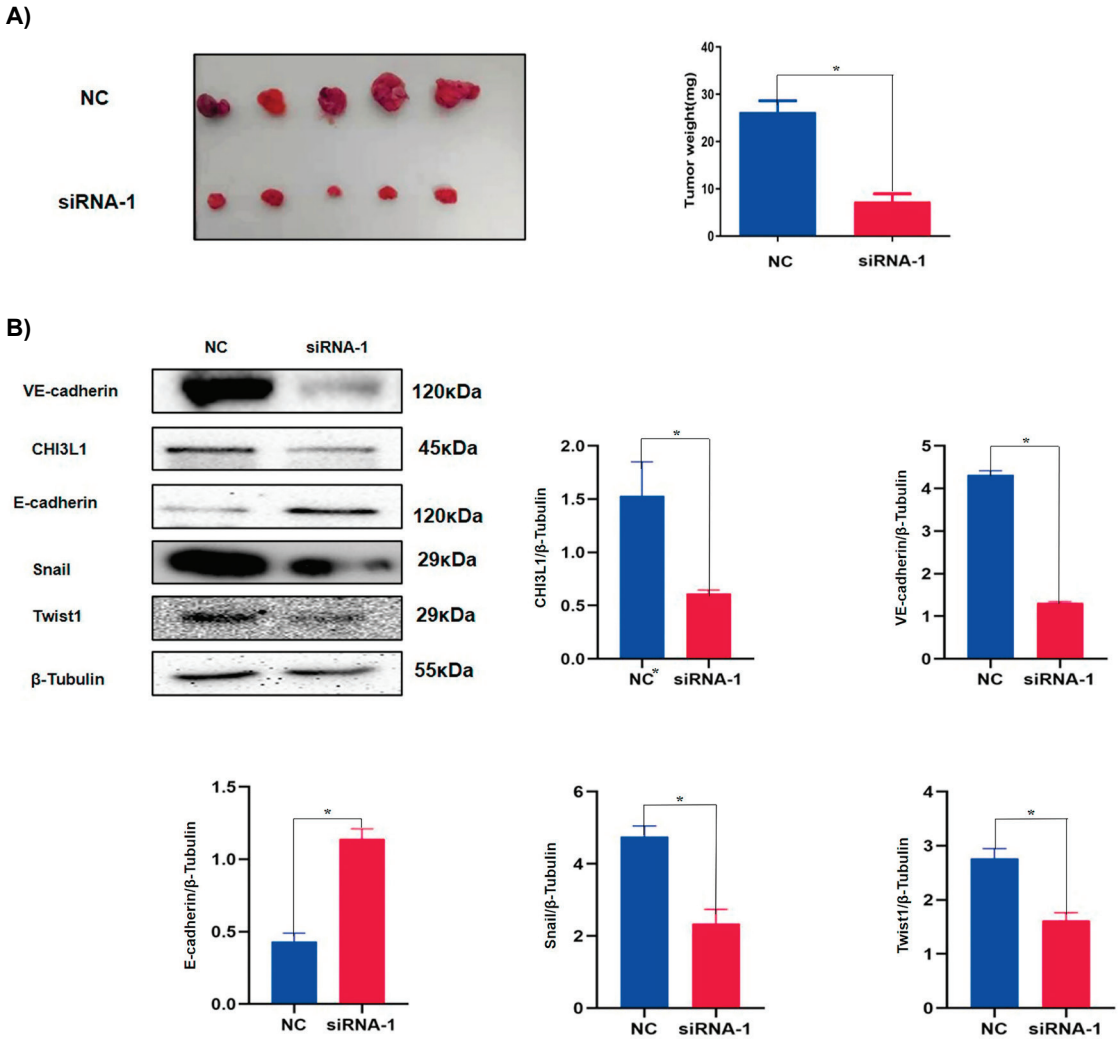

Figure 5. Low-expression of CHI3L1 inhibits tumor growth in vivo. **A)** Representative images of tumors and statistical graph of tumor weights of *in vivo* model. n=5 mice. **B)** Representative images and statistical graph of indicated protein expression levels in tumor tissue. **P*<0.05, siRNA-1 vs control. All the experiments were independently repeated for n=3 times.

