

# Epiplakin1 promotes the progression of esophageal squamous cell carcinoma by activating the PI3K-AKT signaling pathway

Zhongshi Qiao  | Chengcheng Dai | Zhiqian Wang | Zifan Wang | Zheng Wang |  
Tongsong Zhang | Wenjing Niu | Xuezhen Ma

Cancer Center, The Affiliated Qingdao Central Hospital of Qingdao University, The Second Affiliated Hospital of Medical College of Qingdao University, Qingdao, Shandong, China

## Correspondence

Xuezhen Ma, The Affiliated Qingdao Central Hospital of Qingdao University, The Second Affiliated Hospital of Medical College of Qingdao University, No. 127 Siliunan Road, Qingdao, Shandong, 266042, China.  
Email: 18660229289@126.com

## Abstract

**Background:** Epiplakin1 (EPPK1) has been associated with disease progression and unfavorable prognosis of many cancers, but its functional involvement in esophageal squamous cell carcinoma (ESCC) remains to be uncovered.

**Methods:** The Quantitative Real-time PCR (qPCR) assay was employed to determine the expression of EPPK1 in ESCC tissues and cells. CCK-8 assay, colony forming assay, wound healing assay, and transwell invasion assay were utilized to evaluate the effects of EPPK1 on cell proliferation, migration, and invasion capacity in ESCC cells using small interfering ribonucleic acids. Flow cytometry was performed to estimate the cell apoptotic rate caused by silencing of EPPK1. The proteins related to epithelial-to-mesenchymal transition (EMT), apoptosis, and activation of the phosphatidylinositol 3-kinase/serine threonine protein kinase 1 (PI3K/AKT) signaling pathway were measured by western blot.

**Results:** The expression of EPPK1 was dramatically increased in ESCC tissues and cells compared to that in relative controls. Additionally, silencing of EPPK1 suppressed ESCC cell growth, colony formation, migration, invasion, and EMT, while promoting ESCC cell apoptosis. Furthermore, EPPK1 induced ESCC cell progression via mediating the PI3K/AKT signaling pathway.

**Conclusion:** EPPK1 promotes ESCC progression by modulating the PI3K/AKT signaling pathway and could serve as a potential target for ESCC treatment.

## KEYWORDS

EPPK1, ESCC, PI3K/AKT pathway, tumor progression

## INTRODUCTION

Esophageal cancer (EC) is ranked the eighth leading cancer and EC is the sixth most common cause of cancer-related mortality.<sup>1,2</sup> Recent studies report nearly 330 000 new cases and over 270 000 deaths from EC every year.<sup>3</sup> Esophageal squamous cell carcinoma (ESCC) is the major type of EC and exhibits the highest incidence in Southeastern and Central Asia.<sup>4,5</sup> Moreover, the morbidity as well as mortality of ESCC in China presents significant regional variations, which are reportedly higher in several provinces, including Hebei and Shanxi.<sup>6</sup> Given that early detection and effective clinical treatments are still limited, the prognosis of patients

diagnosed with ESCC is extremely poor, with a 5-year survival rate of 9–11%.<sup>7–9</sup> Despite the great efforts made in the past, the detailed molecular triggers of ESCC carcinogenesis and progression are still obscure. Therefore, more exploration needs to be conducted to better understand the molecular basis of these processes, which are critical for early diagnosis and treatment of ESCC patients.

The progression of tumors is controlled by complex and intertwined regulatory networks, in which many key genes play important roles. Epithelial-to-mesenchymal transition (EMT), the classical process related to trans-differentiation, drives tumor cells to invade and leads to tumor metastasis.<sup>10–12</sup> Emerging evidence has revealed that various

signaling pathways are tightly associated with tumor progression, including EMT, such as the Notch, Hedgehog, and TGF- $\beta$  pathways.<sup>13</sup> Moreover, the phosphatidylinositol 3-kinase/serine threonine protein kinase 1 (PI3K/AKT) pathway has also been proved to accelerate the progression and EMT of cancers.<sup>14</sup> Mutations of several key genes have been noticed in this pathway, such as phosphatase and tensin homolog (PTEN), phosphatidylinositol-4, 5-bisphosphate 3-kinase, catalytic subunit alpha (PI3KCA), and AKT.<sup>15–17</sup> Previous findings have proved the vital effects of the PI3K/AKT signaling pathway on progress, including proliferation, apoptosis, and survival of mammalian cells.<sup>18</sup> Changes in the activity of the PI3K/AKT pathway affect the development of multiple cancers, including ESCC.<sup>19</sup> However, investigation of more upstream regulatory genes of the PI3K/AKT signaling pathway still attracts much attention.

Epiplakin1 (EPPK1) belongs to the plakin family and has been determined as the human epidermal self-antigen, which is universally expressed in esophagus and several other organs.<sup>20,21</sup> As a common cell linker protein, EPPK1 is located in the junction of the cytoplasmic membranes and is capable of integrating cytoskeleton to myofilaments.<sup>22</sup> Emerging evidence has illustrated that EPPK1 is involved in the progression of diverse cancers such as liver cancer,<sup>23</sup> cervical cancer,<sup>24</sup> and bladder urothelial cancer.<sup>25</sup> Nevertheless, it is still unclear whether EPPK1 plays roles in ESCC. In this investigation, we found that EPPK1 may promote ESCC tumor progression by mediating the PI3K/AKT signaling pathway.

## MATERIALS AND METHODS

### Clinical samples and cell lines

The complementary DNA (cDNA) array of human ESCC (14 samples) was obtained from Outdo Biotech. Human esophageal epithelial cells (HET-1A) and ESCC cells (KYSE150, EC9706, ECA109, TE-10) were cultured in Dulbecco's modified eagle medium (DMEM; BasalMedia) with 10% fetal bovine serum (FBS; Thermo Fisher). All cells were maintained in an incubator (Thermo Fisher) containing 5% CO<sub>2</sub> at 37°C.

### Cell transfection

Three small interfering ribonucleic acids (siRNAs; GenePharma) were used to target EPPK1, with scramble siRNA as the control (GenePharma). The Lipofectamine 2000 reagent (Thermo Fisher) was used to transfect cells according to user instructions.

