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miR-15b, a diagnostic biomarker and therapeutic target, inhibits oesophageal cancer progression by regulating the PI3K/AKT signalling pathway

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Abstract. MicroRNA (miR)-15b is an important regulator in several types of cancer, such as gastric cancer, colorectal cancer and oesophageal squamous cell carcinoma. The PI3K/AKT signalling pathway has been implicated in the growth and metastasis of oesophageal cancer (EC). The aim of the present study was to investigate the biological effects of miR-15b in EC, as well as the underlying mechanism involving the PI3K/AKT signalling pathway. The present study included 74 patients with EC and 74 healthy volunteers. The expression of miR-15b in peripheral blood mononuclear cells (PBMCs) and EC cell lines was evaluated via reverse transcription-quantitative PCR. The receiver operating characteristic curve was plotted to determine the diagnostic significance of miR-15b. EC cell viability, apoptosis, migration and invasion were analysed by conducting MTT, flow cytometry and transwell assays, respectively. Protein expression levels were analysed via western blotting. The results indicated that PBMCs isolated from patients with EC had lower miR-15b expression levels compared with PBMCs isolated from healthy volunteers. In patients with EC, miR-15b expression was strongly associated with tumour size, lymph node metastasis, TNM stage, fibrous membrane invasion and histologic grade. The results of the gain/loss-of-function *in vitro* experiments indicated that miR-15b inhibited EC cell viability, migration and invasion, facilitated EC cell apoptosis and attenuated the PI3K/AKT signalling pathway in EC109 and TE10 cells. Treatment of EC cells with the PI3K/AKT pathway agonist recilisib displayed the opposite effects, blocking the inhibitory function of miR-15b mimic on EC cell viability, migration and invasion. In summary, the results indicated that miR-15b suppressed EC cell viability, migration and invasion, and promoted EC cell apoptosis by inhibiting the PI3K/AKT signalling pathway.

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Introduction

Oesophageal cancer (EC) is a common gastrointestinal tumour that can be caused by complex genetic variants or an unhealthy lifestyle (1,2). In 2016, 16,910 new cases of EC and 15,910 deaths caused by EC were recorded in the United States (3). Current treatments for EC, including surgical resection and radiotherapy, have greatly improved the prognosis of patients with EC; however, due to delayed diagnosis and frequent metastasis, the 5-year survival rate (at 41.7%) remains unsatisfactory (4). Therefore, identifying novel therapeutic targets for EC is crucial to improve clinical outcomes and allow for earlier diagnosis.

MicroRNAs (miRNAs/miRs) are highly conserved, small (18-22 nucleotides) RNA molecules (5). Emerging evidence has demonstrated that miRNAs serve vital roles in various biological processes, such as cell differentiation, viability, apoptosis and the cell cycle, and a number of miRNAs regulate the progression of various types of cancer, such as lung cancer and colon cancer (6,7). The correlation between miRNAs and tumours was first reported in 2002 (8). Several miRNAs, such as miR-206 (9), miR-203a (10) and miR-543 (11), have been reported to be involved in the progression of EC. Previous studies have also demonstrated that miRNAs, which can function as tumour suppressors or cancer-promoting factors, could be useful for predicting cancer prognosis (12-15). The potential of miR-15b has been evaluated in neuroblastoma (12), as well as in colorectal (13) and gastric cancer (14). In addition, Wang *et al* (15) demonstrated that miR-15b is sponged by long intergenic non-protein coding RNA-regulator of reprogramming, which promotes the development of oesophageal squamous cell carcinoma (ESCC). Based on The Cancer Genome Atlas database, a previous study reported that miR-15b is a novel biomarker for ESCC (16). The results of the

aforementioned studies suggested that miR-15b may serve as a potential biomarker in EC.

The PI3K/AKT signalling pathway serves an important role in regulating various cellular processes, including cell proliferation, apoptosis, the cell cycle and epithelial-mesenchymal transition (17-19). Increasing evidence suggests that the PI3K/AKT signalling pathway also serves a critical role during the progression of EC (20,21). Sheng *et al* (20) reported that protease-activated receptor-2 increases EC cell migration and invasion by activating the PI3K/AKT signalling pathway. In addition, peroxiredoxin 1 knockdown suppresses EC cell proliferation and induces apoptosis by inactivating the PI3K/AKT signalling pathway (21). However, whether the PI3K/AKT signalling pathway is related to the malignant behaviours regulated by miR-15b in EC is not completely understand.

Materials and methods

Study subjects. In the present study, 74 patients (<60, n=30; ≥60, n=44) with EC who had undergone surgery and 74 healthy volunteers (<60, n=30; ≥60, n=44) who had undergone a physical examination between January 2016 and December 2018 were recruited in Shandong Cancer Hospital (Jinan, China). Both patients and healthy volunteer groups comprised of 43 males and 31 females. The age range of the 74 patients was 35 to 66 years. The age range of 74 healthy volunteers matched the age distribution of the patient group. Patients with EC had not been treated with radiotherapy or chemotherapy prior to surgery. Written informed consent was obtained from all participants. The present study was approved by the Ethics Committee of Shandong Cancer Hospital (approval no. 2017024).

Isolation of PBMCs and cell lines. Following a 12-h fast, peripheral blood was collected into EDTA anticoagulant tubes. PBMCs were extracted using Ficoll-Hypaque (450 x g, 20˚C for 20 min), washed by HBSS and resuspended in PBS ($1.0x10^6$ cells/ml).