Compared with control cells in the KYSE-150 and TE1 cell lines, the apoptosis rate of the siRNA-1 (KYSE-150: 6.03% ± 0.94%; TE1: 5.95% ± 0.81%) and siRNA-2 (KYSE-150: 5.84% ± 0.91%; TE1: 5.96% ± 0.76%) groups was enhanced significantly when compared with the NC group (KYSE-150: 2.26% ± 0.95%; TE1: 2.31% ± 1.03%; $p < 0.001$). In this experiment, the MTT method was used to detect changes in cell proliferation (**Fig. 3B**). After reducing the expression of CHI3L1, the proliferation ability was significantly lower in the siRNA-1 (KYSE-150: 0.71 ± 0.05; TE1: 0.82 ± 0.02) and siRNA-2 (KYSE-150: 0.67 ± 0.05; TE1: 0.69 ± 0.06) groups in the KYSE-150 and TE1 cell lines than in the NC group (KYSE-150: 1.0 ± 0.13; TE1: 1.0 ± 0.03; $p < 0.001$). These results suggest that inhibiting the expression of CHI3L1 reduced the growth and proliferation of ESCC cells and increased their apoptosis rate.

4.5. Expression of Messenger Ribonucleic Acid and Different Molecular Proteins in Oesophageal Squamous Cell Carcinoma Cells in Each Group

To further explore the mechanism, this experiment detected VE-cadherin, E-cadherin, Snail and Twist1

protein and mRNA expression levels after reducing CHI3L1 (**Fig. 4A–4C**).

Epithelial cadherin, Snail and Twist1 protein are the markers of EMT. Some studies have reported that Snail and Twist1 protein expression increased, whereas E-cadherin expression decreased, when EMT was enhanced (21). In KYSE-150 and TE1 cell lines, compared with the NC group, CHI3L1 (KYSE-150: NC: 0.91 ± 0.01; siRNA-1: 0.59 ± 0.01; siRNA-2: 0.78 ± 0.01; TE1: NC: 0.94 ± 0.11; siRNA-1: 0.52 ± 0.01; siRNA-2: 0.50 ± 0.05), VE-cadherin (KYSE-150: NC: 1.16 ± 0.01; siRNA-1: 0.45 ± 0.01; siRNA-2: 0.68 ± 0.01; TE1: NC: 1.17 ± 0.01; siRNA-1: 0.47 ± 0.01; siRNA-2: 0.59 ± 0.01), Snail (KYSE-150: NC: 0.95 ± 0.21; siRNA-1: 0.26 ± 0.08; siRNA-2: 0.29 ± 0.09; TE1: NC: 1.20 ± 0.01; siRNA-1: 0.76 ± 0.01; siRNA-2: 0.69 ± 0.01) and Twist1 (KYSE-150: NC: 1.08 ± 0.01; siRNA-1: 0.71 ± 0.01; siRNA-2: 0.77 ± 0.01; TE1: NC: 0.60 ± 0.01; siRNA-1: 0.41 ± 0.01; siRNA-2: 0.28 ± 0.01) protein expression in the siRNA-1 and siRNA-2 groups were significantly higher ($p < 0.01$), and the expression of E-cadherin (KYSE-150: NC: 0.20 ± 0.01; siRNA-1: 0.57 ± 0.01; siRNA-2: 0.61 ± 0.01; TE1: NC: 0.58 ± 0.03; siRNA-1: 0.88 ± 0.01; siRNA-2: 0.86 ± 0.01) protein was significantly lower ($p < 0.01$) (Figure 4B). Moreover, in the KYSE-150 and TE1 cell lines, CHI3L1 (KYSE-150: NC: 1.21 ± 0.26; siRNA-1: 0.46 ± 0.03; siRNA-2: 0.52 ± 0.02; TE1: NC: 1.00 ± 0.16; siRNA-1: 0.45 ± 0.06; siRNA-2: 0.51 ± 0.05), VE-cadherin (KYSE-150: NC: 1.73 ± 0.25; siRNA-1: 0.46 ± 0.01; siRNA-2: 0.53 ± 0.01; TE1: NC: 1.06 ± 0.12; siRNA-1: 0.47 ± 0.01; siRNA-2: 0.45 ± 0.01), Snail (KYSE-150: NC: 1.53 ± 0.19; siRNA-1: 0.73 ± 0.09; siRNA-2: 0.62 ± 0.09; TE1: NC: 0.99 ± 0.11; siRNA-1: 0.82 ± 0.01; siRNA-2: 0.79 ± 0.01) and Twist1 (KYSE-150: NC: 1.60 ± 0.20; siRNA-1: 0.58 ± 0.27; siRNA-2: 0.54 ± 0.25; TE1: NC: 0.99 ± 0.16; siRNA-1: 0.48 ± 0.21; siRNA-2: 0.45 ± 0.08) mRNA expression in the siRNA-1 and siRNA-2 groups were lower than in the NC group ($p < 0.05$) (**Fig. 4C**). The expression of E-cadherin (KYSE-150: NC: 1.00 ± 0.08; siRNA-1: 1.97 ± 0.26; siRNA-2: 1.78 ± 0.18; TE1: NC: 1.00 ± 0.05; siRNA-1: 1.34 ± 0.14; siRNA-2: 1.28 ± 0.11) mRNA in the siRNA-1 and siRNA-2 groups was higher than in the NC group ($p < 0.05$) (**Figu. 4C**). Together, the results suggest that reducing the expression of CHI3L1 could inhibit the expression levels of VE-cadherin, Snail and Twist1 and could increase the expression level of E-cadherin. Chitinase-3-like protein 1 could promote the migration and invasion ability of EC and regulate the expression of VE-cadherin.

