

# Reduced EBP50 expression or mis-localization of the EBP50 protein is associated with the malignant progression of esophageal squamous cell carcinoma

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**Abstract. – PURPOSE:** The aim of this study was to examine the significance of EBP50 (ezrin-radixin-moesin binding phosphoprotein 50) expression in esophageal squamous cell carcinoma (ESCC).

**MATERIALS AND METHODS:** Real-time PCR (qRT-PCR), western blotting, and immunohistochemical staining were performed to detect EBP50 expression in pairs of ESCCs and matched non-tumor tissues, and the relationships between EBP50 expression and other clinical factors in ESCC were analyzed. An iRNA targeting EBP50 was transfected into EC9706 cells. MTT and plate colony assays were performed to assess the effects of EBP50 down-regulation on cell growth, and flow cytometry was used to evaluate the influence of inhibiting EBP50 on cell cycle progression.

**RESULTS:** The real-time PCR (qRT-PCR), western blotting, and immunohistochemical staining results showed that EBP50 expression was significantly lower in ESCCs compared to matched non-tumor tissues. In addition, decreased EBP50 expression correlated with differentiation, T stage, lymph node (LN) metastasis, and poor prognosis in patients with ESCC. The down-regulation of EBP50 may significantly promote the growth and proliferation of EC9706 cells while accelerating cell cycle progression from the G1 to S phase.

**CONCLUSIONS:** EBP50 expression was decreased in ESCC, indicating that EBP50 might play a significant role in the malignant progression of ESCC and be a prognostic marker for patients with ESCC.

*Key Words:*

EBP50, ESCC, Prognostic marker, Malignant Progression, Prognosis.

## Introduction

Esophageal cancer, more than 90% of which is classified as esophageal squamous cell carcinoma (ESCC), ranks 6<sup>th</sup> in the worldwide cancer-related incidence rate<sup>1</sup>, with more than 50% of ESCC occurring in China<sup>2</sup>. Recent progress in surgery, chemotherapy, and molecularly targeted therapy has led to enhanced therapeutic effects. However, patients with ESCC still have a poor prognosis due to the highly aggressive nature of the cancer and the lack of an ideal therapeutic method. Therefore, screening for pivotal prognostic markers in ESCC can lead to the diagnosis, therapy, and prognostic ability of ESCC.

Ezrin-radixin-moesin binding phosphoprotein 50 (EBP50, also known as NHERF1) is located on human chromosome 17q25.1 and encodes a 50-kD microvillar scaffolding protein with two tandem PSD-95/Discs Large/ZO-1 (PDZ) domains and a carboxyl (C)-terminal EB region<sup>3,4</sup>. EBP50 was found to be present, mostly localizing at the plasma membrane, in a variety of cells and tissues, including kidney proximal tubule epithelial cells, terminal lung bronchioles, luminal epithelia, and proliferative endometrium<sup>5,6</sup>. However, EBP50 expression was found to be apically decreased in the normal duct epithelial cells of the nonlactating mammary gland<sup>7</sup>. Indeed, NHERF1/EBP50 controls lactation by establishing basal membrane polarity complexes with the prolactin receptor<sup>8</sup>. In 2003, Shibata et

al<sup>9</sup> found that EBP50 was over-expressed in hepatocellular carcinoma (HCC) and promoted malignant HCC progression via the stabilization of  $\beta$ -catenin for transcriptional regulation and the enhancement of Wnt signaling. Elevated EBP50 expression was also observed as a cytoplasmic accumulation in breast carcinomas in comparison to corresponding normal tissues<sup>10</sup>. However, subsequent studies have shown that an alteration in the apical membrane localization of EBP50 is correlated with the malignant progression of colorectal cancer<sup>11</sup> and that of the aberrant nuclear localization of EBP50 is promoted in the carcinogenesis of colorectal cancer<sup>12</sup>. Our previous study revealed that EBP50 expression is decreased during the malignant progression of human pancreatic cancer<sup>13</sup>, whereas EBP50 over-expression resulted in increased 5-FU-induced apoptosis in gastric cancer cells through Bax- and Bcl-2-triggered mitochondrial pathways<sup>14</sup>. Another previous study in our laboratory showed that the over-expression of EBP50 can inhibit the growth of SMMC7721 cells and promote apoptosis by modulating  $\beta$ -catenin and E-cadherin<sup>15</sup>, indicating that EBP50 might play a suppressive role in cancer cells.

Nonetheless, to date, the expression and role of EBP50 in human ESCC has remained unclear. Thus, to evaluate the significance of EBP50 expression in ESCC, real time-PCR (qPCR), western blotting, and immunohistochemical staining were performed in this study. EBP50 expression in ESCC and matched non-tumor tissues was detected, and the relationships between EBP50 expression and patient clinical data, including age, gender, location, TNM stage, lymph node, and prognosis, were analyzed. An siRNA targeting EBP50 was transfected into EC9706 cells, and MTT, plate colony, and flow cytometry assays were performed to investigate the effects of EBP50 on EC9706 cells.