Human EC cell lines (EC109 and TE10) were obtained from The Cell Bank of Type Culture Collection of the Chinese Academy of Sciences and cultured in RPMI-1640 medium (Invitrogen; Thermo Fisher Scientific, Inc.) containing 10% FBS (Invitrogen; Thermo Fisher Scientific, Inc.). HEEpiCs were obtained from ScienCell Research Laboratories, Inc. and cultured in EpiCM2 medium containing 10% FBS, 5% EGF and 5% penicillin/streptomycin (ScienCell Research Laboratories, Inc.). Cells were incubated at 37˚C in a humidified atmosphere containing 5% CO_2.

Cell transfection. To adjust miR-15b expression levels in EC cells, miR-15b mimics (forward: UAGCAGCACAUCAUG GUUUACA, reverse: UAAACCAUGAUGUGCUGCUAUU), miR-15b inhibitor (forward: UGUAAACCAUGAUGUGCU GCUA), and corresponding mimic negative control (mimics NC, forward: UUCUCCGAACGUGUCACGUTT, reverse: ACGUGACACGUUCGGAGAATT) and inhibitor negative control (inhibitor NC, forward: CAGUACUUUUGUGUA GUACAA) were designed and synthesized by Invitrogen; Thermo Fisher Scientific, Inc.. The non-interference group was used as the blank control. Cells ($2x10^6$) were transfected with the above agents (miR-15b mimics, mimics NC, miR-15b inhibitor and inhibitor NC; 100 nM) for 48 h using the Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. To determine the association between the PI3K/AKT signalling pathway and miR-15b in EC, transfected EC cells ($2x10^6$) were cultured in RPMI-1640 medium containing a PI3K/AKT signalling pathway agonist, recilisib (10 μM; Sigma-Aldrich; Merck KGaA) at 37˚C for at least 48 h. The cells were then harvested to perform the following experiments.

Table I. Primers used for reverse transcription-quantitative PCR.

Gene	Sequence (5'→3')
MircoRNA-15b	F: TAGCAGCACATCATGGTTTACA
	R: TGCGTGTCGTGGAGTC
U6	F: GCTTCGGCAGCACATATACTAAAAT
	R: CGCTTCACGAATTTGCGTGTCAT

F, forward; R, reverse.

RNA extraction and reverse transcription-quantitative PCR (RT-qPCR). After transfection for 24 h, total RNA was extracted from cells using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.). The concentration of total RNA was measured using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Inc.). Total RNA was reverse transcribed into cDNA at 42˚C for 45 min using the MiScript Reverse Transcription kit (Qiagen GmbH). Subsequently, qPCR was performed using the MiScript SYBR-Green PCR kit (Qiagen GmbH) and an ABI7500 system (Applied Biosystems; Thermo Fisher Scientific, Inc.). The thermocycling conditions were: 94˚C for 10 min, followed by 40 cycles at 94˚C for 10 sec, 60˚C for 20 sec and 72˚C for 1 min. The sequences of the primers used in the present study are listed in Table I. Expression levels were quantified using the $2^{-\Delta\Delta Cq}$ method (22) and normalized to the internal reference gene U6.

Protein isolation and western blotting. At 48 h post-transfection, total protein was extracted from the cells using RIPA lysis buffer (Beyotime Institute of Biotechnology) and denatured by incubation in 5X loading buffer (Invitrogen; Thermo Fisher Scientific, Inc.) at 95˚C for 10 min. BCA Protein Assay kit (Abcam) was used to detect the protein concentrations. Protein (20 μg per lane) was separated via SDS-PAGE (15%) and transferred to PVDF membranes, which were blocked with 5% skimmed milk for 2 h at 25˚C. Subsequently, the membranes were incubated overnight at 4˚C with primary antibodies targeted against the following: Bcl-2 (1:1,000; cat. no. AF6139; Affinity Biosciences), Bax (1:1,000; cat. no. AF0120; Affinity Biosciences), PI3K (1:1,000; cat. no. AF6241; Affinity Biosciences), phosphorylated (p)-PI3K (1:1,000; cat. no. AF3242; Affinity Biosciences), AKT (1:1,000; cat. no. 4685; Cell Signaling Technology, Inc.) and p-AKT (1:2,000; cat. no. 4060; Cell Signaling Technology, Inc.). After washing three times with TBST (Tween-20; 0.05%) for 5 min, the membranes were incubated with horseradish