4.6. Inhibition of Tumour Growth in vivo by Knockdown of Chitinase-3-Like Protein 1

In-vivo animal experiments have shown that reducing the expression of CHI3L1 could significantly inhibit the growth of tumour cells. In the nude mouse animal model, the weight of tumour cells was lower in the siRNA-1 group (26.25 ± 2.363 mg) than in the NC group (7.250 ± 1.708 mg; $p < 0.05$) (**Fig. 5A**). Simultaneously, it was confirmed that CHI3L1 (NC: 1.5 ± 0.39; siRNA-1: 0.71 ± 0.04), VE-cadherin (NC: 4.2 ± 0.09; siRNA-1: 1.4 ± 0.02), Snail (NC: 4.7 ± 0.25; siRNA-1: 2.3 ± 0.33) and Twist1 (NC: 2.7 ± 0.28; siRNA-1: 1.6 ± 0.25) in siRNA-1 tumour tissues were significantly reduced compared with the NC group ($p < 0.05$) (**Fig. 5B**).

5. Discussion

In recent years, the relationship between CHI3L1 and cancer has attracted a great deal of attention. Chitinase-3-like protein 1 could induce cell carcinogenesis and tumour angiogenesis and regulate the tumour microenvironment, thereby affecting tumour growth, metastasis and response to treatment (24). Chitinase-3-like protein 1 was significantly increased in patients with a variety of malignant tumours. Moreover, CHI3L1 increased the degree of malignant disease progression and poor prognosis (25-28). In the present study, it was confirmed that the expression of CHI3L1 in ESCC was increased than normal tissues. The high expression of CHI3L1 promoted cancer tissues to invade and develop lymph node metastasis, which led to the later pTNM staging of the tumour. The study authors suggested that CHI3L1 could guide the progression of ESCC. The results of this study were consistent with previous studies (25-28).

At present, research on the relationship between CHI3L1 and EC mainly pertains to serology, and there are few studies at the tissue level. Zheng *et al*. (29) specified that CHI3L1 combined with squamous cell carcinoma antigen (SCCA) could significantly improve the sensitivity of the traditional EC tumour markers, carcinoembryonic antigen, cytokeratin 19 fragment and

SCCA in detecting EC. Xing *et al*. (30) demonstrated for the first time that CHI3L1 serum could be used as a prognostic indicator for EC. Huang *et al*. (11) found that the level of plasma CHI3L1 was higher in the EC group than CTRL group. Simultaneously, CHI3L1 was found to be related to the lymph node metastasis of EC, clinical staging and invasion. Combined with the high level of CHI3L1 expression in the serum and tissues of EC, it was confirmed that CHI3L1 could be used as an oncogene to promote the occurrence and progression of EC.

This study showed that CHI3L1 could be an independent prognostic factor for evaluating the survival of ESCC. Its high expression may indicate that the patient has a poor prognosis and a shortened survival time.