## Materials and Methods

### *Tissues and Cell Lines*

Ninety-eight pairs of archived paraffin-embedded ESCCs and matched non-tumor tissues were obtained from patients who underwent radical esophagectomy at the Renmin Hospital of Wuhan University, Wuhan, Hubei Province, People's Republic of China, from January 2004 to December 2006. For qPCR analyses, 30 pairs of ESCCs and matched non-tumor tissues were ob-

tained from surgical samples between January 2012 and July 2012. For western blot analyses, 10 pairs of ESCCs and matched non-tumor tissues were also collected. The diameters of the tissues for the qPCR and western blot analyses were approximately 1.5-2.0 cm, and each was immediately preserved in liquid nitrogen until use. All the samples were clinically and histopathologically confirmed, and the patients' clinical data were acquired from their medical records. The study was authorized by the Ethics Committee of the Hospital. Written consent for the use of the tissues was also obtained from all the patients prior to their surgery. Patients without complete follow-ups were excluded from this study, and the end points were death or December 2011.

The human ESCC cell lines EC109 and RC9706 were obtained from the Chinese Academy of Medical Science (Beijing, China) and maintained in our Department. The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) 1640 with 10% fetal bovine serum at 37°C in an incubator with 5% CO<sub>2</sub>.

### *qRT-PCR Analyses*

The Trizol reagent (Invitrogen, Carlsbad, CA, USA) was used for total RNA extraction, according to the manufacturer's protocol, and a light cycler system (Roche, Mannheim, Germany) was used for qRT-PCR following a previously described method<sup>16</sup>. The primer sequences for  $\beta$ -actin (internal control) were forward, 5'-gtgacgttgacatccg-3', and reverse, 5'-gagcgtttgtgtacct-3'; the primer sequences for EBP50 were forward, 5'-gatcgattgtggaggtgaa-3', and reverse, 5'-ggagatgttgaagtctag ga-3'. Each sample was detected three times, and the 2<sup>- $\Delta\Delta$ CT</sup> method was performed to analyze the differences in EBP50 expression between ESCCs and matched non-tumor tissues, as previously described<sup>17</sup>.

### *Western Blot Analyses*

Protein from the tissues and cell lines was extracted using Total Cell Protein Lysis Buffer (Biyuntian, Beijing). The protein samples were separated on 10% SDS-polyacrylamide gels and transferred onto an NC (nitrocellulose) membrane (Millipore, Billerica, MA, USA). Ten-percent fat-free milk in tris buffered saline (TBS) (0.25% Tween20) was used for blocking for 1 h, and the NC membrane was incubated with anti-EBP50 (Novus International, Saint Charles, MO, USA; 1:1000) and anti- $\beta$ -actin (Sigma, St Louis,

MO, USA; 1:3000) antibodies overnight at 4°C. The membrane was incubated with HRP-conjugated goat anti-mouse (BIOS 1:3000) and anti-rabbit (BIOS 1:2000) antibodies for 60 minutes, and enhanced chemiluminescence (ECL-kit, Santa Cruz Biotechnology, Santa Cruz, CA, USA) was used for visualization.

### **Immunohistochemical Staining**

Immunohistochemical staining was performed to detect EBP50 expression in each section using the streptavidin-biotin method following the method of a previous study<sup>18</sup>. The sections were deparaffinized in xylene, hydrated in alcohol, and rehydrated in 3 ml/l H<sub>2</sub>O<sub>2</sub>. The sections were subjected to heat-induced antigen retrieval in citrate solution at 100°C for 10 min. A polyclonal rabbit antibody against EBP50 (Novus, USA) was used at a 1:500 dilution overnight at 4°C. After three washes in PBS-T, the sections were incubated with a goat anti-rabbit secondary antibody for 40 min at room temperature. Again, after three washes, DAB (3,3'-Diaminobenzidine) detection was used for visualization.

### **Determination of the Results**

Because EBP50 shows a significant physiological function at the membrane, the results of the immunohistochemical staining were determined by two experienced pathologists after considering the staining intensity and proportion at the membrane of ESCCs or esophageal squamous epithelial cells. Each section was assigned a score according to the sum of the intensity (no=0, weak=1, moderate=2, and strong=3) and the proportion of the expression (0%=0, 0-30%=1, 30-60%=2, and >60%=3). EBP50 expression was finally evaluated according to the following combined scores: 0, negative (-); 1-2, weak expression; 3-4, moderate expression (+); and 5-6, strong expression (++)

### **EBP50 siRNA Plasmid Transfection**

EBP50-targeting siRNA (Santa Cruz, CA, USA) and a non-targeting control siRNA with G418 resistance were transfected into EC9706 cells using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA), according to a previous study<sup>12</sup>; G418 was used for 2 weeks to select the stably transfected cells. The stably transfected cell lines were named Con-EC9706 (transfected with the non-targeting control siRNA) and Si-EC9706 (transfected with the EBP50-targeting siRNA).

