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The Expression and Prognostic Impact of the PI3K/AKT/mTOR Signaling Pathway in Advanced Esophageal Squamous Cell Carcinoma

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Abstract

The abnormal phosphatase and tensin homolog expression and activated phosphoinositide-3 kinase/Protein kinase B (AKT)/mammalian target of rapamycin signaling pathway are involved in the progression of esophageal squamous cell carcinoma. By assessing the expression pattern of key components in the phosphoinositide-3 kinase/AKT/mammalian target of rapamycin signaling pathway by immunohistochemistry in tumor and nontumor esophageal mucosa from patients with esophageal squamous cell carcinomas, we aimed to carefully explore the relationship between the various protein expressions and clinicopathological factors, as well as patient outcome. A total of 145 tumor and 145 nontumor samples from patients with esophageal squamous cell carcinoma, collected from HuaShan Hospital (Shanghai, China) were evaluated. Clinical characteristics, the targeted protein expressions (including phosphatase and tensin homolog, phosphoinositide-3 kinase, AKT, p-AKT, mammalian target of rapamycin, p-mTOR, p70S6 kinase 1, p-P70S6K1, elongation initiation factor 4E binding protein-1, and p-4E-BP1, and survival rate were analyzed. Among them, phosphoinositide-3 kinase, AKT, p-AKT, mammalian target of rapamycin, p-mTOR, elongation initiation factor 4E binding protein-1, p70S6 kinase 1, and p-P70S6K1 proteins were significantly upregulated in tumor tissue. Conversely, phosphatase and tensin homolog was largely downregulated in tumor tissue, notably in pT3-T4 tumors. Low expression of phosphatase and tensin homolog whereas high expression of mammalian target of rapamycin signaling components in tumors was closely related to the presence of lymph node metastases and advanced TNM stage (all $P < .05$). Moreover phosphatase and tensin homolog, mammalian target of rapamycin, and p70S6 kinase 1 were correlated with overall survival as well as p-mTOR was correlated with progression-free survival (all $P < .05$). Overexpression of mammalian target of rapamycin was proved to be an independent adverse prognostic factor for overall survival in esophageal squamous cell carcinomas. Our results suggest that the phosphoinositide-3 kinase/AKT/mammalian target of rapamycin signaling pathway is activated in esophageal squamous cell carcinoma, with the low expression of phosphatase and tensin homolog and the high expression of the mammalian target of rapamycin component proteins (both total and phosphorylated) in tumor tissue. Our result might offer a new strategy for specific targeted therapy and prognostic assessment in esophageal cancer.

Keywords

esophageal squamous cell carcinoma, PI3K/AKT/mTOR signaling pathway, PTEN, Prognosis

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Abbreviations

4E-BP1, elongation initiation factor 4E binding protein-1; ESCC, esophageal squamous cell carcinoma; IHC, immunohistochemistry; mTOR, mammalian target of rapamycin; p70S6K, p70S6 kinase; PET, positron emission tomography; PI3K, phosphoinositide-3 kinase; PTEN, phosphatase and tensin homolog.

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Introduction

Esophageal cancer is the eighth common cancer and the sixth leading cause of cancer mortality in the worldwide.[1] Esophageal squamous cell carcinoma (ESCC) is the dominant histological subtype in East Asian countries.[2] More than 250 000 new diagnosed esophageal cancer cases were reported in China per year, accounting for half of the world.[3] In recent years, although the development of multiple therapy approaches includes surgery, chemotherapy, and radiotherapy, the prognosis remains poor for patients with ESCC who undergo esophagectomy and lymph node dissections.[4,5] The limited improvement in outcomes achieved by these conventional therapies urges us to seek new strategies, especially the possibility on novel oncogene signaling pathways that affect the development of ESCC.

Encouragingly, genomics profiling studies of esophageal cancer have revealed that the phosphoinositide-3 kinase (PI3K)-AKT-mammalian target of rapamycin (mTOR) pathway is a key signal pathway involved in the regulation of diverse key cellular processes, which could stimulate cell proliferation, apoptosis, and migration.[6] Mammalian target of rapamycin is the central protein which interacts with several proteins to form 2 multiprotein complexes, known as mTOR complex 1 (mTORC1) and complex 2 (mTORC2).[7] Activation of mTOR regulates a number of its downstream effectors important in cellular growth, such as p70S6 kinase (p70S6K) and elongation initiation factor 4E binding protein-1 (4E-BP1), resulting in enhanced translation of subset of genes that are required for protein synthesis and cell growth, inhibition of cell apoptosis, and acceleration of cell proliferation, which finally lead to a tumorigenesis.[8-10] Phosphatase and tensin homolog (PTEN) is the primary negative regulator of PI3K/Akt/mTOR signaling pathway.