The authors of the present study designed two siRNAs that inhibited the expression of CHI3L1 and transfected them into KYSE-150 and TE1 cell lines. The results of real-time PCR and western blot analysis showed that the CHI3L1 mRNA and protein expression in the CHI3L1-transfected siRNA-1 and siRNA-2 groups were significantly reduced compared with the NC group; the difference was statistically significant. This again confirmed that, as an oncogene, CHI3L1 plays a role in EC. Invasion and metastasis are important biological characteristics of malignant tumours, and they are also a significant cause of patient death. In this study, transwell invasion chamber and wound healing experiments were used to detect the invasion ability and scratch healing ability of the KYSE-150 and TE1 cells. The results showed that the cell invasion and scratch healing ability of the siRNA-1 and siRNA-2 groups were lower compared with the NC group. This indicated that inhibiting the expression of CHI3L1 in the KYSE-150 and TE1 cells could inhibit their invasion and metastasis ability. The proliferation ability of tumour cells is one of the most important features. Compared with the NC group, the apoptosis rate of the siRNA-1 and siRNA-2 groups was significantly decreased, whereas the proliferation rate was significantly increased. This indicated that CHI3L1 could inhibit the apoptosis of EC cells and promote the proliferation of EC cells.

Similarly, Liu *et al*. (31) confirmed that CHI3L1 was overexpressed in colon cancer cells. The results of cell proliferation curves revealed that the relative proliferation rate of cells after overexpression of CHI3L1 was significantly increased. According to the report, after silencing CHI3L1 using siRNA, the reduction of CHI3L1 levels in the glioma U87MG cell line could significantly reduce cell viability (32). Similarly, Ngernyuang *et al*. (33) revealed that CHI3L1 played a crucial role in angiogenic mimicry, which may help cervical cancer tumour cells to infiltrate more easily. This confirmed that high expressions of CHI3L1 could promote invasion, metastasis and proliferation and inhibit cancer cell apoptosis in EC combined with the above experiments; however, its specific mechanism remains unclear.

Tumours can maintain a small size for a long time in an avascular state in the early stage. When the diameter of the tumour is >2 mm, the tumour cells form capillaries to provide nutrition for the tumour, which can make the tumour grow rapidly. The progress of solid tumours is extremely important (34). Vascular epithelial cadherin was one of the regulatory factors that affect tumour angiogenesis, and it was an important adhesion molecule that mediates tumour metastasis in the formation of blood vessels (35). Typically, VE-cadherin was only expressed in endothelial cells; however, it was also expressed in highly aggressive tumour cells in the study of melanoma, which suggested that it may be involved in the occurrence and development of malignant tumours (36,37). In this study, the VE-cadherin protein expression level was detected in 120 paraffin specimens of ESCC and 60 normal oesophageal tissue specimens, and it was highly expressed in ESCC. As the tumour volume increases, the invasion depth increases, the lymph node metastasis increased, the pTNM staging was delayed, and the expression of VE-cadherin increases in ESCC. The results suggested that the highly expressed VE-cadherin promoted the invasion and metastasis of ESCC. Simultaneously, the high expression of VE-cadherin indicated a poor prognosis for patients with ESCC. Over-expression of VE-cadherin could promote the formation of blood vessels in malignant tumours and promote their growth and progression (22,23,35). The above literature reports were consistent with the results of VE-cadherin detection in ESCC in the present experiment.

Studies on human breast cancer, colon cancer and gliomas have shown that CHI3L1 could promote and participate in tumour angiogenesis (38,39). In gliomas, CHI3L1 could promote tumour cells to express VEGF, promoting new blood vessel formation (15). In colon

cancer, the high expression of CHI3L1 in tumour cells could increase the secretion of pro-angiogenesis factors IL-8 and MCP-1, thereby enhancing tumour angiogenesis (13). In gastric cancer, the up-regulation of the CHI3L1 gene promoted the invasiveness of gastric cancer (40). In ovarian cancer, LINC00963 promoted ovarian cancer progression via the miR-378g/CHI3L1 axis (41). In non-small cell lung cancer (NSCLC), CHI3L1 regulates EMT and migration/invasion (42), and it has been shown that CHI3L1 could induce angiogenesis and angiogenic mimicry in patients with cervical cancer (33). The relationship between CHI3L1 and VE-cadherin in ESCC has not been reported. The Pearson correlation analysis showed that the CHI3L1 was closely related to VE-cadherin, suggesting that the two together promote ESCC.