### **MTT Assay**

The growth ability of the EC9706, Con-EC9706, and Si-EC9706 cells were assessed using MTT assays, as described previously<sup>12</sup>. Briefly, cells in the log-phase stage were collected and plated in 96-well plates (1×10<sup>4</sup> cells/well). Then, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, 5 mg/ml) solution was added into each well and incubated for 4 h. After the supernatant was removed, 150 μl DM-SO (dimethyl sulfoxide) was added to dissolve the crystals with agitation for 10 min. The absorbance (OD) values were detected using an ELISA reader at a wave length of 490 nm. Each experiment was repeated three times.

### **Flow Cytometry Assay**

The cell cycle was assessed using a flow cytometry assay, as previously described<sup>12</sup>. EC9706, Con-EC9706, and Si-EC9706 cells were collected, washed three times with ice-cold PBS (phosphate buffered saline), fixed in 70% ethanol, and stained with propidium iodide. The cell cycle phases were then analyzed by flow cytometry, and the cell proliferation indices (PIs) were determined as  $PI = (S + G2)/(S + G2 + G1)$ .

### **Plate Colony Formation**

EC9706, Con-EC9706, and Si-EC9706 cells were prepared in suspension and inoculated in 6-well flat-bottomed plates at a density of 500 cells/well, and the plates were slightly shaken. The cells were cultured for 8 days in DMEM 1640 with 10% fetal bovine serum at 37°C in an incubator with 5% CO<sub>2</sub>. After three washes with PBS and fixation in methanol for 10 min, the cells were stained with Giemsa's solution for 10 min. The prepared clones, with > 50 cells, were counted using an optical microscope. Each experiment was repeated three times.

### **Statistical Analysis**

The SPSS 17.0 software package (SPSS Inc., Chicago, IL, USA) was used to obtain *p*-values, with *p* < 0.05 considered to be statistically significant. A two-tailed *t*-test was performed for the comparison between ESCC and matched non-tumor tissues, and the Kruskal-Wallis test was used to analyze the relationships among the clinicopathological variables, followed by a one-way ANOVA method for the analysis of the differences between three comparisons: the MTT, colony formation, and cell cycle analyses. The survival curves of the patients were detected us-

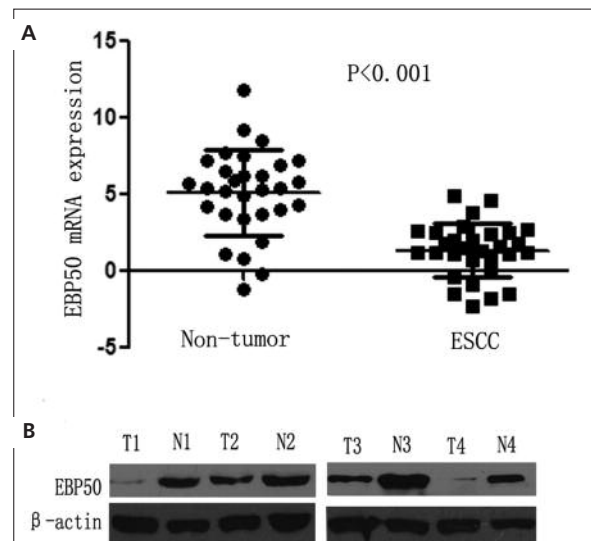
ing a Kaplan-Meier analysis and compared using the log rank test. A Cox regression was used for a multivariate analysis.