Previous studies suggested that the abnormal expression of PTEN and the activation of PI3K/AKT/mTOR signaling pathway were involvedin tumorigenesis and affect ESCC patient prognosis.[11,12] However, the limited number of key components of mTOR signaling pathway (less than 3) were examined in these previous studies. In this study, we aimed to investigate the expression level of most well-known components of proteins in the PI3K/AKT/mTOR signaling pathway including PTEN, PI3K, and both total and phosphorylated fraction of AKT, mTOR, P70S6K1, and 4E-BP1 and expect to reveal and pick up the various biomarker proteins correlated closely with clinicopathologic factors and patient prognosis.

Materials and Methods

Patients and Tissue Samples

We selected an ESCC tissue and a paired nontumor esophageal mucosa sample from 145 patients who had advanced ESCC and underwent esophagectomy and lymph node dissections at the department of cardiothoracic surgery of the Huashan Hospital during January 1, 2006, to December 31, 2008. Patients, who had palliative resections, underwent neoadjuvant or R1/R2 resections were excluded from the analysis. Written informed consent was obtained from all participants. The institutional review board of Huashan hospital, Fudan University, reviewed and approved the study.

Clinical Data and Follow-Up

Routine preoperative staging included fibrogastroscopy, computed tomography of the chest and abdomen, ultrasound imaging of the abdomen and neck, and positron emission tomography (PET)-computed tomography (CT) scanning. Preoperative nutritional risk assessment, cardiac function tests, and pulmonary function test were also performed in all patients to make sure they had the physiological ability to undergo esophagectomy.

Patients with esophageal cancer were staged according to the seventh edition American Joint Committee on Cancer (AJCC) staging system. The standard follow-up schedule contained the following: patients were followed every 3 months for 2 years, every 6 months for the next 3 years, and then annually. The outcome is all-cause mortality and defined as time from the data of surgery to the data of death. Routine postoperative tests contained fibrogastroscopy, ultrasound, CT/PET-CT, and radionuclide bone scan.

Reagents

Antibodies recognizing PTEN (9559S), mTOR, phospho-mTOR (Ser2448), PI3K P110a (C73F8), AKT, phospho-Akt (Thr308), p70S6K1, phospho-p70S6K1 (Thr389), p4E-BP1, phospho-p4E-BP1 (Thr70) were purchased from Cell Signaling Technology (Danvers, Massachusetts). All were rabbit polyclonal antibodies.

Immunohistochemistry

Was performed immunohistochemical staining using the rabbit or mouse DAKO ChemMate EnVision system and a Peroxidase/DAB kit (DAKO, Carpinteria, California). The sample containing paraffin was sliced into serial sections with a width of 5.0 μm. Each section was deparaffinized for 1 hour at 60°C in xylene and rehydrated in serial-graded ethanol before being stored overnight in citrate buffer (0.01 M; pH 6.0) at 75°C for antigen retrieval. Endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide in methanol. The sections were incubated at 4°C overnight with a primary antibody and then recovered at 37°C for 20 minutes. The slides were incubated with the secondary antibody for 30 minutes at room temperature and then were developed by DAB. Nuclear staining was carried out with hematoxylin. Phosphate-buffered saline was added as the primary antibody in the negative control. Normal esophageal tissue, serving as the negative controls, and esophageal cancer tissue (known to express PI3K/AKT/mTOR signaling pathway proteins) serving as positive controls were processed in the same way. However, normal esophageal tissue serves as the positive controls, and esophageal cancer tissue serves as negative controls for PTEN.

Evaluation of Immunohistochemistry staining

The results were interpreted by 2 independent pathologists who were blinded to the specific diagnosis and prognosis for each case. If there was disagreement, the final conclusion was reached through discussion. For each sample, at least 5 fields were chosen randomly and at least 500 cells were required for scoring in each field. Staining extent of immunostaining was scored according to the percentage of positive-stained cells as follows: 0, none; 1, up to 10% positive cells; 2, 10% to 50% positive cells; 3, 51% to 80% positive cells; and 4, positive cells > 80%. The intensity of staining was as follows: 0, none; 1, low intensity (light yellow); 2, moderate intensity (yellow); and 3, strong intensity (reddish brown). The score was calculated as grade in stain intensity × grade in coloration rate, which ranges from 0 to 12. The scores ≥4 were defined as positive overexpression. Low-expression group included negative expression or low-expression samples with overall score of 0 to 3. Both the esophageal cancer tissue and the normal esophageal tissue use the same scoring criteria.