As the leading cause of tumour death, tumour metastasis is a multi-step and sequential process. It has undergone EMT, and the expression of adhesion molecules on the surface of tumour cells is down-regulated to obtain the ability of movement and migration, thereby promoting the occurrence of metastasis (43). Therefore, the expression levels of proteins were tested in relation to EMT. In the KYSE-150 and TE1 cell lines, after inhibiting the expression of CHI3L1, the expression levels of Snail and Twist1 expression decreased, whereas the E-cadherin expression level increased. At the same time, in the nude mouse model, Snail and Twist1 expression was also reduced, whereas the E-cadherin protein level increased. This experiment confirmed that the high-level expression of CHI3L1 may be involved in the EMT of EC cells. It has been reported that in NSCLC CL1-1 and CL1-5 cell lines, CHI3L1 could promote tumour cell invasion and metastasis. In the *in-vivo* nude mouse tumour formation experiments, cell lines with a high expression of CHI3L1 formed more lung tumour nodules and further confirmed that CHI3L1 could promote tumour progression by regulating EMT (44). However, how CHI3L1 regulates VE-cadherin, as well as EMT of ESCC cells, is currently unknown, and further exploration is needed.

The formation of tumour plasticity VM was closely related to VE-cadherin (21). During VM formation, highly aggressive epithelial tumour cells could obtain a mesenchymal phenotype through EMT over-expression (42). The authors of the present study found that the high expression of CHI3L1 was related to the expression of VE-cadherin in ESCC. By targeting the inhibition of CHI3L1, VE-cadherin expression and the transformation of tumour epithelium to mesenchyme could be inhibited, thereby inhibiting tumour progression. It was speculated that in ESCC, CHI3L1 may modulate tumour EMT-related molecules to make the epithelium acquire a mesenchymal phenotype, reduce epithelial adhesion and promote VE-cadherin levels and tumour neovascularisation, such as VM.

There are some limitations to this study. First, it found that CHI3L1 could affect the growth and invasion ability of ESCC by affecting EMT, but the molecular mechanism of how CHI3L1 specifically affects the EMT of ESCC requires further investigation. Second, the knockdown of CHI3L1 in ESCC reduced the VE-cadherin protein expression, and its molecular mechanism also requires exploration. Future research will also further explore the molecular mechanism through which CHI3L1 impacts ESCC.

6. Conclusion

The present study found that high expression of CHI3L1 promoted EMT in ESCC, which led to the easier infiltration, growth and promotion of tumour enlargement and further led to pTNM staging. The *in-vitro* experiment showed that the targeted inhibition of CHI3L1 expression could reduce the invasion, migration and proliferation ability of ESCC cells and promote apoptosis of tumour cells. In the nude mouse model, reducing the expression of CHI3L1 was found to inhibit tumour growth. The study also found that decreased CHI3L1 may reduce the expression of VE-cadherin, inhibit the EMT of the oesophageal epithelial tissue and mediate tumour angiogenesis. Therefore, the authors proposed that the goal of tumour anti-angiogenesis could be achieved by targeted inhibition of CHI3L1 expression, which could inhibit the occurrence and progression of ESCC.

Acknowledgments

No funding or sponsorship was received for this study or publication of this article.

Ethics approval and consent to participate

The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted

in accordance with the Declaration of Helsinki (as revised in 2013). This study was conducted with approval from the Ethics Committee of Bengbu Medical College (No.055).

Author Contributions

QYZ, ZWJ and LQC contributed equally to this work. CZG, WGW and ML participated in the research design. QYZ and ML performed the research and wrote the manuscript. WN and ML contributed towards critically revising the manuscript.

Funding

This work was funded by the Natural Science Key Project of Anhui Provincial Department of Education (KJ2019A0344, KJ2021A0760, KJ2021A0814, 2022 AH051521)

Competing interests

The authors declare that they have no competing interests.