## Results

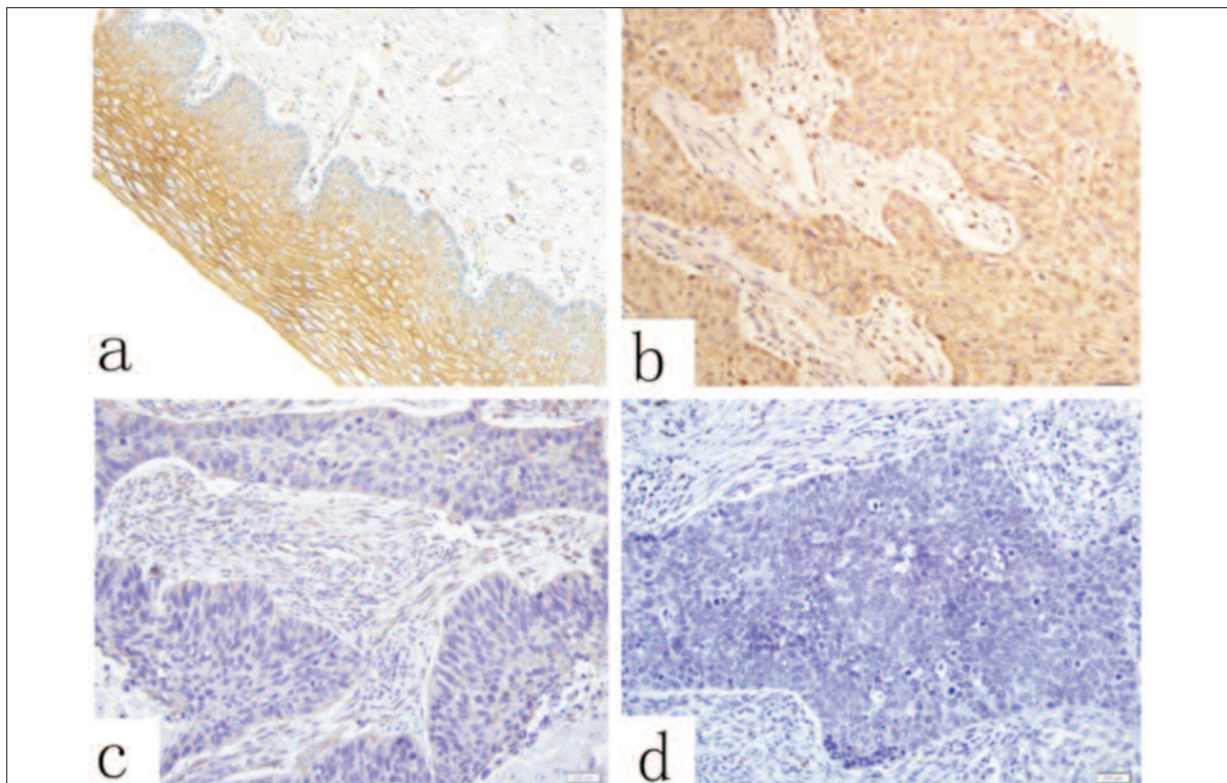
### Decreased EBP50 Expression in ESCC

The mean values of EBP50 mRNA expression were  $1.33 \pm 1.76$  in the ESCC samples and  $5.14 \pm 2.79$  in the matched non-tumor tissues. A statistical analysis showed that the expression of EBP50 mRNA in ESCC tissues was significantly lower than in matched non-tumor tissues ( $p < 0.01$ ; 95% confidence interval, 2.459 to 5.154) (Figure 1a). The results of the western blot analyses showed that, for 9 of the 10 patients, EBP50 protein expression was lower in the ESCC samples than in the matched non-tumor tissues (Figure 1b).

As shown in Figure 2a, EBP50 is mostly expressed in the apical membrane of normal esophageal epithelial cells and not in the nucleus and/or cytoplasm, if at all, in ESCC tissues (Fig-



**Figure 1.** Real time-PCR and western blot analyses of EBP50 expression in ESCCs and non-tumor tissues. **A**, The results of real time-PCR showed that EBP50 expression was significantly decreased in ESCC tissues compared to matched non-tumor tissues ( $p < 0.001$ ). **B**, Western blot analyses indicated that EBP50 expression was markedly lower in ESCC tissues compared to matched non-tumor tissues; four representatives are shown.  $\beta$ -actin was used as the loading control.



**Figure 2.** Expression of EBP50 in ESCC and non-tumor tissues. **a**, Strong expression in the cell membrane of the esophageal squamous epithelium. **b**, Moderate expression in the cytoplasm and nucleus of ESCC tissues. **c**, Weak expression in the cytoplasm of ESCC tissues. **d**, Negative expression in ESCC tissues. Original magnification,  $\times 200$ .

**Table I.** EBP50 expression in ESCC and matched non-tumor tissues.

Group	Total	EBP50 expression				<i>p</i> value
		-	±	+	++	
Non	98	8 (8.2%)	15 (15.3%)	40 (40.8%)	35 (35.7%)	< 0.001
ESCC	98	30 (30.6%)	42 (42.9%)	20 (20.4%)	6 (6.1%)	

ures 2 b, c, and d). With regard to the non-tumor tissues, a lack of immunostaining was observed in 8 patients (8.2%), weak expression in 15 patients (15.3%), moderate expression in 40 patients (40.8%), and strong expression in 35 patients (35.7%). In contrast, a lack of expression was observed in the ESCC tissues from 30 patients (30.6%), weak expression in 42 patients (42.9%), moderate expression in 20 patients (20.4%), and strong expression in 6 patients (6.1%). Statistical analyses revealed that EBP50 expression was significantly lower in ESCCs compared to the matched non-tumor tissues (Table I;  $p < 0.001$ ).

