HPV Infection and its Relationship with Expression of PIK3CA, PIK3CB in Esophageal Squamous Cells Carcinoma

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Research Article

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Abstract

Background: Esophageal squamous cell carcinoma (ESCC) is a highly lethal cancer. Human papillomavirus (HPV) is currently considered as a potential risk factor for ESCC, but this assumption is still contradictory. P16INK4a (p16) staining can be used as an alternative marker of HPV oncogene activity. This study was to investigate the role of HPV in esophageal cancer and to compare the relationship between HPV status and PIK3CA, PIK3CB.

Methods: Immunohistochemistry, Western blot, and RT-PCR analyses were performed to detect the expression of p16, PIK3CA, PIK3CB and p53 in 156 cases of esophageal squamous cell carcinoma. The patients were followed up by telephone or clinic.

Results: The positive rates of p16, PIK3CA, PIK3CB and p53 in esophageal squamous cell carcinoma were significantly higher than those in normal esophageal mucosa. The overexpression of p16 was closely related to tumor location, TNM stage, differentiation and lymph node metastasis. In addition, the expression of p16 was positively correlated with the expression of PIK3CA, but not with the expression of PIK3CB and p53. Survival analysis showed that p16 was a good prognostic marker, while PIK3CA and p53 were poor prognostic markers.

Conclusions: HPV infection is associated with ESCC. The imbalance of PI3K/Akt signaling pathway is closely related to HPV infection and prognosis. Detection of p16 and PIK3CA is of great significance in evaluating the prognosis and optimizing the treatment of esophageal squamous cell carcinoma.

Background

Esophageal cancer (EC) represents the eighth highest incidence of cancer worldwide (456,000 cases/year) and is usually diagnosed at an advanced stage, showing rapid progression and extremely poor prognosis [1, 2]. The two main histological types of EC are squamous cell carcinoma and adenocarcinoma and 90% of cases are esophageal squamous cell carcinoma (ESCC). Despite advances in medical technology, such as early screening and surgery combined with chemotherapy or radiotherapy, the prognosis of EC is still very poor [3]. Therefore, elucidating the underlying pathogenesis of EC and identifying effective biological agents are urgently needed.

Viral infections, such as Epstein Barr virus (EB virus), nasopharyngeal carcinoma, and Burkitt's lymphoma, are closely related to tumorigenesis. Studies confirmed that HPV is a risk factor for the occurrence and development of multiple malignant tumors. In particular, HPV16 and HPV18 infections are closely related to the occurrence of multiple tumors [4, 5]. For example, cervical cancer is closely related to HPV infection. The detection of HPV infection is currently used as an important indicator for the diagnosis of cervical intraepithelial neoplasia and the judgement of cancerization [6]. HPV infection is closely related to ESCC, but its tumorigenic mechanism is not clear [7].

Persistent HPV infection upregulates the expression of oncoproteins, especially E6 and E7. Both proteins lead to genetic instability through the inactivation of p53 and pRB, which is a negative regulator of cyclin-dependent kinase inhibitor p16INK4a (p16). The inactivation of pRB results in the upregulation of p16 [8]. Therefore, p16 is usually used as an alternative biomarker for HPV infection. In more than 90% of HPV-positive oropharyngeal cancer, p16 was overexpressed. The immunohistochemical evidence of p16 overexpression has been widely used to establish the correlation between HPV carcinogenic activity in cervical cancer and dysplasia [9–10]. Several studies have shown that the immunohistochemical detection of p16 overexpression may serve as a surrogate marker of functionally relevant HPV infection [10–11]. This detection is simple, fast, and inexpensive. In our study, p16 expression was detected by immunohistochemistry instead of by HPV detection.