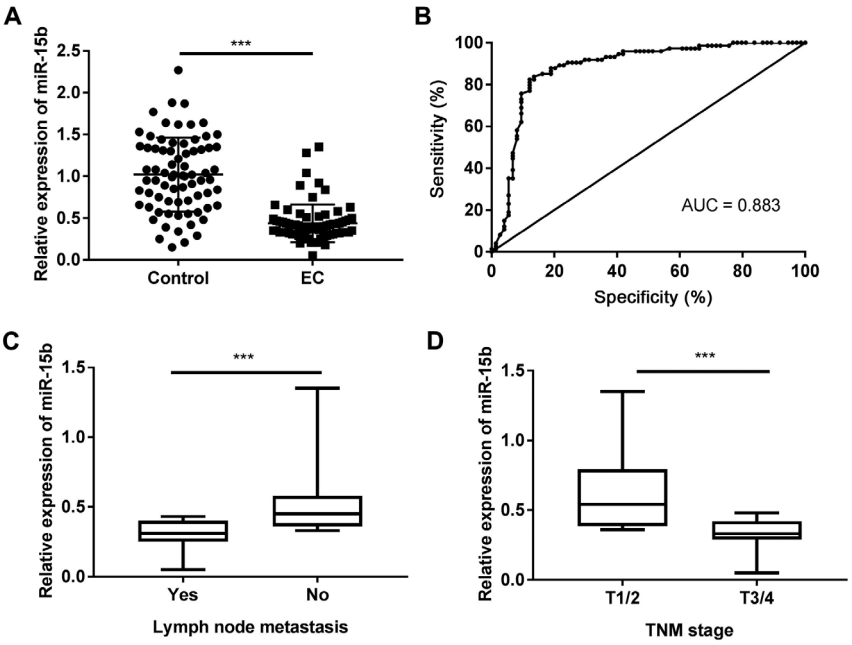

Figure 1. miR-15b is downregulated in PBMCs isolated from patients with EC and serves an important role for the prognosis of EC. (A) Reverse transcription-quantitative PCR was conducted to measure miR-15b expression levels in PBMCs isolated from patients with EC (n=74) and healthy volunteers (n=74). (B) The diagnostic value of miR-15b in EC was determined by receiver operating characteristic curve (AUC=0.883). (C) Analysis of miR-15b expression levels in EC tissues with lymph node metastasis. (D) Analysis of miR-15b expression levels and the TNM stage in EC tissues. ***P<0.001. miR, microRNA; PBMC, peripheral blood mononuclear cells; EC, oesophageal cancer; AUC, area under the curve.

peroxidase-conjugated secondary antibody anti-mice immunoglobulin G (1:5,000; cat. no. 14708; Cell Signaling Technology, Inc.) for 1 h at 25˚C. GAPDH (1:1,000; cat. no. MA5-15738; Thermo Fisher Scientific, Inc.) was used as the internal reference. The membranes were developed using Chemiluminescence reagents (Thermo Fisher Scientific, Inc.) with a Gel-Pro analyzer (version 4.0; Media Cybernetics, Inc.).

MTT assay. The effect of miR-15b on EC cell viability was assessed by performing the MTT assay. At 24 h post-transfection, cells were collected and seeded ($2x10^3$ cells/well) into 96-well plates. At the indicated time point, 20 μl MTT reagent (5 mg/ml; Sigma-Aldrich; Merck KGaA) was added to each well and incubated at 37˚C with 5% CO_2 for 4 h. DMSO (150 μl; Sigma-Aldrich; Merck KGaA) was added to each well and the optical density at 450 nm was measured using an ELX800 microplate reader (Perkin Elmer, Inc.).

Flow cytometry analysis. The early apoptosis of EC cells was evaluated using the Annexin V-PI apoptosis detection kit (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. Briefly, $1\text{-}5x10^5$ cells were resuspended in 500 μl binding buffer and stained with 5 μl Annexin V-EGFP and PI at 4˚C for 15 min in the dark. Subsequently, 400 μl binding buffer was added to the cells and apoptosis was assessed using a FACScan flow cytometer (version 2.0; Becton, Dickinson and Company).

Transwell invasion and migration assays. Cell invasion was assessed using Transwell chambers (Corning, Inc.) pre-coated (at 37˚C for 30 min) with Matrigel® (BD Biosciences). Briefly, transfected EC cells were resuspended in serum-free medium and 200 μl cell suspension ($1x10^5$ cells) was placed in the upper chamber. RPMI-1640 medium containing 10% FBS (600 μl) was added to the lower chamber. Following incubation for 24 h at 37˚C with 5% CO_2, cells on the upper surface were removed using a cotton swab. Invading or migratory cells were fixed at room temperature for 20 min with 3.7% formaldehyde and stained with 0.1% crystal violet (at 37˚C for 15 min). Stained cells were observed in five randomly selected fields using a light microscope (magnification, x200). To assess migration, the Transwell chambers were not pre-coated with Matrigel® and the cell density in the upper chamber was $3x10^4$ cells/200 μl.

Statistical analysis. Data are presented as the mean ± SD. Statistical analyses were performed using GraphPad Prism software (version 7.0; GraphPad Software, Inc.). The Student's t-test was used to analyse differences between two groups. One-way ANOVA followed by Tukey's post hoc test was used to analyse differences among multiple groups. The association between miR-15b expression levels and clinical data of patients with EC were analysed using the χ^2 test. P<0.05 was considered to indicate a statistically significant difference. The diagnostic analysis was performed through ROC curve analysis with healthy controls as true negative cases and EC patients as true positive cases. Each experiment was performed at least three times.

Results

miR-15b expression levels are decreased in PBMCs isolated from patients with EC. To explore the biological function of miR-15b in EC, RT-qPCR was performed to measure miR-15b expression levels in PBMCs isolated from patients with EC and healthy volunteers. The results indicated that miR-15b expression levels were significantly lower in patients with EC compared with healthy volunteers (P<0.001; Fig. 1A). The

Table II. Association between microRNA-15b expression and clinical features of patients with oesophageal cancer.