#### **Suppression of EBP50 Expression is Associated With Tumor Progression**

We then investigated the correlation between EBP50 expression and the patient's clinical data.

As shown in Table II, lower EBP50 expression significantly correlated with differentiation ( $p = 0.04$ ), T stage ( $p = 0.002$ ), and LN metastasis ( $p = 0.03$ ). However, there was no significant correlation of EBP50 expression with patient age, gender, or location. These data indicate that the suppression of EBP50 expression is associated with malignant progression in patients with ESCC.

#### **Survival Analysis**

We further analyzed the prognostic value of EBP50 expression in addition to patient clinical features. The total survival rate of all patients was 31.6% (31/98). The survival rate of patients with a lack of EBP50 expression was 13.3% (4/30), with weak expression was 26.2% (11/42), with moderate expression was 60.0% (4/6), and with strong expression was 66.7% (4/6). A Kaplan-Meier

**Table II.** Correlations of EBP50 expression with clinical features in ESCC tissues.

Feature	N	Result				<i>p</i>
		-	±	+	++	
Age						0.66
> 53	49	16	21	8	4	
≤ 53	49	14	21	12	2	0.80
Sex						
Men	66	19	28	14	5	0.80
Women	32	11	14	6	1	
Differentiation						0.04
Well	22	4	7	8	3	
Moderate	31	12	11	5	3	
Poor	45	30	42	20	6	0.002
T stage						
I	5	1	1	2	1	
II	34	6	14	12	2	
III	55	24	24	5	2	
IV	4	3	1	1	0	0.03
LN Metastasis						
No	33	11	8	11	3	0.03
Yes	65	19	34	9	3	
Location						0.28
Up	30	6	12	9	3	
Middle	40	15	15	7	3	
Low	28	9	15	4	0	

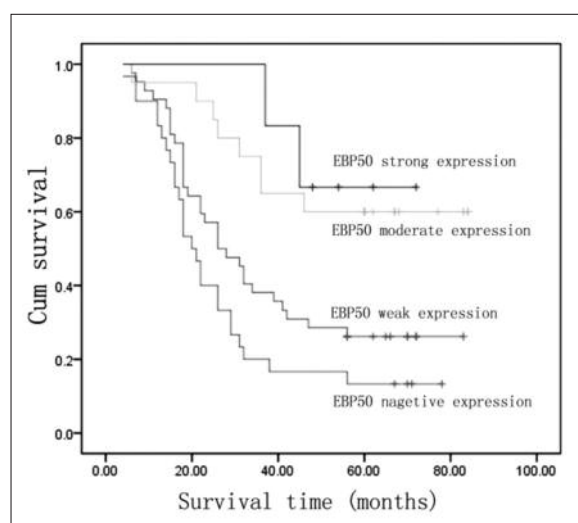
analysis showed that the patients with lower EBP50 expression had a shorter survival time compared to those with higher EBP50 expression ( $p = 0.001$ ; Figure 3). Univariate analyses showed that the patients with advanced T stage ( $p = 0.001$ ) and LN metastasis ( $p = 0.02$ ) had a poorer prognosis (Table III). Furthermore, the results of multivariate analyses revealed that EBP50 ( $p = 0.04$ ) and T stage ( $p < 0.001$ ) were two independent prognostic factors for ESCC patients (Table IV).

### Knockdown of EBP50 Promotes ESCC cell Growth

A plate colony experiment was performed to detect the effects of EBP50 suppression on the colony formation ability of EC9706 cells. As shown in Figure 4d, the Si-EC9706 cell colony number was significantly increased compared to the EC9706 and Con-EC976 cells ( $p < 0.05$ ; Figure 4 d and e).

### Decreased EBP50 Expression Promotes cell Cycle Progression

A flow cytometry assay was performed to detect the impact of EBP50 inhibition on the EC9706 cell cycle. As shown in Figure 5a, the percentages of cells in the G1, S, and G2 stages were  $32.4 \pm 5.2\%$ ,  $49.6 \pm 7.1\%$ , and  $17.3 \pm 3.0\%$  for the Si-EC9706 cells, respectively;  $59.2 \pm 6.2\%$ ,



**Figure 3.** Survival analysis of EBP50 expression correlated with the postoperative survival times of patients. Patients with lower expression of EBP50 exhibit significantly shorter postoperative survival times compared to those with higher EBP50 expression ( $p < 0.001$ ).