Abnormal signal transduction pathway is one of the important mechanisms underlying tumorigenesis and tumor development and has become a popular research topic in the fields of tumorigenesis, target therapy, and drug resistance. The roles of viral infection in cell signaling pathway and oncogene activation are also popular topics in viral tumorigenic mechanism research. HPV infection can activate the PI3K/AKT signal transduction pathway, upregulate PIK3CA and PIK3CB expression levels, and worsen tumors [12]. The relationship between the PI3K/AKT pathway and tumorigenesis has attracted much attention in recent years [13]. The oncogene PIK3CA is a key molecule with high mutation rate in the PI3K/AKT pathway. PIK3CA mutation exists in 30% of solid tumors, is related to tumorigenesis, and affects targeted therapy [14–16]. Few studies have investigated PIK3CB. In-depth studies on the correlation between HPV infection and PIK3CA and PIK3CB mutations in EC and their effects on the occurrence, development, and prognosis of EC are also still lacking.

Therefore, studying the correlation between HPV infection and PIK3CA and PIK3CB mutations in EC is important for the detection of high-risk factors, judgment of prognosis, and prevention and treatment of EC.

Materials And Methods

Clinical specimens and patients' data

A total of 156 ESCC tissue samples at different stages were collected from the Department of Pathology of Binzhou Medical University Hospital between January 2011 and May 2013. Tissue samples were routinely fixed in 10% buffered neutral formalin. Cancer tissues were cut into wedge shapes, whereas normal tissues were cut at least 5 cm away from the tumor margin. All ESCC patients were clinically and pathologically proven to have not received preoperative chemotherapy or radiotherapy. All specimens were collected with the informed consent of the patients, and the Ethical Committee of Binzhou Medical University Hospital approved the protocols used in this study. Clinicopathologic classification was performed according to the National Comprehensive Cancer Network classification parameters. Demographic and clinicopathological parameters were prospectively recorded using a chart review. Patients were followed-up annually by telephone or at outpatient clinics until July 2020 or death.

Immunohistochemistry and scoring

Before staining, paraffin-embedded tissue blocks were cut into 4 μm -thick sections. The sections were deparaffinized in an oven at 60 °C for 2 h and then rehydrated with two and three changes of xylene and ethanol, respectively. Antigen retrieval was performed using the microwave retrieval method. Endogenous peroxidase activity was quenched through incubation with 3% hydrogen peroxide for 10 min at room temperature. Nonspecific binding was blocked by incubating the sections with 10% normal goat serum in PBS for 30 min at room temperature. Without washing, the sections were incubated with rabbit monoclonal antibody against human PIK3CA (1:150, Abcam, Cambridge, MA, USA), PIK3CB (1:250, Abcam, Cambridge, MA, USA), p16 (Fuzhou Maixin Biotech Co.), and p53 (Fuzhou Maixin Biotech Co.) at 4 °C overnight. The sections were then incubated with horseradish peroxidase-conjugated secondary goat anti-rabbit antibody (Abcam, San Francisco, USA) for 1h at room temperature. They were washed with PBS and treated with the metal-enhanced DAB substrate kit (Thermo Scientific, USA) to visualize the antigen–antibody complex. Two researchers who were unaware of the clinicopathological status of the specimens, scored each section separately. The percentage of stained cells on each section was scored as follows: 0 (less than 5%), 1 (5%–25%), 2 (26%–50%), and 3 (> 50%). Staining intensity was scored as follows: 0 (no staining), 1 (weak staining), 2 (moderate staining), and 3 (strong staining). The final score of each specimen was calculated by the multiplying stained cell score with the staining intensity score. Thus, the final score ranged from 0 to 9. Low expression was defined as a final score of <4, whereas a score ≥ 4 was determined to be high expression.

Western blotting analysis

Tissue samples were homogenized in SDS buffer containing the protease inhibitor PMSF. The homogenate on ice for 20 min, then at 4 °C for 30 minutes was centrifuged at 12000 rpm. Collect the supernatant and add a similar volume of 2 $\times$ SDS buffer. Boil the mixture for 10 minutes and store at -20°C . The protein extract (50 μg) was separated by SDS-PAGE, and then transferred to a polyvinylidene fluoride membrane (Millipore, USA). The membrane was blocked with 5% skim milk in Tris buffered saline containing 0.1% Tween-20 for 90 minutes at room temperature. Then immunoblotting was used to detect PIK3CA (1:1000, Abcam), PIK3CB (1:1000, Abcam), p16 (1:500, Abcam), p53 (1:500, Abcam) and β -actin (1: 1000, Abcam). Use horseradish peroxidase secondary antibody (1:5000, Abcam) to detect protein bands and display them with enhanced chemiluminescence reagents. Each band is quantified by densitometry, and the results are expressed in terms of the relative expression of each protein in different samples.