### Real-time PCR

Total RNA of tissues and cells was isolated with Trizol (Thermo Fisher) according to manufacturer instructions.

Total RNA as template was synthesized to cDNA by reverse transcription kit (Vazyme) and then the cDNA was the template for real-time PCR using ChamQ SYBR qPCR Master Mix (Vazyme) in the StepOnePlus™ Real-Time PCR System (Thermo Fisher).

### Cell viability assay

ESCC cells planted in 96-well plates ( $1 \times 10^4$  cells/well) were transfected with siRNAs or scramble. After 48 h, 10  $\mu$ l of Cell Counting Kit-8 (CCK-8) reagent (Dojindo Laboratories) was added to each well and incubated for 2 h. The absorbance value at 450 nm was recorded by a microplate reader (Molecular Devices).

### Colony forming assay

Three hundred ESCC cells transfected with siRNAs or scramble were seeded in six-well plates. After 14 days, cell colonies formed and they were then fixed with methanol and stained with crystal violet (Solarbio). Images were captured with a microscope (Olympus) at 4 $\times$  magnification.

### Wound healing assay

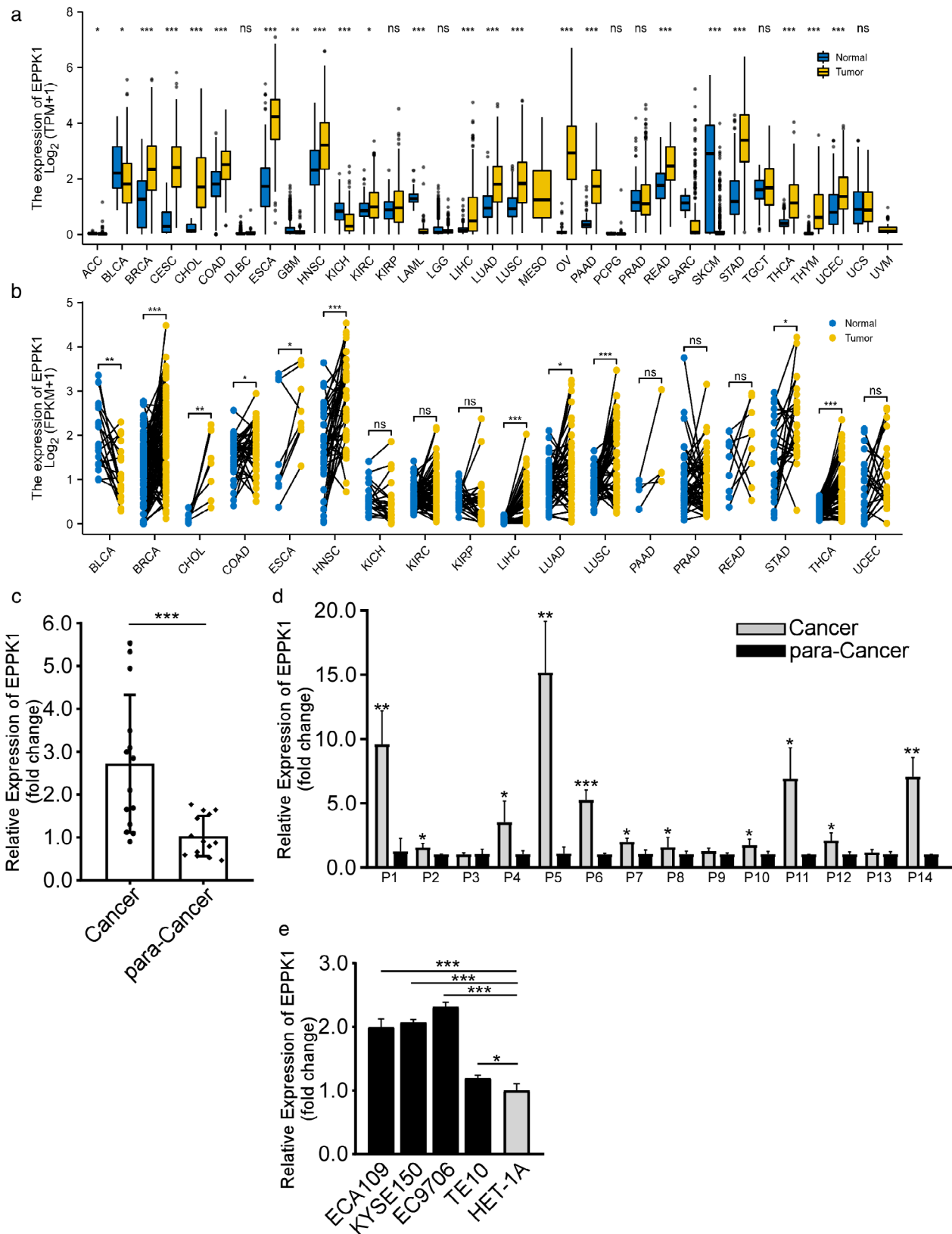
ESCC planted in 12-well plates ( $2 \times 10^5$  cells/well) were transfected with siRNAs or scramble. When the cells were confluent, a scratch-wound was generated by a pipette tip. Images of the scratches were taken at 0 and 24 h under microscope (Olympus) at 4 $\times$  magnification.

### Transwell invasion assay

A 24-well Milli cell chamber (Corning) that was coated with 30  $\mu$ l of Matrigel (Corning) was used for the invasion assay. First,  $3 \times 10^4$  cells transfected with siRNAs or scramble were seeded in the coated chambers containing 150  $\mu$ l of serum-free medium. The chambers were placed in a well of 24-well plates which was filled with 600  $\mu$ l of medium with 10% FBS per well, then the plates were kept at 37°C for 24 h. After that, the invasion cells were fixed with methanol and stained with crystal violet (Solarbio), then five fields per chamber was observed for counting under a microscope (Olympus) at 10 $\times$  magnification.

### Western blot

ESCC cells planted in six-well plates ( $1 \times 10^6$  cells/well) were transfected with siRNA or scramble. After 48 h, the cells were collected using RIPA buffer (Thermo Scientific) supplemented with proteinase inhibitor cocktail (Merck).