		MicroRNA-15b expression		
Variable	Total	Low	High	P-value
Age				0.344
<60	30	13	17	
≥60	44	24	20	
Gender				0.48
Male	43	20	23	
Female	31	17	14	
Tumour size				0.013[a]
<2 cm	24	7	17	
≥2 cm	50	30	20	
Lymph node metastasis				0.034[a]
Yes	31	20	11	
No	43	17	26	
TNM stage				0.027[a]
T1/2	25	8	17	
T3/4	49	29	20	
Fibrous membrane invasion				0.008[b]
Yes	47	29	18	
No	27	8	19	
Histologic grade				0.049[a]
Well	26	9	17	
Modest	30	15	15	
Poor	18	13	5	

[a]P<0.05 and [b]P<0.01.

receiver operating characteristic curve revealed that the area under the curve of the EC and control groups distinguished by miR-15b was 0.883 with a cut-off value of 0.525, 82.43% sensitivity and 87.84% specificity (Fig. 1B). Patients with EC were then divided into two groups (high and low expression) according to the median miR-15b expression level (0.46). The association between miR-15b expression and various clinical parameters was determined and the results are presented in Table II. miR-15b expression was associated with tumour size, lymph node metastasis, TNM stage (23), fibrous membrane invasion and histologic grade (P<0.05). Moreover, patients with EC with lymph node metastasis displayed significantly lower miR-15b expression levels compared with patients without lymph node metastasis (P<0.001; Fig. 1C). In addition, miR-15b expression levels were significantly lower in patients with T3/4 stage EC compared with patients with T1/2 stage EC (P<0.001; Fig. 1D).

miR-15b reduces EC cell viability and induces apoptosis. To determine the function of miR-15b in EC, the role of miR-15b in EC cell viability and apoptosis was assessed. First, miR-15b expression levels in HEEpiC and EC cells (EC109 and TE10) were measured via RT-qPCR. Compared with HEEpiC cells, EC109 and TE10 cells expressed significantly lower levels of miR-15b (P<0.001; Fig. 2A). After transfection of miR-15b mimics/NC and miR-15b inhibitor/NC into EC109 and TE10 cells, transfection efficiency was assessed via RT-qPCR. The results indicated that miR-15b mimics significantly increased miR-15b expression in contrast to the mimics NC group, whereas miR-15b inhibitor significantly decreased miR-15b expression in comparison to the inhibitor NC group (P<0.01; Fig. 2B). The overexpression and knockdown cell lines were then used in further experiments to assess the function of miR-15b in EC. The MTT assay results revealed that miR-15b overexpression significantly inhibited EC109 cell viability in contrast to the mimics NC group, whereas miR-15b knockdown significantly enhanced TE10 cell viability in comparison to the inhibitor NC group (P<0.01; Fig. 2C). Flow cytometry analysis suggested that miR-15b overexpression significantly promoted EC109 cell apoptosis, increased Bax expression and decreased Bcl-2 expression in contrast to the mimics NC group, whereas miR-15b knockdown displayed the opposite effects on TE10 cells (P<0.01; Fig. 2D and E).

miR-15b reduces EC cell migration and invasion. Transwell assays were performed to assess the effects of miR-15b on EC cell migration and invasion. miR-15b overexpression significantly decreased EC109 cell migration and invasion (P<0.05),