Real-time PCR analysis

Trizol reagent (Invitrogen, China) was used to extract total RNA from fresh frozen tumor specimens and corresponding non-cancerous tissues. The content of RNA was measured by the absorbance at 260nm, and the purity was estimated by the ratio of absorbance at A260/280. Total RNA was reverse transcribed using PrimeScript RT kit (Takara, DRR037A, China). The real-time PCR reaction was performed on the CFX96TM real-time PCR detection system C1000 (Applied Biosystems, China). For real-time PCR

reactions based on SYBR GREEN I, each 25μL reaction mixture contains 2μL primer pairs, 2μL cDNA, 12.5μL SYBR Premix Ex Taq II and 8.5μL ddH₂O to obtain a final volume of 25μL.

Statistical analysis

All statistical analyses were conducted with the SPSS 22.0 software (SPSS Inc., Chicago, USA). The expression of protein and mRNA was expressed by mean ± standard deviation. The comparison between the two groups was performed by independent sample t test. The correlation of p16 with PIK3CA, PIK3CB, p53 expression and clinical parameters was analyzed using X and Fisher’s exact tests. Overall survival (OS) was plotted using the Kaplan-Meier method. The analyses of prognostic factors for OS were determined using multivariate Cox proportional hazards regression method. *P* value ≤0.05 was considered statistically significant.

Results

Expression levels of p16, PIK3CA, PIK3CB and p53 in ESCC samples

Immunohistochemistry was used in human ESCC and matched adjacent non-tumor tissues to explore the expression levels of p16, PIK3CA, PIK3CB and p53. The immunohistochemical results are shown in Fig. 1. The expression levels of p16, PIK3CA and PIK3CB and p53 were positive in the cytoplasm, nuclei, and cytoplasm and nuclei of the ESCC samples, respectively. The positive ratios of p16, PIK3CA, PIK3CB, p53 and were 30.8%, 20.50%, 15.4% and 65.4%, respectively. As shown in Table 1, p16, PIK3CA, PIK3CB and p53 expression levels were significantly higher in the ESCC samples than in the non-tumor tissues (*P*< 0.05). In addition, Western blot analysis and real-time PCR results showed that p16, PIK3CA, PIK3CB and p53 expression was significantly higher in the CRC samples than in the non-tumor tissues (*P*< 0.05) (Fig. 2).

Table 1
Expression of p16, PIK3CA, PIK3CB and p53 protein in esophageal squamous cell carcinoma and normal mucosa

group	Normal mucosa	Cancer	<i>P</i> value
p16	0(0/156)	30.8(48/156)	0.001*
PIK3CA	2.5(4/156)	20.5(32/156)	0.001*
PIK3CB	1.3(2/156)	15.4(24/156)	0.001*
p53	1.9(3/156)	65.4 (102/156)	0.000*

*indicate statistically significant ($p < 0.05$)

Correlation of p16 with PIK3CA, PIK3CB, p53 and different clinicopathologic parameters

The correlation between p16 expression levels of the ESCC samples and a set of clinicopathologic parameters, including age, gender, tumor location, TNM stage, differentiation grade, and lymph node metastasis was analyzed (Table 2). The expression of p16 was significantly correlated with esophageal location, differentiation grade, TNM stage, and lymph node metastasis. The expression rate of p16 in the cervical/upper part (64.71%) of the esophagus was significantly higher than that in the middle (30.36%) and low (24.10%) parts. In addition, p16 expression was higher in well-differentiated ESCC (43.14%) than in moderately and poorly differentiated ESCCs (27.40% and 18.75%, respectively). The p16 expression levels in stages I and II (37.5%) were higher than those in stages III and IV (20%). The expression of p16 was lower in the presence of lymph node metastasis (20.97%) than in its absence (37.23%). PIK3CA expression was positively correlated with p16 ($r_s = 0.175$, $P = 0.027$). However, PIK3CB and p53 expression levels were not correlated with p16 expression.