**FIGURE 1** EPPK1 decreased in ESCC tissues and cells. (a) The expression level of EPPK1 in tumor tissues and normal tissues obtained from the TCGA + GTEx database. (b) The expression level of EPPK1 in paired cancer and normal tissues obtained from the TCGA database. (c) The mRNA level of EPPK1 in ESCC tissues and para-carcinoma tissues measured by qPCR ( $p < 0.05$ ). (d) The mRNA level of EPPK1 in 14 pairs of ESCC tissues and para-carcinoma tissues ( $p < 0.05$ ). (e) The mRNA level of EPPK1 in ESCC and normal cells measured by qPCR ( $p < 0.05$ ). Values represent means  $\pm$  SD,  $n = 3$ . \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ . P, patient

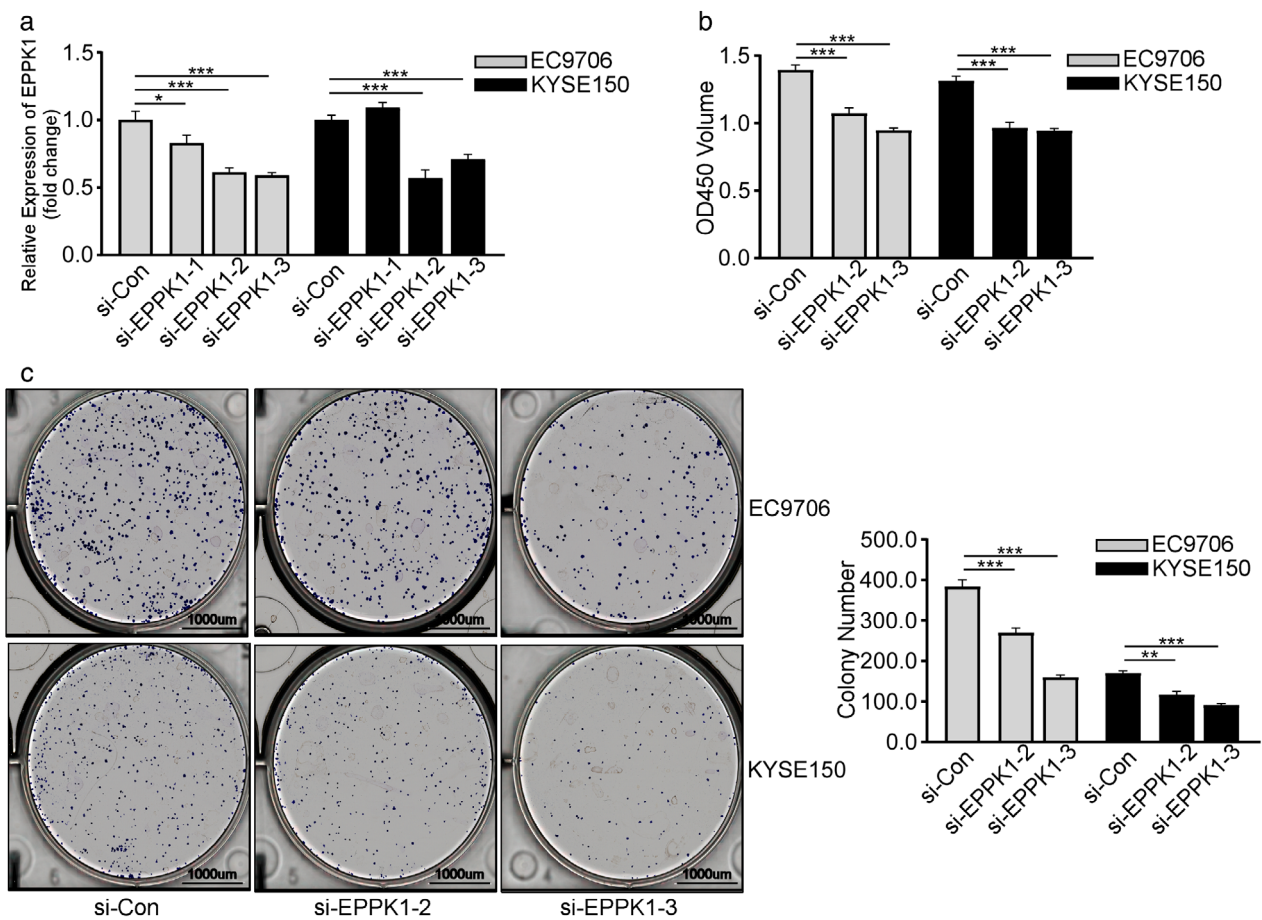
The concentration of protein was determined by bicinchoninic acid (BCA) protein assay kit (Thermo Fisher). The heat-denatured proteins were separated with 12% SDS-PAGE, transferred onto polyvinylidene fluoride membrane (Thermo Fisher), then incubated by bovine albumin (BSA) and primary antibodies Caspase-3 (1:1000 dilution; CST), Caspase-3 (1:1000 dilution; Santa Cruz), B-cell lymphoma-2 (BCL2) (1:1000 dilution; Santa Cruz), poly ADP-ribose polymerase (PARP) (1:1000 dilution; CST), N-Cadherin (1:1000 dilution; CST), Vimentin (1:1000 dilution; CST), E-Cadherin (1:1000 dilution; Proteintech), phospho-phosphatidylinositol 3-kinase (p-PI3K) (1:1000 dilution; CST), PI3K (1:1000 dilution; CST), phospho-protein kinase B (p-AKT) (1:1000 dilution; CST), AKT (1:1000 dilution; CST), and  $\beta$ -actin (1:5000 dilution; Merck) overnight at 4°C. Then membrane was incubated with horseradish peroxidase (HRP) conjugated secondary antibody (1:5000; Jackson ImmunoResearch) and an enhanced chemiluminescence (ECL) solution (Thermo Fisher) was used to observe the protein bands with the Gel Imaging System (GE Healthcare).