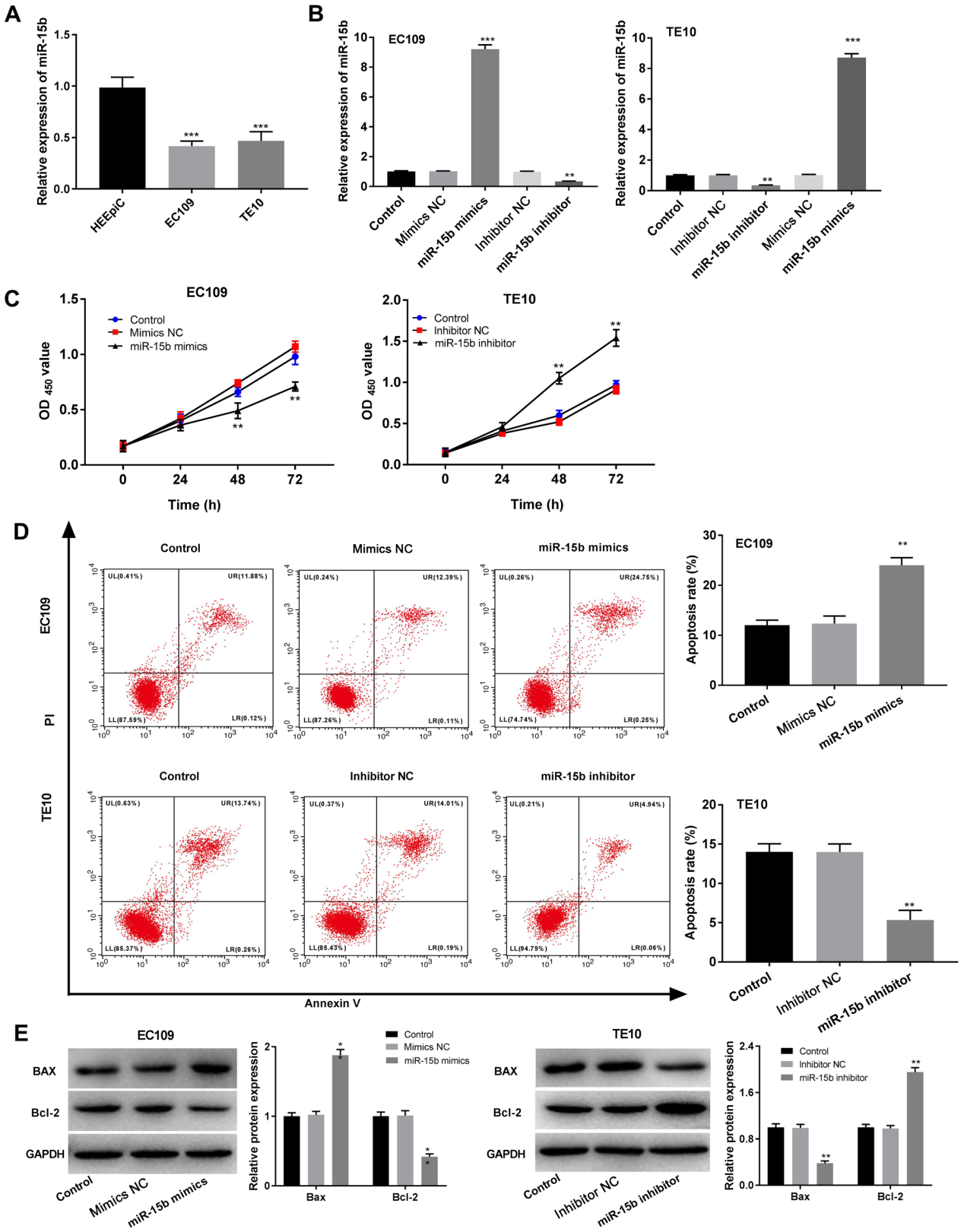

Figure 2. miR-15b reduces EC cell viability and induces apoptosis. (A) miR-15b expression levels in HEEpic, EC109 and TE10 cells. ***P<0.001 vs. HEEpiC (B) Transfection efficiency of miR-15b mimic and miR-15b inhibitor in EC109 and TE10 cells. **P<0.01, ***P<0.001 vs. control. (C) The MTT assay was conducted to assess EC cell viability. (D) EC cell apoptosis was determined by flow cytometry analysis. (E) Relative protein expression levels of Bax and Bcl-2 in EC cells. **P<0.01 vs. control. miR, microRNA; EC, oesophageal cancer; NC, negative control; OD, optical density; PI, propidium iodide.

whereas miR-15b knockdown significantly increased TE10 cell migration and invasion (P<0.01) compared with the corresponding NC cells (Fig. 3).

miR-15b inhibits the PI3K/AKT signalling pathway in EC. A previous study demonstrated that miR-15b upregulation suppressed the PI3K/AKT signalling pathway in ovarian