Consent for publication

not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

References

1. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. *CA Cancer J Clin*. 2015;**65**:87-108. doi: 10.3322/caac.21262
2. Wei G, Luo H, Sun Y, Li J, Tian L, Liu W, *et al*. Transcriptome profiling of esophageal squamous cell carcinoma reveals a long noncoding RNA acting as a tumor suppressor. *Oncotarget*. 2015;**6**:17065-17080. doi: 10.18632/oncotarget.4185
3. Di Pietro M, Canto MI, Fitzgerald RC. Endoscopic Management of Early Adenocarcinoma and Squamous Cell Carcinoma of the Esophagus: Screening, Diagnosis, and Therapy. *Gastroenterology*. 2018;**154**:421-436. doi: 10.1053/j.gastro.2017.07.041
4. Bussink AP, Speijer D, Aerts JM. Evolution of mammalian chitinase(-like) members of family 18 glycosyl hydrolases. *Genetics*. 2007;**177**:959-970. doi: 10.1534/genetics.107.075846
5. Lee CG, Da Silva CA, Dela Cruz CS, Ahangari F, Ma B, Kang MJ, *et al*. Role of chitin and chitinase/chitinase-like proteins in inflammation, tissue remodeling, and injury. *Annu Rev Physio1*. 2011;**73**:479-501. doi: 10.1146/annurev-physiol-012110-142250
6. Johansen JS, Williamson MK, Rice JS, Price PA. Identification of proteins secreted by human osteoblastic cells in culture. *J Bone Miner Res*. 1992;**7**:501-512. doi: 10.1002/jbmr.5650070506
7. Kronborg G, Ostergaard C, Weis N, Nielsen H, Obel N, Pedersen SS, *et al*. Serum level of YKL-40 is elevated in patients with Streptococcus pneumoniae bacteremia and is associated with the outcome of the disease. *Scand J Infect Dis*. 2002;**34**:323-326. doi: 10.1080/00365540110080233
8. Pizano-Martínez O, Yañez-Sánchez I, Alatorre-Carranza P, Miranda-Díaz A, Ortiz-Lazareno PC, García-Iglesias T, *et al*. YKL-40 expression in CD14 liver cells in acute and chronic injury. *World J Gastroenterol*. 2011;**17**:3830-3835. doi: 10.3748/wjg.v17.i33.3830
9. Mizoguchi E. Chitinase 3-like-1 exacerbates intestinal inflammation by enhancing bacterial adhesion and invasion in colonic epithelial cells. *Gastroenterol*. 2006;**130**:398-411. doi: 10.1053/j.gastro.2005.12.007
10. Greenberg J, Zarnegar R. Exploring the role of chitinase-3-like protein 1 in recurrence patterns among patients with differentiated thyroid cancer. *J Pathol*. 2020;**252**:343-345. doi: 10.1002/path.5550
11. Huang R, Yao J, Ding X, Liu J, Fan C, Duan H, Ye H. Plasma YKL-40: a Potential Biomarker for Tumor Invasion in Esophageal Cancer. *Clin Lab*. 2020;**66**(3). doi: 10.7754/Clin.Lab.2019.190528
12. Johansen JS, Jensen BV, Roslind A, Nielsen D, Price PA. Serum YKL-40, a new prognostic biomarker in cancer patients? *Cancer Epidemiol Biomarkers Prev*. 2006;**15**:194-202. doi: 10.1158/1055-9965.EPI-05-0011
13. Kawada M, Seno H, Kanda K, Nakanishi Y, Akitake R, Komekado H, *et al*. Chitinase 3-like 1 promotes macrophage recruitment and angiogenesis in colorectal cancer. *Oncogene*. 2012;**31**:3111-3123. doi: 10.1038/onc.2011.498
14. Salvatore V, Focaroli S, Teti G, Mazzotti A, Falconi M. Changes in the geneexpression of co-cultured human fibroblast cells and osteosarcoma cells: the role of micro-environment. *Oncotarget*. 2015;**6**:28988-28998. doi: 10.18632/oncotarget.4902
15. Francescone RA, Scully S, Faibish M, Taylor SL, Oh D, Moral L, *et al*. Role of YKL-40 in the angiogenesis, radioresistance, and progression of glioblastoma. *J Biol Chem*. 2011;**28**:15332-15343. doi: 10.1074/jbc.M110.212514
16. Huang J, Gu Z, Xu Y, Jiang L, Zhu W, Wang W. CHI3L1 (Chitinase 3 Like 1) upregulation is associated with macrophage signatures in esophageal cancer. *Bioengineered*. 2021;**12**(1):7882-7892. doi: 10.1080/21655979.2021.1974654
17. Finney AC, Funk SD, Green JM, Yurdagul A Jr, Rana MA, Pistorius R, *et al*. EphA2 Expression Regulates Inflammation and Fibroproliferative Remodeling in Atherosclerosis. *Circulation*. 2017;**136**:566-582. doi: 10.1161/CIRCULATIONAHA.116.026644
18. Bittner M, Meltzer P, Chen Y, Jiang Y, Seftor E, Hendrix M, *et al*. Molecular classification of cutaneous malignant melanoma by gene expression profiling. *Nature*. 2000;**406**:536-540. doi: 10.1038/35020115
19. Bartolomé RA, Torres S, De Val SI, Escudero-Paniagua B, Calviño E, Teixidó J, *et al*. VE-cadherin RGD motifs promote metastasis and constitute a potential therapeutic target in melanoma and breast cancers. *Oncotarget*. 2017;**8**:215-227. doi: 10.18632/oncotarget.13832
20. Tang NN, Zhu H, Zhang HJ, Zhang WF, Jin HL, Wang L, *et al*. Hif-1α Induces Ve-Cadherin Expression and Modulates Vasculogenic Mimicry in Esophageal Carcinoma Cells. *World*