## Flow cytometry assay

ESCC cells planted in six-well plates ( $1 \times 10^6$  cells/well) were transfected with siRNAs or scramble. After 48 h, cells were trypsinized and collected, then re-suspended with 500  $\mu$ l of binding buffer containing 5  $\mu$ l of Annexin V-FITC and propidium iodide (PI) reagent (Vazyme). Finally, the cells were analyzed using a flow cytometer (BD Biosciences).

## Statistical analysis

All data were provided as mean  $\pm$  standard deviation (SD) using SPSS 13.0 in addition to Graphpad Prism 7.0 software. An unpaired two-tailed *t*-test was introduced to finish the contrast of data between two groups while one-way ANOVA was employed to compare multiple groups followed by the post hoc Tukey's test. Differences were considered statistically significant when *p* values were  $< 0.05$ .



**FIGURE 2** Epiplakin1 regulated the proliferation of ESCC cells. (a) The mRNA level of EPPK1 measured by qPCR ( $p < 0.05$ ). (b) Cell viability was detected by CCK-8 assay ( $p < 0.05$ ). (c) Colony formation was detected by soft agar assay ( $p < 0.05$ ). Values represent means  $\pm$  SD,  $n = 3$ . \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$

## RESULTS

### EPPK1 was increased in ESCC tissues and cells

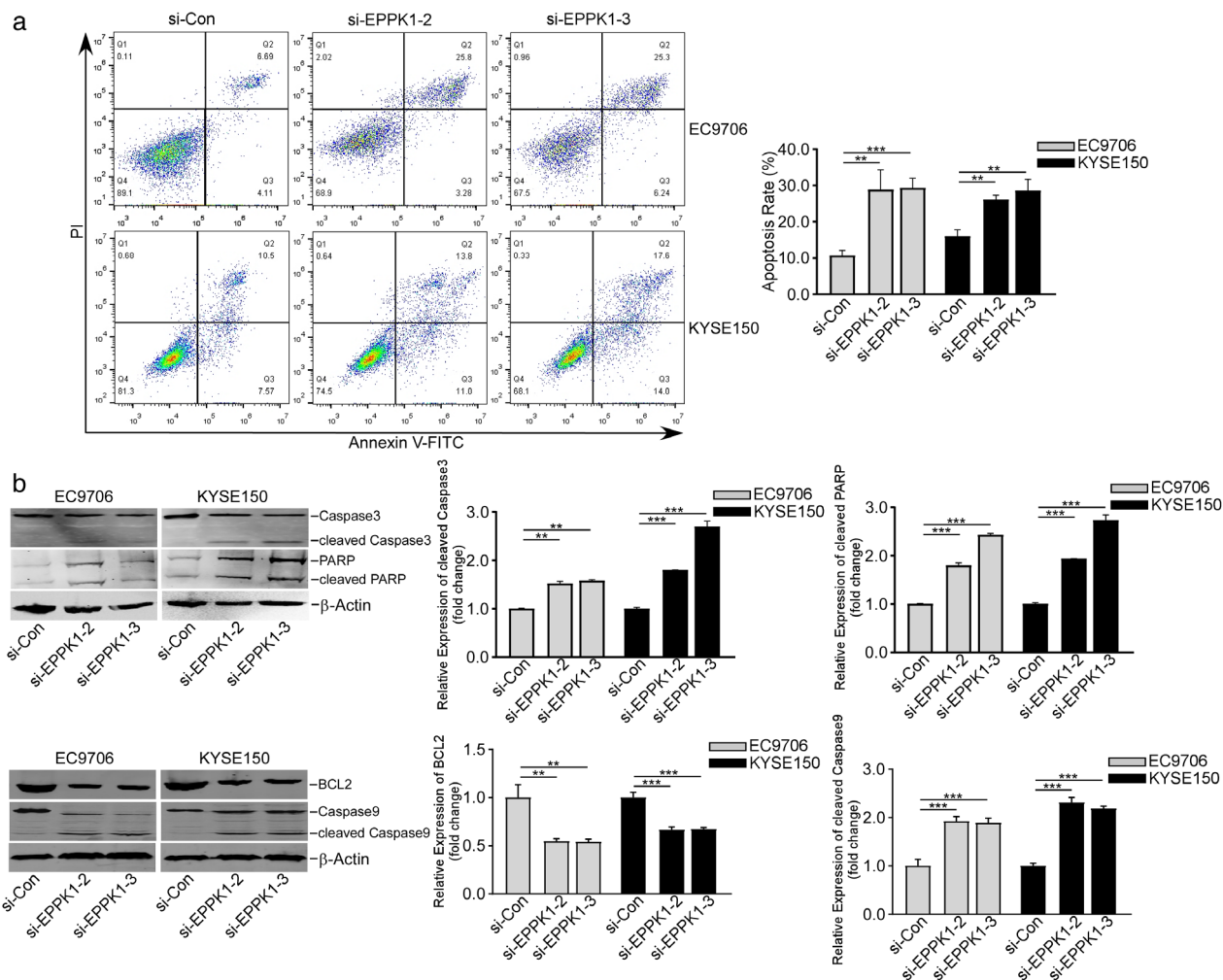
By analyzing the expression profile of EPPK1 from The Cancer Genome Atlas (TCGA) and The Genotype-Tissue Expression (GTEx), the EPPK1 expression of cancer tissues was significantly found to be higher than that in normal tissues in 22 out of 33 tumor types, such as ESCC (Figure 1a). By analyzing the transcriptome of paired cancer and normal tissues in TCGA, the EPPK1 expression in cancer tissues was found to be significantly higher than that in normal tissues in 11 out of 18 tumor types, including ESCC (Figure 1b).

To further validate these results, qRT-PCR was employed to detect the expression of EPPK1 in 14 pairs of ESCC tissues and para-carcinoma tissues. The level of EPPK1 was higher in ESCC tissues than that in para-carcinoma tissues ( $p < 0.05$ , Figure 1c). Additionally, the EPPK1 expression was significantly increased in 11 out of 14 ECSS samples ( $p < 0.05$ ; Figure 1d). Furthermore, EPPK1 was

more highly expressed in ESCC cells than in esophageal epithelial cells ( $p < 0.05$ ; Figure 1e). Taken together, these data showed that EPPK1 was increased in ESCC.