Statistical Analysis

All statistical analysis was performed with SPSS 16.0. The associations between immunohistochemistry (IHC) expression and clinicopathological variables were examined using χ^2 test and Fisher exact test for categorical variables and the Student *t* test for continuous variables. The Kaplan-Meier survival curve and log-rank tests were performed to estimate the survival function across groups. Cox proportional hazard regression model for multivariate analysis was performed to identify the independent prognostic factors. The level of significance was set to $P < .05$.

Table 1. The Staining Scores of Proteins of the mTOR Signaling Pathway Expression in ESCC and Normal Esophageal Mucosa Tissues.

Factors	ESCC, N (%)	Normal Mucosa Tissue, N (%)	X^2	*P*
PTEN				
Low	84 (57.9)	42 (29.0)	24.756	.000
High	61 (42.1)	103 (71.0)		
PI3K				
Low	124 (85.5)	116 (93.8)	5.354	.021
High	21 (14.5)	9 (6.2)		
AKT				
Low	82 (56.6)	104 (71.7)	7.256	.007
High	63 (43.4)	41 (28.3)		
p-AKT				
Low	77 (53.1)	97 (66.9)	5.747	.017
High	68 (46.9)	48 (33.1)		
mTOR				
Low	74 (51.0)	91 (62.8)	4.064	.044
High	71 (49.0)	54 (37.2)		
p-mTOR				
Low	67 (46.2)	93 (64.1)	9.425	.002
High	78 (53.8)	52 (35.9)		
4E-BP1				
Low	87 (60.0)	105 (72.4)	4.994	.025
High	58 (40.0)	40 (27.6)	3.639	.056
p-4E-BP1				
Low	93 (64.1)	77 (53.1)		
High	52 (35.9)	68 (58.6)		
P70S6K1				
Low	77 (53.1)	100 (69.0)	7.670	.006
High	68 (46.9)	45 (31.0)		
p-P70S6K1				
Low	86 (59.3)	104 (71.7)	4.945	.026
High	59 (40.7)	41 (28.3)		

Abbreviations: ESCC, esophageal squamous cell carcinoma; 4E-BP1, elongation initiation factor 4E binding protein-1; mTOR, mammalian target of rapamycin; P70S6K1, P70S6 kinase 1; PI3K, phosphoinositide-3 kinase; PTEN, phosphatase and tensin homolog.

Results

The Expression of the PI3K/AKT/mTOR Signaling Pathway Proteins in ESCC and Normal Esophageal Mucosa Tissues

The expression of the PI3K/AKT/mTOR signaling pathway proteins were detected by IHC in the study. The proteins investigated were PTEN, PI3K, AKT, p-AKT, mTOR, p-mTOR, P70S6K1, p-P70S6K1, 4E-BP1, and p-4E-BP1. The staining intensity of upregulated and downregulated proteins is shown in Table 1. The significantly upregulated proteins in ESCC were PI3K ($x^2 = 5.354$, $P = .021$), AKT ($x^2 = 7.256$, $P = .007$), p-AKT ($x^2 = 5.747$, $P = .017$), mTOR ($x^2 = 4.064$, $P = .044$; Figure 1), p-mTOR ($x2 = 9.425$, $P = .002$), 4E-BP1 ($x^2 = 4.994$, $P = .025$), P70S6K1 ($x^2 = 7.670$, $P = .006$), and