$25.3 \pm 4.2\%$ , and  $15.3 \pm 3.2\%$  for the EC9706 cells, respectively; and  $58.6 \pm 7.3\%$ ,  $25.9 \pm 3.2\%$ , and  $15.2 \pm 2.9\%$  for the Con-EC9706 cells, respectively. Statistical analyses showed that the percent of Si-EC9706 cells was significantly decreased in

**Table III.** Univariate analyses of factors in patients with ESCC.

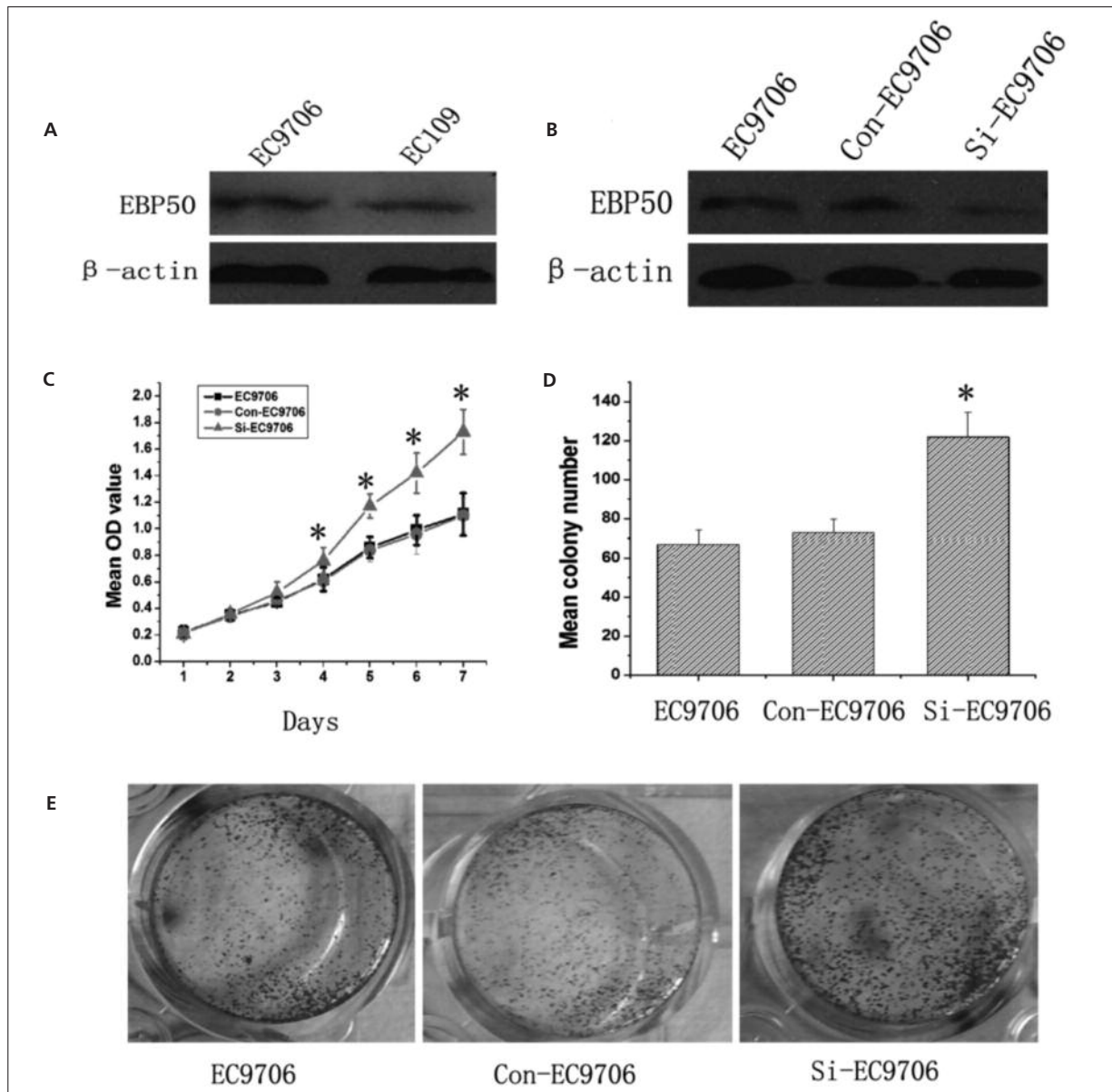
Factor	Survival rate	95% CI	$p$
Age			0.79
> 53	32.7% (16/49)	23.8-41.6	
≤ 53	30.6% (15/49)	22.8-39.2	
Sex			0.12
Men	36.4% (24/66)	22.0-50.9	
Women	21.9% (7/32)	20.5-31.5	
Differentiation			0.31
Well	40.9% (9/22)	28.1-53.9	
Moderate	35.5% (11/31)	19.2-52.8	
Poor	24.4% (11/45)	18.1-33.9	
T stage			0.001
I	80% (4/5)		
II	55.9% (19/34)		
III	14.5% (8/55)	17.5-26.5	
IV	0	3.3-32.7	
LN Metastasis			0.02
No	48.5% (16/33)		
Yes	23.1% (15/65)	19.1-32.9	
Location			0.31
Up	40.9% (9/22)	28.1-53.9	
Middle	35.5% (11/31)	19.2-52.8	
Low	24.4% (11/45)	18.2-33.9	

CI: 95% Confidence Interval.