J Gastroenterol. 2014;**20**(47):17894-17904. doi: 10.3748/wjg.v20.i47.17894
21. Hendrix MJ, Seftor EA, Meltzer PS, Gardner LM, Hess AR, Kirschmann DA, *et al*. Expression and functional significance of VE-cadherin in aggressive human melanoma cells: role in vasculogenic mimicry. *Proc Natl Acad Sci U S A*. 2001;**98**:8018-8023. doi: 10.1073/pnas.131209798
22. Azzi S, Hebda JK, Gavard J. Vascular permeability and drug delivery in cancers. *Front Oncol*. 2013;**3**:211. doi: 10.3389/fonc.2013.00211
23. Irani S, Dehghan A. Expression of Vascular Endothelial-Cadherin in Mucoepidermoid Carcinoma: Role in Cancer Development. *J Int Soc Prev Community Dent*. 2017;**7**:301-307. doi: 10.4103/jispcd.JISPCD_323_17
24. Subramaniam R, Mizoguchi A, Mizoguchi E. Mechanistic roles of epithelial and immune cell signaling during the development of colitis-associated cancer. *Cancer Res Front*. 2016;**2**:1-21. doi: 10.17980/2016.1
25. Fuksiewicz M, Kotowicz B, Rutkowski A, Achinger-Kawecka J, Wagrodzki M, Kowalska MM. The Assessment of Clinical Usage and Prognostic Value of YKL-40 Serum Levels in Patients with Rectal Cancer Without Distant Metastasis. *Technol Cancer Res Treat*. 2018;**17**:1533033818765209. doi: 10.1177/1533033818765209
26. Mitsuhashi A, Matsui H, Usui H, Nagai Y, Tate S, Unno Y, *et al*. Serum YKL-40 as a marker for cervical adenocarcinoma. *Ann Oncol*. 2009;**20**:71-77. doi: 10.1093/annonc/mdn552
27. Rusak A, Jablonska K, Piotrowska A, Grzegrzolka J, Nowak A, Wojnar A, *et al*. The Role of CHI3L1 Expression in Angiogenesis in Invasive Ductal Breast Carcinoma. *Anticancer Res*. 2018;**38**:3357-3366. doi: 10.21873/anticanres.12602
28. Steponaitis G, Skiriutė D, Kazlauskas A, Golubickaitė I, Stakaitis R, Tamašauskas A, *et al*. High CHI3L1 expression is associated with glioma patient survival. *Diagn Pathol*. 2016;**11**:42. doi: 10.1186/s13000-016-0492-4
29. Zheng X, Xing S, Liu XM, Liu W, Liu D, Chi PD, *et al*. Establishment of using serum YKL-40 and SCCA in combination for the diagnosis of patients with esophageal squamous cell carcinoma. *BMC Cancer*. 2014;**14**:490. doi: 10.1186/1471-2407-14-490
30. Xing S, Zheng X, Zeng T, Zeng MS, Zhong Q, Cao YS, *et al*. Chitinase 3-like 1 secreted by peritumoral macrophages in esophageal squamous cell carcinoma is a favorable prognostic factor for survival. *World J Gastroenterol*. 2017;**23**:7693-7704. doi: 10.3748/wjg.v23.i43.7693
31. Liu K, Jin M, Ye S, Yan S. CHI3L1 promotes proliferation and improves sensitivity to cetuximab in colon cancer cells by down-regulating p53. *J Clin Lab Anal*. 2020;**34**:e23026. doi: 10.1002/jcla.23026