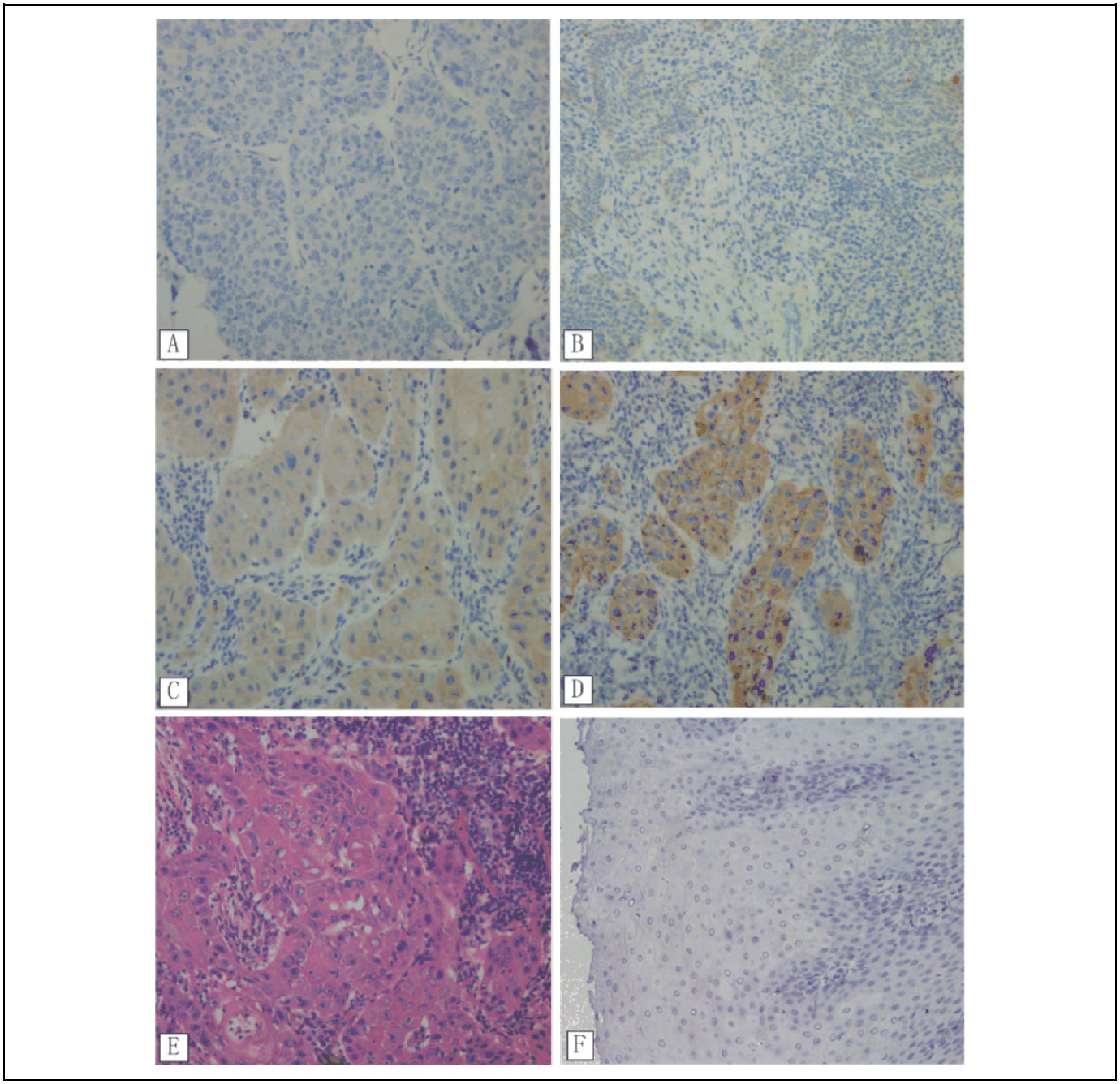

Figure 1. Immunohistochemistry analysis of mTOR expression in ESCC tissues. (A) Nearly negative expression, (B) low expression, (C) moderate expression, (D) high expression, (E) Hematoxylin and Eosin (H&E) staining of ESCC tissue, and (F) mTOR negative expression in normal esophageal mucosa tissues; all figures ×200. ESCC denotes esophageal squamous cell carcinoma; mTOR, mammalian target of rapamycin.

p-P70S6K1 ($x^2 = 4.945$, $P = .026$). In contrast, the expression of PTEN was largely downregulated in tumor tissues ($x^2 = 24.756$, $P < .001$).

Patients' Clinical Characteristics, Survival Analysis, and Subgroup Analysis

The clinical characteristics of the 145 patients are summarized in Table 2. There were 111 men and 34 women with the median age of 59 years (range 36-81 years). The median tumor length was 4.0 cm (ranged 0.4-9.0 cm). The 5-year overall and progression-free survival rates of these 145 patients were 36.1% and 31.4%, respectively.

Correlations of the clinical characteristics and the PI3K/AKT/mTOR signaling pathway protein expression levels with overall survival and progression-free survival are shown in Table 2. Univariate analysis demonstrated the prognostic factors for overall survival (OS) were tumor length ($P = .002$),

Table 2. Patient Characteristics and Univariate Analysis.