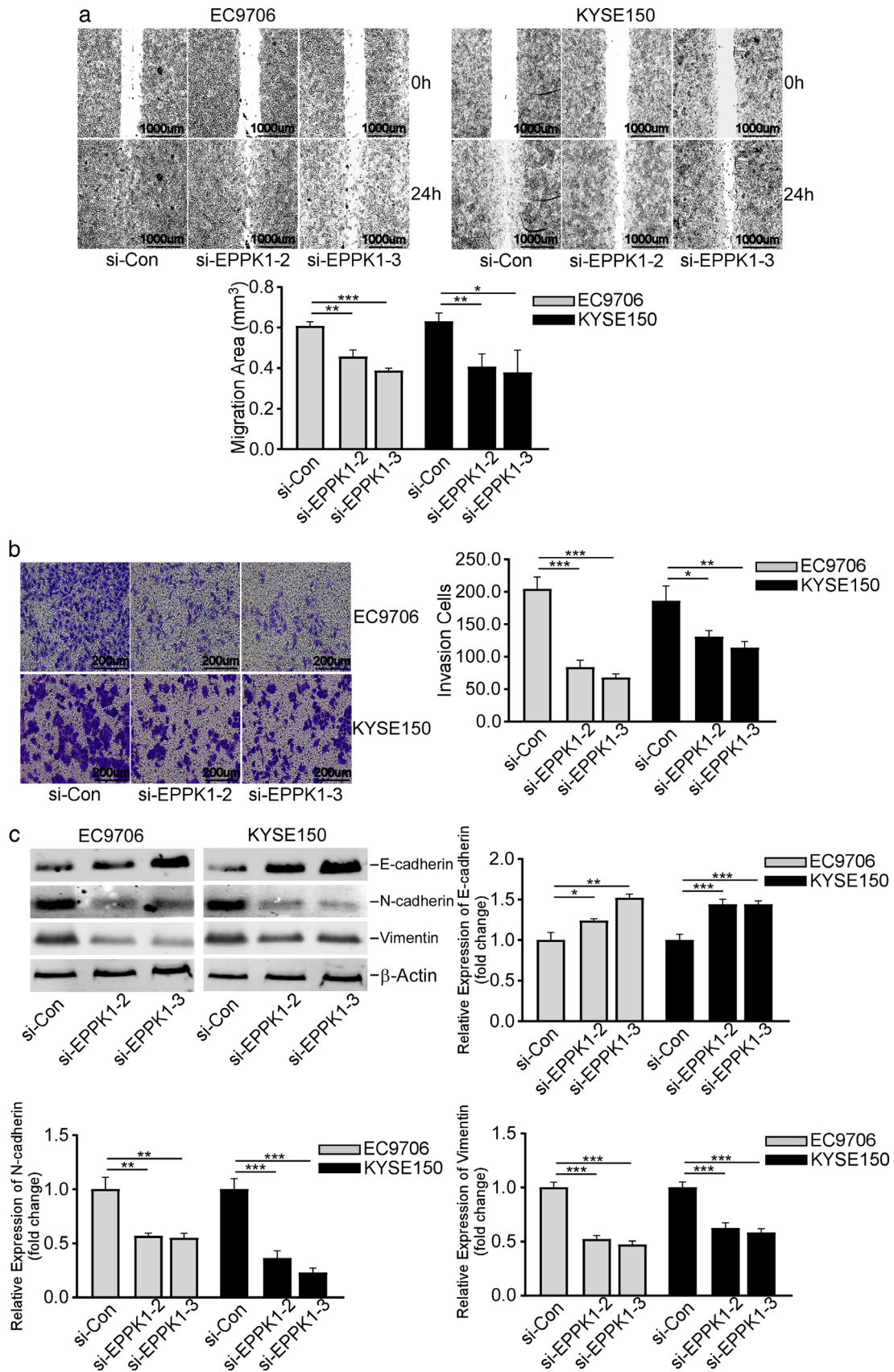
### EPPK1 promoted the proliferation of ESCC cells

To elucidate the roles of EPPK1 in ESCC cells, KYSE150 and EC9706 cell lines were selected to conduct the function experiments. First, three siRNAs targeting EPPK1 at locations of 5341, 5571 and 6449, which were named as siEPPK1-1, siEPPK1-2 and siEPPK1-3, were transfected into KYSE150 and EC9706 cells, and the messenger RNA (mRNA) level of EPPK1 was determined to confirm the suppression efficiency. Then siEPPK1-2 and si-EPPK1-3 were selected for subsequent in vitro experiments ( $p < 0.05$ ; Figure 2a). Subsequently, CCK-8 assay illustrated that EPPK1 knockdown suppressed the proliferation of KYSE150 and EC9706 cells significantly ( $p < 0.05$ ; Figure 2b).



**FIGURE 3** Epiplakin1 affected ESCC cell apoptosis. (a) Cell apoptosis detected by flow cytometry assay ( $p < 0.05$ ). (b) The apoptosis-related protein level of Caspase3, Caspase9, BCL2, and PARP measured by western blot with the indicated antibodies ( $p < 0.05$ ). Values represent means  $\pm$  SD,  $n = 3$ .

\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$



**FIGURE 4** Epiplakin1 regulated the migration and invasion via EMT progression. (a) The wound healing rate of ESCC cells detected by wound healing assay ( $p < 0.05$ ). (b) The invasion of ESCC cells detected by transwell assay ( $p < 0.05$ ). (c) The proteins related to EMT measured by western blot ( $p < 0.05$ ). Values represent means  $\pm$  SD,  $n = 3$ . \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$

Moreover, silencing EPPK1 obviously reduces the colony formation ability of KYSE150 and EC9706 cells ( $p < 0.05$ ; Figure 2c). These results suggest that knockdown of EPPK1 notably inhibits ESCC cell growth.