Table 2
Epidemiological data and statistical analysis of the correlation between the positivity of p16 and the different data analyzed

Parameters	p16 expression		Total	r value	P value
	Positive	Negative	N = 156		
	N = 48	N = 108			
Age (years)					
< 60	20(42%)	33(31%)	53(34%)	0.108	0.176
≥ 60	28(58%)	75(69%)	103(66%)		
Gender					
Male	29(60%)	81(75%)	110(71%)	0.146	0.086
Female	19(40%)	27(25%)	46(29%)		
Tumor location					
Cervical/Upper	11(23%)	6(5%)	17(11%)	0.256	0.004*
Middle	17(35%)	39(36%)	56(36%)		
Low	20(42%)	63(59)	83(53%)		
TNM stage					
I-II	36(75%)	60(56%)	96(62%)	0.169	0.047*
III-IV	12(25%)	48(44%)	60(38%)		
Differentiation grade					
Well	22(46%)	29(27%)	51(33%)	-0.196	0.045*
Moderate	20(41.5%)	53(49%)	73(47%)		
Poor	6(12.5)	26(24%)	32(20%)		
Depth of invasion					
Mucosa and submucosa	10(21%)	16(15%)	26(17%)	0.123	0.303
Myometrium	15(31%)	26(24%)	41(26%)		
Adventitia	23(48%)	66(61%)	89(57%)		
Lymph node metastasis					
Yes	13(27%)	49(45%)	62(40%)	-0.170	0.035*
No	35(73%)	59(55%)	94(60%)		

Parameters	p16 expression		Total	r value	P value
	Positive	Negative	N = 156		
	N = 48	N = 108			
PIK3CA					
Positive	15(31%)	17(16%)	32(21%)	0.175	0.027*
Negative	33(69%)	91(84%)	124(79%)		
PIK3CB					
Positive	11(23%)	13(12%)	24(15%)	0.138	0.138
Negative	37(77%)	95(88%)	132(85%)		
P53					
Positive	30(62.5%)	72(67%)	102(65%)	0.040	0.614
Negative	18(37.5%)	36(33%)	54(35%)		

*indicate statistically significant ($p < 0.05$)

Correlation between PIK3CA, PIK3CB, p53 and p16 expression levels and patient OS

The median survival time of 156 patients was 44 months, and their 5-year survival rate was 28.2%. The 5-year survival rates of patients with positive and negative p16 expression levels were 39.6% and 23.1%, respectively ($P < 0.05$, Fig. 3B). Kaplan–Meier analysis (Fig. 3) revealed that p16, PIK3CA, p53, depth of invasion, TNM stage, and lymph node metastasis could be relevant predictive factors of OS. The 5-year survival rates of patients with positive and negative p16 expression levels were 39.6% and 23.1%, respectively ($P < 0.01$, Fig. 3A). The 5-year survival rates of patients with positive and negative PIK3CA expression levels were 18.7% and 30.6%, respectively ($P < 0.05$, Fig. 3B). The 5-year survival rates of patients with positive and negative p53 expression levels were 23.5% and 37.1%, respectively ($P < 0.05$, Fig. 3D). Thus, p16-positive ESCC was a distinct entity with a favorable prognosis compared with p16-negative ESCC. PIK3CA and p53 were markers of poor prognosis in ESCC. Cox regression analysis showed that p16 and PIK3CA expression levels, differentiation grade, and depth of invasion were independent prognostic factors of ESCC (Table 3).