		OS		PFS	
Factors	N	5-Year OS (%)	*P*	5-Year PFS (%)	*P*
Age, year					
≤60	78	46.2	.102	36.8	.140
>60	67	33.2		25.9	
Sex					
Male	111	32.8	.165	27.9	.240
Female	34	48.0		44.1	
Tumor length					
<3 cm	37	53.5	**.002**	61.5	**.002**
≥3 cm	108	30.0		23.9	
Differentiation					
Well	17	40.3	**.006**	38.6	**.032**
Moderately	90	39.4		30.7	
Poorly	38	24.0		24.7	
T stage					
T1	13	82.5	**.000**	72.9	**.001**
T2	34	48.9		37.2	
T3	64	31.7		28.5	
T4	34	18.8		19.1	
N stage					
N0	55	47.4	**.000**	52.1	**.000**
N1	55	33.5		32.5	
N2	30	5.3		5.8	
N3	5	0		0	
PTEN					
Low	84	29.7	**.044**	24.2	.140
High	61	45.7		43.7	
PI3K					
Low	124	34.4	.932	31.5	.800
High	21	45.5		35.1	
AKT					
Low	82	38.2	.250	31.5	.168
High	63	33.3		30.6	
p-AKT					
Low	77	39.1	.064	34.5	.072
High	68	32.8		27.8	
mTOR					
Low	74	46.3	**.011**	39.2	**.010**
High	71	25.6		23.2	
p-mTOR					
Low	67	47.6	.053	49.2	**.015**
High	78	28.9		21.3	
4E-BP1					
Low	87	41.3	.074	33.1	.159
High	58	28.5		27.9	
p-4E-BP1					
Low	93	35.7	.373	37.8	.279
High	52	35.8		24.9	
P70S6K1					
Low	77	43.3	**.009**	40.7	**.007**
High	68	27.8		21.4	
p-P70S6K1					
Low	86	41.7	.106	38.4	.171
High	59	27.5		21.7	

Abbreviations: 4E-BP1, elongation initiation factor 4E binding protein-1; mTOR, mammalian target of rapamycin; OS, overall survival; P70S6K1, P70S6 kinase 1; PFS, progression-free survival; PI3K, phosphoinositide-3 kinase; PTEN, phosphatase and tensin homolog. Bold values mean significant difference.

differentiation ($P = .006$), T-stage ($P < .001$), N-stage ($P < .001$), PTEN expression ($P = .044$), mTOR expression ($P = .011$), and P70S6K1 expression ($P = .009$). The prognostic factors of the PI3K/AKT/mTOR pathway proteins for progression-free survival were mTOR expression ($P = .010$), p-mTOR expression ($P = .015$), and P70S6K1 expression ($P = .007$). Kaplan-Meier survival curves are shown in Figure 2.

The multivariate analyses indicated differentiation, T-stage, N-stage, and mTOR expression (hazard ratio = 1.662; 95% confidence interval: 1.030-2.681; $P = .037$) were independently associated with overall survival (Table 3). For progression-free survival, none of these pathway proteins had proved to be an independent prognostic factor.

In order to evaluate the impact of prognostic protein biomarkers expressions (PTEN, mTOR, p-mTOR, and P70S6K1) on different tumor stages, we divided patients into subgroups on the basis of N-stage (N0 vs N1-3). In the subgroup of N0, patients with high expression of mTOR ($X^2 = 12.354$, $P < .001$) and p-mTOR ($X^2 = 4.549$, $P = .033$) had significantly worse OS, but no statistical significance was found in the subgroup of N1 to N3. And there were no statistical significance of PTEN and P70S6K1 expressions in each subgroup.

Relationship Between Clinical Characteristics and the Expression of PI3K/AKT/mTOR Signaling Pathway Proteins

It was interesting to explore the relationship between clinical characteristics and the prognostic protein biomarkers of the PI3K/AKT/mTOR signaling pathway (Table 4). We found a low expression of PTEN ($P = .001$) in pT3 and pT4 tumors, whereas the high expression of P70S6K1 ($P = .003$) in pT3 and pT4 tumors. Patients with a high expression of p-mTOR ($P = .008$) or P70S6K1 ($P = .005$) tended to have tumors of more than 3 cm in maximum diameter. The high expression of mTOR ($P = .026$), p-mTOR ($P = .002$), and P70S6K1 ($P < .001$) were related to lymph node metastases. Consistently low expression of PTEN ($P = .003$) was related to lymph node metastases in ESCC. Especially, the low expression of PTEN ($P = .017$) or the high expression of mTOR ($P = .022$), p-mTOR ($P = .010$), and P70S6K1 ($P = .028$) were correlated with advanced TNM stage.