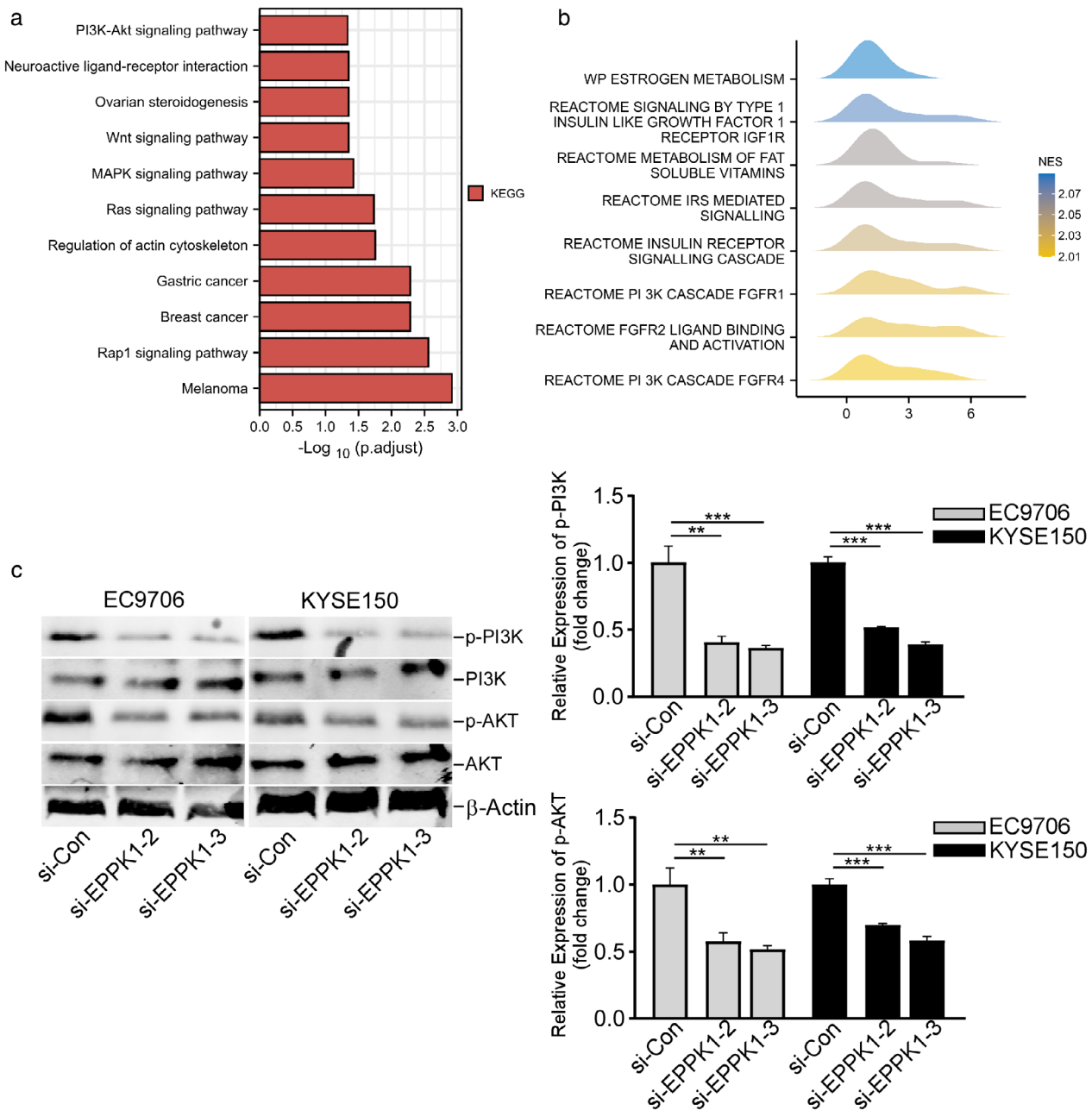
### EPPK1 inhibits ESCC cells apoptosis

To explore whether EPPK1 affects ESCC cell apoptosis, the flow cytometry assay was employed to detect cell apoptotic rates. EPPK1 silencing dramatically accelerated the apoptosis of KYSE150 and EC9706 cells ( $p < 0.05$ ; Figure 3a). For

apoptosis-related proteins, silencing EPPK1 dramatically increased the cleavage of Caspase3, Caspase9, and PARP, and decreased the BCL2 in ESCC cells ( $p < 0.05$ ; Figure 3b). Collectively, these findings verify that EPPK1 significantly inhibits ESCC cell apoptosis.

### EPPK1 promotes ESCC cell migration and invasion via EMT

To further explore the effects of EPPK1 on the migration and invasion of ESCC cells, we conducted wound healing



**FIGURE 5** Epiplakin1 knockdown inhibited the PI3K/AKT signaling pathway. (a) KEGG analysis was accomplished by the clusterProfiler package in R. (b) GSEA revealed significant differences (false discovery rate, FDR  $q$  value  $< 0.05$ ; nominal, NOM  $p$  value  $< 0.05$ ) in enrichment of the Molecular Signatures Database (MSigDB) Collection (c2.cp.v7.2.symbols.gmt). (c) The proteins related to the PI3K/AKT signaling pathway were measured by western blot with the indicated antibodies ( $p < 0.05$ ). Values represent means  $\pm$  SD,  $n = 3$ . \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$

assay and transwell invasion assay. The wound healing rate was inhibited by silencing EPPK1 in KYSE150 and EC9706 cells, indicating that EPPK1 knockdown decreased the migration capacity of ESCC cells ( $p < 0.05$ ; Figure 4a). In addition, silencing EPPK1 notably downregulated the cells' invasion ability ( $p < 0.05$ ; Figure 4b). To explore the functional links between EPPK1 and EMT, the change of proteins associated with EMT was determined after EPPK1 was silenced. The data illustrated that EPPK1 knockdown significantly reduced N-Cadherin and Vimentin levels but increased E-Cadherin in both KYSE150 and EC9706 cells ( $p < 0.05$ ; Figure 4c), implying that EPPK1 silencing suppresses EMT progression. All these results suggest that EPPK1 promotes ESCC cell migration and invasion via EMT progression.