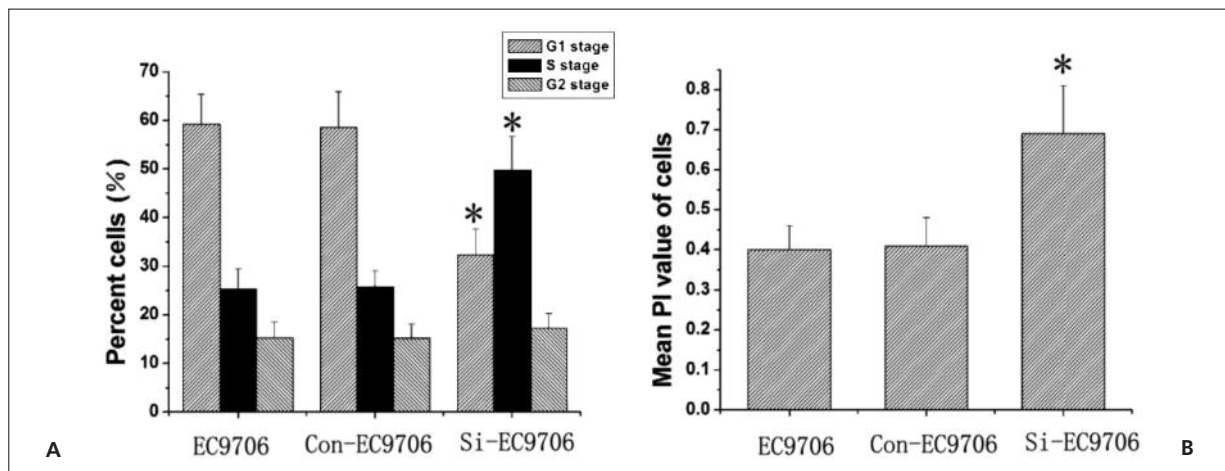
**Table IV.** Multivariate analyses of factors in patients with ESCC.

Factor	Hazard ratio	<i>p</i> value
T stage	13.43	0.001
LN metastasis	2.4	0.12
EBP50 expression	8.26	0.04

the G1 stage but was significantly increased in the S stage compared to the EC9706 and Con-EC9706 cells ( $p < 0.05$ ; Figure 5a). The PI of Si-EC9706 cells was  $0.69 \pm 0.12$ , which was significantly higher than that of EC9706 ( $0.4 \pm 0.06$ ) and Con-EC9706 ( $0.41 \pm 0.07$ ) ( $p < 0.05$ ; Figure 5b). This finding revealed that the inhibition of EBP50 promoted cell cycle progression from the G1 to the S phase in EC9706 cells.



**Figure 4.** The down-regulation of EBP50 expression increases the malignant growth potential of EC9706 cells. **A**, Western blotting demonstrated that EBP50 expression was higher in EC9706 cells compared to EC109 cells. **B**, EBP50 expression was markedly down-regulated by siRNA transfection. **C**, MTT assays showed that the down-regulation of EBP50 promoted the growth of EC9706 cells. **D**, Plate colony formation assays revealed that the colony number of siEC9706 cells was significantly higher than that of EC9706 and Con-EC9706 cells. **E**, Representative plate colony formation results for each cell line are shown. \*Statistical significance ( $p < 0.05$ , EC9706 and Con-EC9706 cells vs. Si-EC9706 cells).



**Figure 5.** Inhibiting EBP50 promotes cell cycle progression from the G1 to S phase in EC9706 cells. **A**, Flow cytometry revealed that the percentage of Si-EC9706 cells was significantly lower in G1 and significantly higher in S stage compared to the respective percentages of EC9706 or Con-EC9706 cells. **B**, The proliferation index (PI) of Si-EC9706 cells was significantly higher than that of EC9706 and Con-EC9706 cells and was determined as  $PI = (S + G2)/(S + G2 + G1)$ . \*Statistical significance ( $p < 0.05$ , EC9707; Con-EC9706 cells vs. Si-EC9706 cells).