The presence of PTEN and other PI3K/AKT/mTOR signaling pathway proteins had significant differences. There were significant linear relations between proteins and their phosphorylated forms. Phosphatase and tensin homolog expression was negative associated with p-AKT ($P < .001$), P70S6K1 ($P < .001$), and p-P70S6K1 ($P = .019$). Mammalian target of rapamycin expression was positive associated with p-AKT ($P = .036$) and p-mTOR expression ($P < .001$). P70S6K1 expression had a positive correlation with p-P70S6K1 ($P < .001$), p-AKT ($P < .001$), and p-4E-BP1 ($P < .001$) expression, as shown in Table 3.

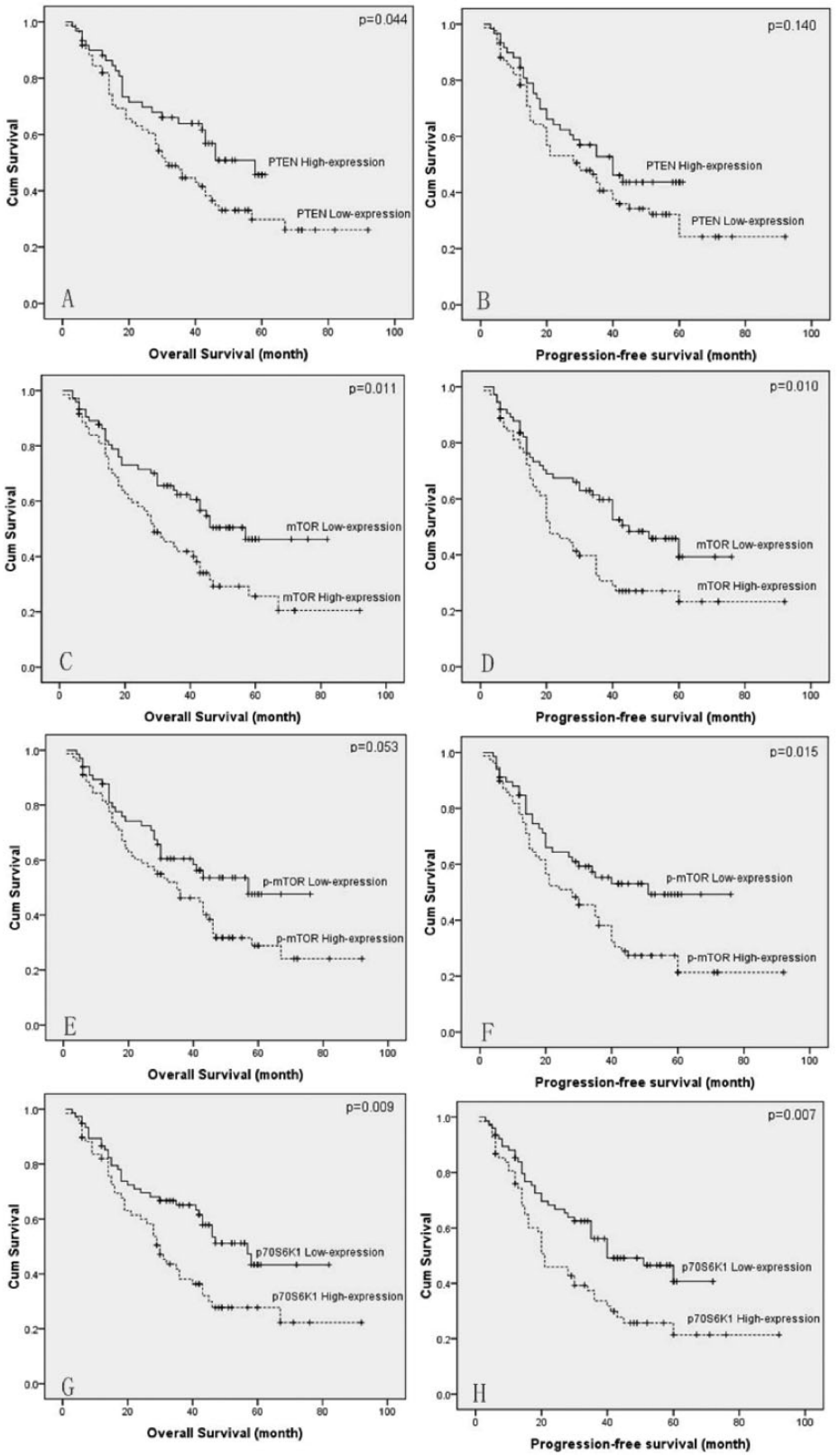

Figure 2. Kaplan-Meier survival curve of patients with advanced ESCC after radical esophageal resection. Overall survival of patients with PTEN expression (A), mTOR expression (C), p-mTOR expression (E), and p70s6k1 expression (G); progression-free survival of patients with PTEN expression (B), mTOR expression (D), p-mTOR expression (F), and p70s6k1 expression (H). ESCC denotes esophageal squamous cell carcinoma; mTOR, mammalian target of rapamycin; PTEN, phosphatase and tensin homolog

Table 3. Multivariate Prognostic Analyses of Overall Survival.