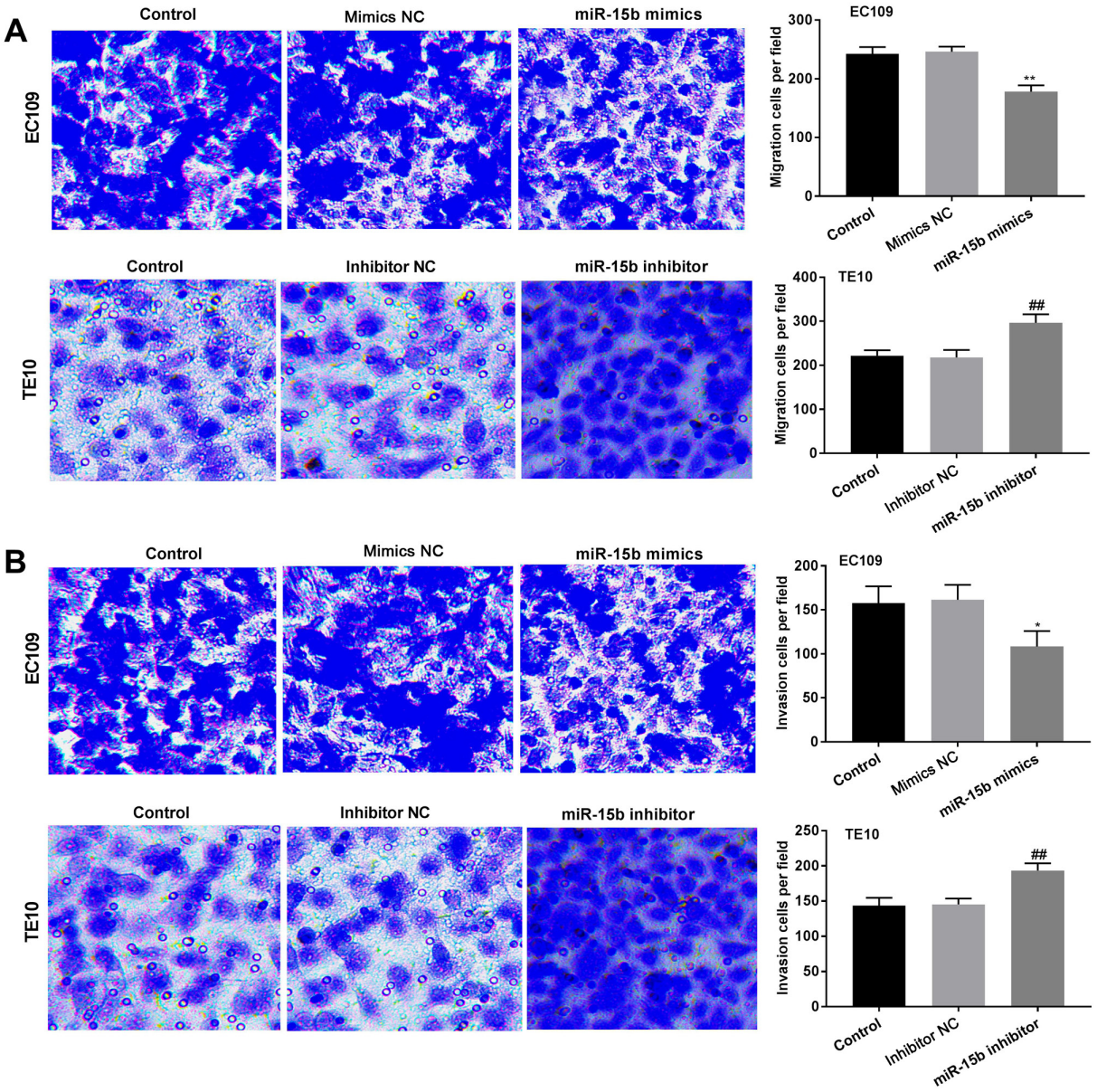

Figure 3. miR-15b attenuates EC cell migration and invasion. Transwell assays were conducted to assess EC109 and TE10 cell (A) migration and (B) invasion. (magnification, x200). *P<0.05, **P<0.01 vs. mimics NC; $^{\#\#}$P<0.01 vs. inhibitor NC. miR, microRNA; NC, negative control.

cancer (23). In addition, activation of the PI3K/AKT signalling pathway increased cancer cell viability and inhibited tumour cell death (24,25). To determine whether the effects of miR-15b in EC were associated with the PI3K/AKT signalling pathway, western blotting was performed. The results indicated that the expression levels of p-PI3K and p-AKT were significantly reduced in miR-15b-overexpression EC109 cells compared to those in the mimics NC EC109 cells, but significantly increased in miR-15b-knockdown TE10 cells compared with the inhibitor NC TE10 cells (P<0.01; Fig. 4A and B). There were no significant differences in the expression levels of PI3K and AKT between miR-15b-overexpression/knockdown cells and the corresponding NC cells (P>0.05; Fig. 4A and B).

miR-15b suppresses the malignant behaviours of EC cells by regulating the PI3K/AKT signalling pathway. To further explore the relationship between miR-15b and the PI3K/AKT signalling pathway during EC progression, TE10 cells were treated with recilisib, a PI3K/AKT signalling pathway agonist. Recilisib treatment significantly increased the ratios of p-PI3K/PI3K and p-AKT/AKT in TE10 cells compared with untreated controls (P<0.01; Fig. 5A). However, miR-15b mimic significantly reduced recilisib-induced increases in p-PI3K/PI3K and p-AKT/AKT ratios in TE10 cells compared with the mimics NC + recilisib group (P<0.01; Fig. 5A). Functional *in vitro* experiments suggested that recilisib significantly promoted TE10 cell viability, migration and invasion, and attenuated TE10 cell apoptosis compared with the control group (P<0.01; Fig. 5B-E). However, miR-15b mimic blocked recilisib-induced cell viability, migration and invasion, and also inhibited the inhibitory effects of recilisib on TE10 cell apoptosis (P<0.01; Fig. 5B-E). Compared with the control group, recilisib treatment significantly decreased Bax expression and increased Bcl-2 expression (P<0.01), which were reversed by miR-15b mimic (P<0.01; Fig. 5F).