Table 3
Univariate and multivariate analysis of clinicopathologic parameters with OS by Cox proportional hazards regression

Parameters	β	SE	Wald	Exp(β)	95%CI		<i>P</i> value
					Lower	Upper	
Age (< 60, $\geq$ 60)	0.219	0.198	1.221	1.245	0.844	1.837	0.269
Gender (male, female)	0.325	0.208	2.453	1.384	0.922	2.079	0.117
Tumor location			3.026				0.220
Tumor location (Cervical/Upper, Middle)	0.307	0.345	0.790	0.736	0.374	1.447	0.374
Tumor location (Middle, Lower)	0.319	0.198	2.590	0.727	0.493	1.072	0.108
Lymph node metastasis (yes, no)	1.194	1.073	1.237	0.303	0.037	2.484	0.266
Differentiation grade			6.747				0.034*
Differentiation grade (Well, Moderate)	0.101	0.289	0.123	0.903	0.513	1.592	0.725
Differentiation grade (Moderate, Poor)	0.539	0.235	5.271	0.583	0.368	0.924	0.022*
Depth of invasion			12.811				0.002*
Depth of invasion (Mucosa and submucosa, Myometrium)	0.959	0.289	10.997	0.383	0.217	0.675	0.001*
Depth of invasion (Myometrium, Epicardial)	0.535	0.237	5.091	0.586	0.368	0.932	0.024*
TNM stage (I-II, III-IV)	0.791	1.071	.546	2.207	0.271	18.000	0.460
p16	-0.524	0.251	4.371	0.592	0.362	0.968	0.037*
PIK3CA	0.796	0.239	11.107	2.217	1.388	3.542	0.001*
PIK3CB	0.340	0.255	1.779	1.405	0.852	2.315	0.182
p53	0.266	0.207	1.642	1.304	0.869	1.958	0.200

*indicate statistically significant ($p < 0.05$)

Discussion

EC is a tumor with high invasive ability. It is usually diagnosed in the late stage due to the lack of specific initial symptoms. For late-stage EC, the main treatment is surgery combined with chemotherapy [17, 18].

However, the cure and survival rates of patients are still very low, and other methods are needed to help predict the survival rate and identify potential responders of a given treatment.

HPV is a kind of DNA virus with small size and no envelope. HPV DNA is often dissociated from chromosome. In malignant tumor, HPV DNA is integrated into host cell DNA, resulting in the loss of negative regulation of HPV E2 gene on HPV E6 and HPV E7 gene initiation, resulting in abnormal expression of E6 and E7 genes, leading to malignant cell proliferation. In 1982, Syrjänen et al. Proposed for the first time that HPV infection may be related to the occurrence of esophageal squamous cell carcinoma[19]. In recent years, some scholars suggested that HPV could be a prognostic indicator of esophageal squamous cell carcinoma[20–22]. In head and neck cancer, patients with HPV-positive cancer have better therapeutic outcome and higher survival rate than patients with HPV-negative cancer [23–25]. A similar correlation possibly exists, because the esophagus may also be infected with HPV. HPV-infected tumors are characterized by a high expression of p16, which is widely considered as an alternative marker for HPV infection [10, 12]. In the present study, the expression rate of p16 in ESCC was 30.8%, which was significantly higher than in normal esophageal mucosa. In the correlation analysis with clinical pathological factors, p16 expression was significantly correlated with esophageal location, and the expression rate of p16 in the cervical/upper part was significantly higher than in the middle and lower parts. In addition, p16 expression was negatively correlated with the degree of differentiation, invasion depth, and lymph node metastasis of ESCC. Thus, p16 was highly expressed in patients with a high degree of differentiation, shallow invasion depth, and no lymph node metastasis. Prognosis analysis showed that the 5-year survival rate of p16-positive group was 39.6%, whereas that of p16-negative group was 23.1%. Cox regression analysis of OS and PFS showed that p16 status was an independent prognostic factor for OS and PFS in patients with ESCC. The present study clearly showed that p16-positive tumor was related to high differentiation, TNM stage, and lymph node metastasis. These three differences may have enhanced the prognosis in patients with p16-positive tumor. Another hypothesis was that HPV infection leads to the high expression of tumor protein E6/E7, which weakens the functions of p53 and RB proteins and promotes genome rearrangement and DNA rearrangement is theoretically more sensitive to radiotherapy and chemotherapy [26–27]. This hypothesis explains the indications of increased survival rate in patients with HPV-positive tumors.