Prognostic Factors	*P* values	HR	95% CI
Tumor length	.348	1.388	0.701-2.749
Differentiation	.042	1.519	1.015-2.274
T-stage	.017	1.489	1.074-2.605
N-stage	.009	1.523	1.109-2.092
PTEN	.585	0.824	0.412-1.649
mTOR	.037	1.662	1.030-2.681
P70S6K1	.566	1.200	0.644-2.237

Abbreviations: CI, confidence interval; HR, hazard ratio; mTOR, mammalian target of rapamycin; P70S6K1, P70S6 kinase 1; PTEN, phosphatase and tensin homolog.

Discussion

The PI3K/AKT/mTOR signaling pathway plays a crucial role in the regulation of multiple cellular functions including cell growth, proliferation, and angiogenesis in numerous solid tumors.[13,14] Particularly, activation of this pathway has been linked to various prognostic clinicopathological parameters, such as the stage of tumor, the degree of tumor differentiation, tumor size, nodal involvement, distant metastasis, and chemosensitivity, which could result a poor survival in gastrointestinal tumors, such as gastric cancer, colon cancer, and so on.[15-16] Previous studies have suggested the activation of PI3K/AKT/mTOR signaling pathway in ESCC specimens and revealed the PI3K pathway activation contributes to the proliferation and survival of esophageal cancer, and mTOR is a key downstream protein kinase of PI3K/AKT signaling pathway.[17-20] For example, Hirashima *et al* examined 167 patients with ESCC and found that 116 (69.5%) patients had the overexpression of p-mTOR. Suppression of p-mTOR could inhibit proliferation and invasion and induce apoptosis in ESCC.[21] Lu *et al* found 94 (63.5%) of 148 patients have high expression of mTOR. Instead, 102 (69.9%) of the 148 patients have low expression of PTEN in ESCC and suggested that mTOR was an independent prognostic factor by multivariate survival analysis.[22] Hou *et al* demonstrated that the expression of mTOR has important clinical significance and inhibition of mTOR pathway by mTOR siRNA can improve the chemotherapy sensitivity of ESCC cells.[23] Although these studies revealed the importance of PI3K/AKT/mTOR signaling pathway in the dissemination of esophageal cancer, the main mTOR signaling components were not included, retaining the question of the significance of mTOR signaling in esophageal cancer. Thus, we systematically determined the expression level of the key proteins in PI3K/AKT/mTOR signaling pathway.

In our study, statistically significant differences were observed for 9 of the 10 proteins investigated. Compared to nontumor tissues, PI3K, AKT, p-AKT, mTOR, p-mTOR, 4E-BP1, P70S6K1, and p-P70S6K1 were all upregulated in tumor tissues. Conversely, PTEN was found to be downregulated in ESCC tumor tissue. Our results, as well as the previous studies, confirmed that the PI3K/AKT/mTOR signaling pathway plays a key role in the process of ESCC carcinogenesis. Moreover, our study strongly suggested that the main components of PI3K/AKT/mTOR signaling pathway proteins are all involved in the process of oncogenesis.

Our study also found the low expression of PTEN was closely related to advanced T-stage, the presence of lymph node metastases, and advanced TNM stage. Phosphatase and tensin homolog is a tumor suppressor and the previous studies have demonstrated its value as an important marker of good prognosis in some gastrointestinal solid tumors, which contains ESCC.[24-26] In our study, we found that PTEN is a major negative regulator of the PI3K/AKT/mTOR signaling pathway. The loss of PTEN expression was inversely correlated with the accumulated expression of the majority of the mTOR signaling pathway proteins. Patients with the loss of PTEN expression had significantly worse overall survival.