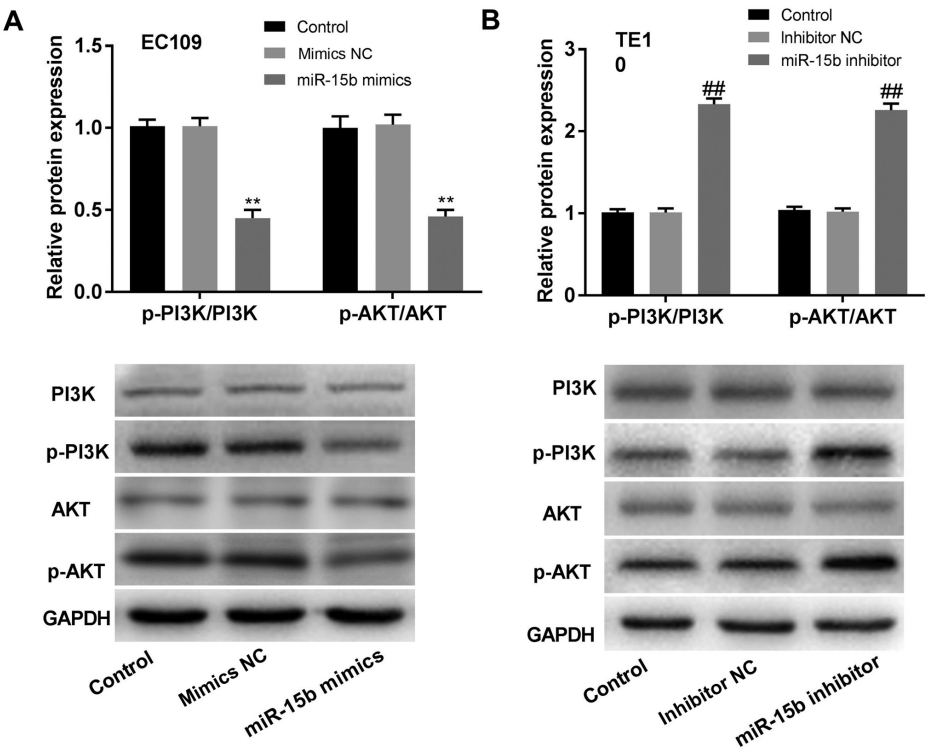

Figure 4. miR-15b impairs the activation of PI3K/AKT signalling pathway in oesophageal cancer. The effect of miR-15b on the PI3K/AKT signalling pathway was investigated via western blotting. p-PI3K/PI3K and p-AKT/AKT ratios in (A) EC109 and (B) TE10 cells. **P<0.01 vs. mimics NC; $^{##}$P<0.01 vs. inhibitor NC. miR, microRNA; p, phosphorylated; NC, negative control.

Discussion

EC is one of the most serious digestive malignancies, resulting in unfavourable prognosis due to tumour metastasis and recurrence (24,25). The present study suggested that miR-15b expression levels were significantly decreased in PBMCs isolated from patients with EC compared with healthy volunteers. In addition, miR-15b expression was significantly associated with tumour size, lymph node metastasis, TNM stage, fibrous membrane invasion and histologic grade in patients with EC. Li *et al* (16) identified miR-15b as a potential biomarker for ESCC, and demonstrated that miR-15b was correlated with tumour histological grade, TNM stage and overall survival. miR-15b was also reported to be related to major clinical features in other types of cancer, such as glioblastoma and cervical carcinoma (26,27). The functional *in vitro* experiments conducted in the present study suggested that miR-15b overexpression inhibited EC cell viability, migration and invasion, and promoted EC cell apoptosis compared with the corresponding NC group. Moreover, the results indicated that the effects of miR-15b were associated with the PI3K/AKT signalling pathway.

miRNAs have received increasing attention as potential therapeutic targets for various tumours, including EC (9,10,11,28). miR-15b is an important RNA molecule that is located on chromosome 3q25 (29). miR-15b levels in PBMCs are correlated with baseline blood glucose concentrations and might serve as a useful indicator of diabetes (30). A previous study demonstrated that miR-15b displays an inhibitory effect on glioblastoma development by inhibiting cell proliferation, cell cycle arrest and invasion (31). In addition, miR-15b upregulation significantly reduces neuroblastoma cell proliferation, migration and invasion by directly targeting MYCN proto-oncogene, bHLH transcription factor (12). Similarly, miR-15b was reported to function as a tumour suppressor by reducing cellular malignant behaviours in osteosarcoma (32) and oral tongue squamous cell cancer (33). Lu *et al* (34) demonstrated that miR-15b inhibits thyroid cancer cell proliferation, migration and invasion by regulating Bcl-2. By contrast, miR-15b displays cancer-promoting effects in other types of cancer. For example, miR-15b upregulation improved resistance to sunitinib, and promoted cell survival and invasion in renal cell carcinoma (35). Moreover, miR-15b-5p serves an oncogenic role in hepatocarcinogenesis by mediating multiple complex regulatory pathways (36). Therefore, it was hypothesized that the function of miR-15b may vary according to the type of cancer. In the present study, miR-15b overexpression reduced EC cell viability, invasion and migration, and promoted EC cell apoptosis compared with the corresponding NC group, which suggested that miR-15b might serve a suppressive role in EC.

Accumulating evidence suggests that the PI3K/AKT signalling pathway is associated with the development of EC (10,37). Activation of the PI3K/AKT signalling pathway can increase cancer cell viability and inhibit tumour cell death (38,39). A number of miRNAs are involved in activating the PI3K/AKT signalling pathway. For example, Zheng *et al* (40) reported that miR-145 could promote apoptosis by suppressing the PI3K/AKT signalling pathway in ESCC. The aggressive phenotype of EC is stimulated by miR-508 via activation of the PI3K/AKT signalling pathway (41). In addition, Pan *et al* (42)