### EPPK1 knockdown inhibits the PI3K/AKT signaling pathway

To explore the potential regulation mechanism of EPPK1, KEGG analysis was accomplished using the clusterProfiler package in R. The KEGG analysis revealed that the EPPK1 were involved in several kinds of cancer types and multiple regulation pathways, including the PI3K-AKT pathway (Figure 5a). Gene Set Enrichment Analysis (GSEA) between low- and high-expression EPPK1 using the clusterProfiler package in R was used to identify EPPK1-related enrichment signaling pathways in ESCC. With high expression of EPPK1, the most significantly enriched signaling pathways based on their normalized enrichment score (NES) revealed differential enrichment of estrogen metabolism, vitamin metabolism, and PI3K-AKT-related terms, including the insulin like growth factor 1 receptor (IGF1R) signaling pathway, the insulin receptor substrate (IRS-mediated signaling pathway, the insulin receptor signaling pathway, and the FGFR pathway (Figure 5b). These regulation pathways triggered a cascade via the PI3K-AKT pathway.

Regarding the vital roles of the PI3K/AKT signaling pathway in tumor progression, such as apoptosis and migration, the load of p-PI3K and p-AKT was examined. As shown in Figure 5c, silencing EPPK1 suppressed both p-PI3K and p-AKT remarkably. These results proved that EPPK1 could activate the PI3K/AKT signaling pathway.

## DISCUSSION

As the leading subtype of EC, ESCC is characterized by a high recurrence rate and poor patient prognosis.<sup>3</sup> Thus, further investigation of the molecular mechanisms underlying ESCC development is urgently needed to facilitate the discovery of more valid therapeutic targets. This study for the first time uncovered that EPPK1 modulates the development of ESCC. Briefly, we discovered that EPPK1 knockdown inhibits ESCC cell proliferation, migration invasion, and EMT progression while inducing cell apoptosis.

Furthermore, silencing EPPK1 blocked the phosphorylation of PI3K and AKT. Our investigation suggests that EPPK1 might be a promising target for treating ESCCs.

Most members of the plakin family abundant in diverse organs and tissues contain a conserved domain, the plakin domain.<sup>26</sup> However, the specific expression level of plakins in various species and organs remains unclear. As a member of the plakin set, EPPK1 has been found to be expressed in multiple tissues and cells, particularly in pancreatic cancers,<sup>27,28</sup> demonstrating that EPPK1 is likely to be a potent regulator for cancer progression. In this investigation, we fixed our attention on the effects of EPPK1 on the development of ESCC, which has not been elucidated up to now. By analyzing the TCGA database, we discovered that EPPK1 expression was higher in ESCC tissues compared with normal tissues. Consistently, tumor and normal tissues from 16 patients with ESCC confirmed the higher expression of EPPK1 in tumor tissues. In a subsequent experiment, EPPK1 was knocked down in KYSE150 and EC9706 cells to verify the roles and molecular mechanisms of EPPK1 on ESCC cell progression. As expected, silencing EPPK1 dramatically inhibited cell viability, migration, and invasion capacity. All these findings primarily demonstrated that EPPK1 might have essential roles in ESCC cell development.

Based on previous studies regarding ESCC cell apoptosis,<sup>29</sup> we utilized flow cytometry as well as protein assay to examine whether EPPK1 affected the apoptosis of ESCC cells. The results show that EPPK1 knockdown efficiently increased cellular apoptosis, suggesting that EPPK1 regulates ESCC cell progress at least partly by inhibiting apoptosis.

Recent studies have revealed that EMT exerts crucial roles in the invasion as well as metastasis of ESCC.<sup>30</sup> Nevertheless, the underlying mechanisms associated with EMT remain confusing. This investigation verified that the suppression of EPPK1 significantly inhibits EMT progression, which indicates that EPPK1 might influence cell migration and invasion by mediating EMT. Pang et al. reported that AKT affected EMT and further regulated the invasion in addition to metastasis of ESCC cells.<sup>31</sup> Consistently, our present study proved that silencing EPPK1 remarkably inhibited the activation of the PI3K/AKT signaling pathway, therefore EPPK1 might mediate EMT by activating the PI3K/AKT signaling pathway.

Despite the merits of the current study, there are some limitations. First, we only explored the in vitro roles of EPPK1 in ESCC cells, therefore more experiments are needed to confirm the effects of EPPK1 on ESCC in vivo. Additionally, the direct target gene of EPPK1 in the PI3K/AKT signaling pathway is also worth exploring in subsequent experiments.

In conclusion, our findings reveal that EPPK1 is able to activate the PI3K/AKT signaling pathway and significantly enhance ESCC cell progression, including cell growth, migration, invasion, and EMT. This investigation first attracted attention on the effects of EPPK1 on ESCC, and presents a potential gene target for diagnosing and curing ESCC in the future.



## CONFLICT OF INTEREST

The authors have no conflicts of interest to report.