32. Wang Y, Wong CW, Yan M, Li L, Liu T, Or PM, *et al*. Differential regulation of the pro-inflammatory biomarker, YKL-40/CHI3L1, by PTEN/Phosphoinositide 3-kinase and JAK2/STAT3 pathways in glioblastoma. *Cancer Lett*. 2018;**429**:54-65. doi: 10.1016/j.canlet.2018.04.040
33. Ngernyuang N, Shao R, Suwannarurk K, Limpaiboon T. Chitinase 3 like 1 (CHI3L1) promotes vasculogenic mimicry formation in cervical cancer. *Pathology*. 2018;**50**:293-297. doi: 10.1016/j.pathol.2017.09.015
34. Folkman J. Tumor angiogenesis: therapeutic implications. *N Engl J Med*. 1971;**285**:1182-1186. doi: 10.1056/NEJM197111182852108
35. Irani S, Dehghan A. The Expression and Functional Significance of Vascular Endothelial-Cadherin, CD44, and Vimentin in Oral Squamous Cell Carcinoma. *J Int Soc Prev Community Dent*. 2018;**8**:110-117. doi: 10.4103/jispcd.JISPCD_408_17
36. Khan KN, Masuzaki H, Fujishita A, Kitajima M, Sekine I, Ishimaru T. Immunoexpression of hepatocyte growth factor and c-Met receptor in the eutopic endometrium predicts the activity of ectopic endometrium. *Fertil Steril*. 2003;**79**:173-181. doi: 10.1016/s0015-0282(02)04535-1
37. Duffy JP, Eibl G, Reber HA, Hines OJ. Influence of hypoxia and neoangiogenesis on the growth of pancreatic cancer. *Mol Cancer*. 2003;**2**:12. doi: 10.1186/1476-4598-2-12
38. Shao R, Hamel K, Petersen L, Cao QJ, Arenas RB, Bigelow C, *et al*. YKL-40, a secreted glycoprotein, promotes tumor angiogenesis. *Oncogene*. 2009;**28**:4456-4468. doi: 10.1038/onc.2009.292
39. Faibish M, Francescone R, Bentley B, Yan W, Shao R. A YKL-40-neutralizing antibody blocks tumor angiogenesis and progression: a potential therapeutic agent in cancers. *Mol Cancer Ther*. 2011;**10**:742-751. doi: 10.1158/1535-7163.MCT-10-0868
40. Abed Kahnamouei S, Baghaei K, Pakzad P, Hashemi M, Zali MR. The role of extracellular matrix proteins in gastric cancer development via epithelial-mesenchymal transition. *Gastroenterol Hepatol Bed Bench*. 2020;**13**(Suppl1):S139-S144.
41. Liu W, Yang YJ, An Q. LINC00963 Promotes Ovarian Cancer Proliferation, Migration and EMT via the miR-378g /CHI3L1 Axis. *Cancer Manag Res*. 2020;**12**:463-473. doi: 10.2147/CMAR.S229083
42. Jefri M, Huang YN, Huang WC, Tai CS, Chen WL. YKL-40 regulated epithelial-mesenchymal transition and migration/invasion enhancement in non-small cell lung cancer. *BMC Cancer*. 2015;**15**:590. doi: 10.1186/s12885-015-1592-3
43. Chaffer CL, Weinberg RA. A perspective on cancer cell metastasis. *Science*. 2011;**331**:1559-1564. doi: 10.1126/science.1203543