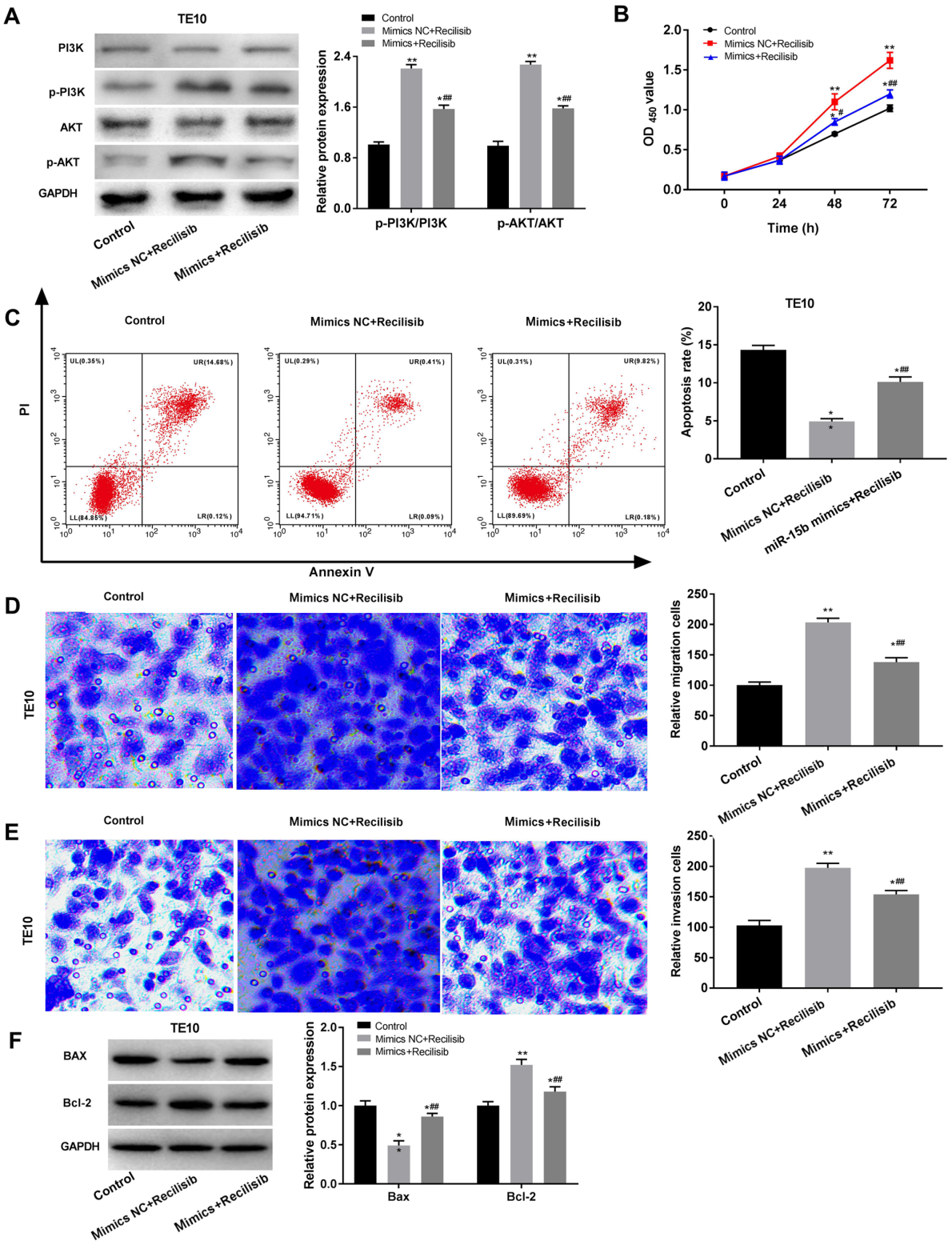

Figure 5. miR-15b suppresses oesophageal cancer cell malignant behaviours by mediating the PI3K/AKT signalling pathway. (A) Western blotting was performed to measure the expression levels of PI3K/AKT signalling pathway-related proteins. TE10 cell. (B) viability, (C) apoptosis, (D) migration and (E) invasion (magnification, x200) were detected by performing MTT, flow cytometry and Transwell assays, respectively. (F) Western blotting was performed to measure the expression levels of Bax and Bcl-2 in TE10 cells. *P<0.05, **P<0.01 vs. control; $^{\#\#}$P<0.01 vs. mimics NC + Recilisib. miR, microRNA; p, phosphorylated; NC, negative control; OD, optical density; PI, propidium iodide.

demonstrated that miR-205 can promote radioresistance by regulating the PI3K/AKT signalling pathway in ESCC, which affects clinical outcomes. miR-15b overexpression alleviated ovarian cancer by inhibiting the PI3K/AKT signalling pathway (43). By contrast, miR-15b participates in the development of peripheral arterial disease by inactivating the PI3K/AKT signalling pathway (44). Therefore, it was speculated that the effects of miR-15b in EC may be related

to the PI3K/AKT signalling pathway. Mechanistically, AKT and PI3K serve vital roles in PI3K/AKT/mTOR signalling (45) as PI3K binds stably to the pleckstrin homology domain of AKT, partially activating it (46). The results of the present study suggested that miR-15b may block activation of the PI3K/AKT signalling pathway in EC cells by reducing p-PI3K and p-AKT expression levels. Some compounds, such as PI3K activators or inhibitors, have been widely used to explore the crosstalk between the PI3K/AKT signalling pathway and various miRNAs in tumorigenesis (47,48). The PI3K/AKT pathway activator recilisib was used in the present study, and the results indicated that recilisib increased cell viability, migration and invasion, and promoted apoptosis in TE10 cells. In addition, recilisib reversed the inhibitory effects of miR-15b overexpression.

In conclusion, in the present study, miR-15b expression levels were significantly lower in PBMCs isolated from patients with EC compared with PBMCs isolated from healthy controls. In addition, miR-15b expression was associated with various clinicopathological characteristics in patients with EC. Functional *in vitro* experiments further indicated that miR-15b may function as a suppressive factor in EC by inhibiting the PI3K/AKT signalling pathway. In summary, the present study improved the current understanding of the mechanism underlying EC and suggested potential novel therapeutic targets for EC.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

JL made substantial contributions to the conception and design of the study. JL, HX, NW and MS made substantial contributions to the acquisition, analysis and interpretation of data, as well as the drafting and revision of the manuscript. All authors agreed to be accountable for the work, read and approved the final version of the manuscript.

Ethics approval and consent to participate

Written informed consent was obtained from all participants. The present study was approved by the Ethics Committee of Shandong Cancer Hospital (Jinan, China; approval no. 2017024).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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