## ORCID

Zhongshi Qiao  <https://orcid.org/0000-0003-2275-8181>

## REFERENCES

- Salehi M, Moradi-Lakeh M, Salehi MH, Nojomi M, Kolahdooz F. Meat, fish, and esophageal cancer risk: a systematic review and dose-response meta-analysis. *Nutr Rev*. 2013;71:257–67.
- Mattiuzzi C, Lippi G. Cancer statistics: a comparison between World Health Organization (WHO) and global burden of disease (GBD). *Eur J Public Health*. 2020;30:1026–7.
- Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2018;68:394–424.
- Abnet CC, Arnold M, Wei WQ. Epidemiology of esophageal squamous cell carcinoma. *Gastroenterology*. 2018;154:360–73.
- Kim JA, Shah PM. Screening and prevention strategies and endoscopic management of early esophageal cancer. *Chin Clin Oncol*. 2017;6:50.
- Guohong Z, Min S, Duenmei W, Songnian H, Min L, Jinsong L, et al. Genetic heterogeneity of oesophageal cancer in high-incidence areas of southern and northern China. *PLoS One*. 2010;5:e9668.
- Wang Q, Zhang W, Liu X, Zhang X, He J, Feng Q, et al. Prognosis of esophageal squamous cell carcinoma patients with preoperative radiotherapy: comparison of different cancer staging systems. *Thorac Cancer*. 2014;5:204–10.
- Kaupilla JH, Mattsson F, Brusselaers N, Lagergren J. Prognosis of esophageal adenocarcinoma and squamous cell carcinoma following surgery and no surgery in a nationwide Swedish cohort study. *BMJ Open*. 2018;8:e021495.
- Cheng YF, Chen HS, Wu SC, Chen HC, Hung WH, Lin CH, et al. Esophageal squamous cell carcinoma and prognosis in Taiwan. *Cancer Med*. 2018;7:4193–201.
- Morikawa M, Derynck R, Miyazono K. TGF-beta and the TGF-beta family: context-dependent roles in cell and tissue physiology. *Cold Spring Harb Perspect Biol*. 2016;8:a021873.
- Menendez-Menendez J, Hermida-Prado F, Granda-Diaz R, Gonzalez A, Garcia-Pedrero JM, Del-Rio-Ibisate N, et al. Deciphering the molecular basis of melatonin protective effects on breast cells treated with doxorubicin: TWIST1 a transcription factor involved in EMT and metastasis, a novel target of melatonin. *Cancers (Basel)*. 2019;11:1011.
- Aiello NM, Kang Y. Context-dependent EMT programs in cancer metastasis. *J Exp Med*. 2019;216:1016–26.
- Huber MA, Kraut N, Beug H. Molecular requirements for epithelial-mesenchymal transition during tumor progression. *Curr Opin Cell Biol*. 2005;17:548–58.
- Guertin DA, Sabatini DM. Defining the role of mTOR in cancer. *Cancer Cell*. 2007;12:9–22.
- Platt FM, Hurst CD, Taylor CF, Gregory WM, Harnden P, Knowles MA. Spectrum of phosphatidylinositol 3-kinase pathway gene alterations in bladder cancer. *Clin Cancer Res*. 2009;15:6008–17.
- Ross RL, Burns JE, Taylor CF, Mellor P, Anderson DH, Knowles MA. Identification of mutations in distinct regions of p85 alpha in urothelial cancer. *PLoS One*. 2013;8:e84411.
- Yardena Samuels KE. Oncogenic PI3K and its role in cancer. *Curr Opin Oncol*. 2006;18(1):77–82.
- Yan W, Ma X, Zhao X, Zhang S. Baicalein induces apoptosis and autophagy of breast cancer cells via inhibiting PI3K/AKT pathway in vivo and vitro. *Drug Des Dev Ther*. 2018;12:3961–72.
- Wang Y, Lyu Z, Qin Y, Wang X, Sun L, Zhang Y, et al. FOXO1 promotes tumor progression by increased M2 macrophage infiltration in esophageal squamous cell carcinoma. *Theranostics*. 2020;10:11535–48.
- Fujiwara S, Takeo N, Otani Y, Parry DA, Kunimatsu M, Lu R, et al. Epiplakin, a novel member of the Plakin family originally identified as a 450-kDa human epidermal autoantigen. Structure and tissue localization. *J Biol Chem*. 2001;276:13340–7.
- Naoko Takeo WW, Matsuo N, Sumiyoshi H, Yoshioka H, Fujiwara S. Structure and heterogeneity of the human gene for epiplakin (EPPK1). *J Invest Dermatol*. 2003;121(5):1224–6.
- Blagoev B, Kratchmarova I, Ong SE, Nielsen M, Foster LJ, Mann M. A proteomics strategy to elucidate functional protein-protein interactions applied to EGF signaling. *Nat Biotechnol*. 2003;21:315–8.
- Shen T, Lu Y, Zhang Q. High squalene epoxidase in tumors predicts worse survival in patients with hepatocellular carcinoma: integrated Bioinformatic analysis on NAFLD and HCC. *Cancer Control*. 2020;27:1073274820914663.
- Guo X, Hao Y, Kamilijiang M, Hasimu A, Yuan J, Wu G, et al. Potential predictive plasma biomarkers for cervical cancer by 2D-DIGE proteomics and ingenuity pathway analysis. *Tumour Biol*. 2015;36:1711–20.
- Hyebin Lee KK, Woo J, Park J, Kim H, Lee KE, Kim H, et al. Quantitative proteomic analysis identifies AHNAK (neuroblast differentiation-associated protein AHNAK) as a novel candidate biomarker for bladder urothelial carcinoma diagnosis by liquid-based cytology. *Mol Cell Proteomics*. 2018;17(9):1788–802.
- Ortega E, Manso JA, Buey RM, Carballido AM, Carabias A, Sonnenberg A, et al. The structure of the plakin domain of plectin reveals an extended rod-like shape. *J Biol Chem*. 2016;291:18643–62.
- Sawamura D, Li K, Chu ML, Uitto J. Human bullous pemphigoid antigen (BPAG1). Amino acid sequences deduced from cloned cDNAs predict biologically important peptide segments and protein domains. *J Biol Chem*. 1991;266(27):17784–90.
- Yoshida T, Shiraki N, Baba H, Goto M, Fujiwara S, Kume K, et al. Expression patterns of epiplakin1 in pancreas, pancreatic cancer and regenerating pancreas. *Genes Cells*. 2008;13:667–78.
- Zabihula B, Yiliyasi M, Lu Y, Salai A. MicroRNA-490-3p inhibits proliferation and stimulates apoptosis of ESCC cells via MAPK1 down-regulation. *Oncol Lett*. 2019;18:3170–6.
- Liu Q, Zheng S, Chen Y, Liu T, Han X, Zhang X, et al. TGF-beta1-induced upregulation of MALAT1 promotes Kazakh's esophageal squamous cell carcinoma invasion by EMT. *J Cancer*. 2020;11:6892–901.
- Pang L, Li Q, Wei C, Zou H, Li S, Cao W, et al. TGF-beta1/Smad signaling pathway regulates epithelial-to-mesenchymal transition in esophageal squamous cell carcinoma: in vitro and clinical analyses of cell lines and nomadic Kazakh patients from Northwest Xinjiang, China. *PLoS One*. 2014;9:e112300.

**How to cite this article:** Qiao Z, Dai C, Wang Z, Wang Z, Wang Z, Zhang T, et al. Epiplakin1 promotes the progression of esophageal squamous cell carcinoma by activating the PI3K-AKT signaling pathway. *Thorac Cancer*. 2022;13:1117–25. <https://doi.org/10.1111/1759-7714.14366>