In contrast to PTEN that acts as a suppressor of this signaling pathway, activation of mTOR could increase growth signals and increase protein synthesis through the phosphorylation and inactivation of 4E-BP1 and P70S6K1. And both the total and phosphorylated proteins had important clinical implications. Our study found patients with a high expression of p-mTOR or P70S6K1 tended to have tumors of more than 3 cm in maximum diameter. Meanwhile, the overexpression of mTOR, p-mTOR, and P70S6K1 in tumors was closely related to the presence of lymph node metastases, advanced TNM stage. p-mTOR expression was correlated with progression-free survival as well as mTOR expression was correlated with both progression-free survival and OS. The above results indicated that the PI3K/AKT/mTOR signaling pathway played a key role in promoting esophageal cancer and might offer new therapeutic avenues for esophageal cancer.

In the future, it will be valuable to increase the sample size to firmly confirm our observation and build up the solid correlation between PI3K/AKT/mTOR signaling and ESCC tumors. Since we only examined the expression levels of the PI3K/AKT/mTOR signaling components were by the IHC, it will be of great interest to further investigate the related gene alterations, transcriptions, and mutations. Finally, by combination of the mTOR signaling specific inhibitors, it will be possible to identify the precise signaling pathway that is involved in the pathogenesis of ESCC and its value for optimizing individual molecular target therapy.

Conclusions

In summary, our study demonstrated that most advanced ESCC tumors showed the activated PI3K/AKT/mTOR signaling pathway with the low expression of PTEN but accumulated expression of the majority of the mTOR signaling pathway proteins (both total and phosphorylated). The level of expression of PTEN, mTOR, p-mTOR, and P70S6K1 were closely related to the presence of lymph node metastases. The expression of PTEN, mTOR, and P70S6K1 were correlated to the TNM stage and overall survival. Therefore, the PI3K/AKT/mTOR signaling pathway had potential value for both the prognostic marker and therapy of ESCCs.

Table 4. Associations Between Clinical Characteristics and the Expression of mTOR Signaling Pathway Proteins.

		PTEN Expression			mTOR Expression			p-mTOR Expression			P70S6K1 Expression		
Factors	N	Low	High	*P*	Low	High	*P*	Low	High	*P*	Low	High	*P*
Age, years				.198			.788			.989			.888
≤60	78	49	29		39	39		36	42		41	37	
>60	67	35	32		35	32		31	36		36	31	
Sex				.360			.518			**.025**			.420
Male	111	62	49		55	56		57	54		61	50	
Female	34	22	12		19	15		10	24		16	18	
Tumor length				.185			.447			**.008**			**.005**
<3 cm	37	18	19		23	14		24	13		27	10	
≥3 cm	108	66	42		51	57		43	65		50	58	
Differentiation				.100			.282			.059			.160
Well	17	9	8		10	7		9	8		12	5	
Moderately	90	54	36		52	38		47	43		44	46	
Poorly	38	21	17		12	26		11	27		21	17	
T stage				**.001**			.308			.422			**.003**
T1	13	4	9		9	4		8	5		9	4	
T2	34	12	22		16	18		18	16		23	11	
T3	64	42	22		35	29		26	38		40	24	
T4	34	26	8		14	20		15	19		10	24	
N stage				**.003**			**.026**			**.002**			**.000**
N0	55	24	31		35	20		36	19		39	16	
N1	55	31	24		28	27		22	33		31	24	
N2	30	25	5		9	21		8	22		4	26	
N3	5	4	1		2	3		1	4		3	2	
TNM stage				**.017**			**.022**			**.010**			**.028**
I	12	3	9		9	3		8	4		10	2	
II	53	28	25		32	21		31	22		31	22	
III + IV	80	53	27		33	47		28	52		36	44	
PTEN							.085						**.000**
Low	84	–	–	–	48	36		–	–	–	25	59	
High	61	–	–		26	35		–	–		52	9	
p-AKT				**.000**			**.036**			.071			**.000**
Low	77	36	41		33	44		41	36		52	25	
High	68	48	20		41	27		26	42		25	43	
p-mTOR				.544			**.000**						.601
Low	67	37	30		45	22		–	–	–	34	33	
High	78	47	31		29	49		–	–		43	35	
p-4E-BP1				.088			.056			.992			**.000**
Low	93	49	44		53	40		43	50		61	32	
High	52	35	17		21	31		24	28		16	36	
p-P70S6K1				**.019**			.167			.272			**.000**
Low	86	43	43		48	38		43	43		57	29	
High	59	41	18		26	33		24	35		20	39	

Abbreviations: mTOR, mammalian target of rapamycin; P70S6K1, P70S6 kinase 1; PTEN, phosphatase and tensin homolog. Bold values mean significant difference.

Authors' Note

Written informed consent was obtained from all individual participated included in the study. Ning Wu and Zunguo Du were co-first authors.

Declaration of Conflicting Interests

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