## Discussion

ESCC is one of the most aggressive cancers worldwide. Although certain biomarkers have been found to be involved in the progression of ESCC<sup>11,19</sup>, other potential biomarkers provide benefits to patients with ESCC. Regardless, it remains a great challenge for clinicians and basic scientists to determine the molecular biomarkers that are correlated with the malignant progression and prognosis of ESCC. To evaluate the clinician significance of EBP50, qPCR, western blotting, and immunohistochemical staining were used in the present study to detect the expression of EBP50 in 98 pairs of ESCCs and matched non-tumor tissues.

Both the qPCR and western blot analyses suggested that EBP50 expression in ESCC was significantly lower than in matched non-tumor tissues. Furthermore, immunohistochemical staining showed that EBP50 was mostly expressed in the apical membrane of normal esophageal epithelial cells, but not in the nucleus and/or cytoplasm, if at all, in ESCC tissues. The results of the unpaired two-tailed *t*-test showed that EBP50 expression was significantly decreased in ESCCs compared to matched non-tumor tissues, indicating that EBP50 expression at the apical membrane might play a critical role in the suppression of ESCC development. These findings are consistent with our previous study, which showed that EBP50 expression was significantly inhibit-

ed and might function as a potential tumor suppressor in pancreatic cancer<sup>13</sup>. However, the present results are partially consistent with the findings of a previous study, as the aberrant nuclear localization of EBP50 was still found to promote colorectal carcinogenesis<sup>4,20</sup>, indicating that the EBP50 subcellular localization might play an important role in the development of human cancers. Decreased EBP50 expression correlated with malignant indices, including differentiation, T stage, and LN metastasis, further suggesting that EBP50 might play a critical role in the malignant progression of human ESCC.

The EBP50 protein contains two tandem PDZ domains and a C-terminal ERM-binding domain, which contribute to its function as an adaptor linking membrane proteins to the underlying actin cytoskeleton<sup>21</sup>. EBP50 can target ion channels, GPCRs, and nuclear proteins depending on its different subcellular compartments and locations<sup>22</sup>. The over-expression and nuclear localization of EBP50 promoted cell growth in HCC cells, and increased EBP50 inhibited the transcriptional activity of  $\beta$ -catenin, indicating that EBP50 in the nucleus might induce an interaction with  $\beta$ -catenin and promote Wnt signaling<sup>9</sup>. Controversially, the over-expression of EBP50 significantly suppressed cellular proliferation and attenuated colony formation by inhibiting ERK activity in breast cancer cells<sup>21</sup>. EBP50 can directly inhibit cell motility and anchorage-independent growth in gene-deficient mouse embry-



onic fibroblasts through the interaction of the PDZ2 domain with  $\beta$ -catenin, which is required for  $\beta$ -catenin localization at cell-cell junctions. In addition, EBP50 is a potential tumor suppressor through its regulation of cyclin E and phosphorylated Rb expression<sup>2</sup>, and the over-expression of EBP50 increases 5-FU-induced apoptosis in gastric cancer cells through Bax and Bcl-2-triggered mitochondrial pathways<sup>3</sup>. Therefore, the functions of EBP50 might differ according to its sub-cellular location, which needs to be confirmed by further studies, such as immunofluorescence in ESCC tissues and cell lines.

The decreased expression of EBP50 in ESCC tissues correlated with malignant progression in patients, indicating that EBP50 expression might present a prognostic value for ESCC. A survival analysis revealed that the decreased expression of EBP50 also correlated with a significantly poorer prognosis compared to higher EBP50 expression, which can be explained by the finding that decreased EBP50 expression is associated with differentiation, T stage, and lymph node metastasis. In addition to the expression of EBP50, advanced T stage and lymph node metastasis also presented prognostic value in patients with ESCC. Furthermore, the multivariate analyses showed that T stage and EBP50 expression were two independent prognostic factors for these ESCC patients. These findings might benefit clinicians in their practice of individualized therapy for ESCC patients. In addition, the present study revealed that the down-regulation of EBP50 could significantly promote the growth and proliferation of EC9706 cells while accelerating the cell cycle progression from the G1 to S phase. These findings were in agreement with those of our previous study, which implicated EBP50 in the progression of human pancreatic cancer<sup>12</sup>. These data further support that EBP50 may influence the proliferation and growth of cancer cells.

## Conclusions

EBP50 expression was decreased in ESCCs and was correlated with tumor differentiation, advanced T stage, lymph node metastasis, and a poor prognosis. The down-regulation of EBP50 promoted the growth and proliferation of EC9706 cells and accelerated cell cycle progression from the G1 to S phase, indicating that EBP50 might be a candidate prognostic biomarker for ESCC.

## Acknowledgements

This study was supported by Fundamental Research Funds from the Central Universities (No. 20103020101000194).

## Conflict of Interest

The Authors declare that there are no conflicts of interest.

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