As one of the important signal transduction pathways in cells, the PI3K/Akt signaling pathway is closely related to the occurrence and development of various human tumors. Its abnormal activation promotes the normal development of cells to cancer cells. An abnormal PI3K/Akt signal transduction is present in many human malignant tumors [28–31]. As a catalytic subunit of PI3K, PI3Kp110 has four members, namely, P110 α , P110 β , P110 δ and P110 γ . P110 α and P110 β , which are encoded by PIK3CA and PIK3CB, respectively, are widely found in cells. They can regulate DNA synthesis and promote normal cell development with normal expression; moreover, they are closely related to the occurrence and development of tumors with abnormal expression [32]. The mutation of PIK3CA gene can increase the expression of p110 α , which receives the EGF signal. In addition, p110 α is activated by Ras protein, phosphorylates downstream Akt protein and continuously activates the PI3K/Akt signal pathway, leading to cell overproliferation. The role of p110 β in tumorigenesis and its mechanism are unclear. In the present

study, the positive rates of PIK3CA and PIK3CB in ESCC were significantly higher than those in the normal mucosa, suggesting the abnormal PIK3CA and PIK3CB expression levels in ESCC. High PIK3CA and PIK3CB expression levels were related to the malignant potential of EC. PIK3CA overexpression resulted in the abnormal activation of the PI3K/Akt pathway. This abnormal activation could promote the growth and proliferation of tumor cells, inhibit apoptosis, promote invasion and metastasis, regulate endothelial growth and angiogenesis and affect the efficacy of chemotherapy by catalyzing a series of protein phosphorylation.

The expression of HPV E6/E7 oncoprotein induces the carcinogenesis of HPV-transformed cells, changing various cell and molecular events by activating the PI3K/Akt signaling pathway. Xie J et al. demonstrated that ALA-PDT treatment inhibits the proliferation of HeLa cells in a dose-dependent manner and effectively reduces HPV viral load via autophagy and apoptosis by regulating the Ras/Raf/MEK/ERK and PI3K/AKT/mTOR pathways [33]. Madera D et al. suggested that inhibiting PIK3CA expression and HPV can slow down the further growth of OSCC [34]. In our study, p16 expression was positively correlated with PIK3CA expression but was not correlated with PIK3CB expression. We believe that the PI3K/Akt/mTOR signaling pathway in HPV-infected cells was activated by mutations in pathway components and the activation of upstream signaling molecules. Activating this pathway contributes to the dysregulation of proliferation and changes in anti-apoptosis and metabolic characteristics and ultimately leads to the malignant transformation of infected cells. This signaling pathway may be a huge therapeutic opportunity and practical challenge for the treatment of HPV-induced cancer. The PI3K/Akt pathway plays a key role in many HPV-related human cancers and the HPV infection expressed in E6/E7 activates the PI3K/Akt signaling pathway by changing various cell and molecular events that lead to cancer.

The differential expression of tumor suppressor protein p53 is one of the most common abnormal expressions in cancer. Mutation is mainly related to cell invasion, metastasis and late-stage tumor. The expression of HPV E6/E7 oncoprotein can induce the transformation of HPV transformed cells, which leads to the degradation and instability of p53 and pRB. In this study, the positive expression rate of p53 protein in esophageal squamous cell carcinoma was 41.1%, which was significantly higher than that in normal tissues and expression of p53 protein was related to the poor prognosis of ESCC. As a tumor suppressor gene, wild-type p53 can regulate cell cycle and avoid cell carcinogenesis. After p53 gene mutates, it loses its regulatory effect on cell growth, apoptosis and DNA repair and increases the tumor's adaptability to hypoxic microenvironment due to the change in its spatial conformation [35, 36]. However, in the present study, no correlation was found between p53 and p16 expression levels, possibly due to the insufficient number of samples.

In conclusion, this study confirmed that HPV infection is associated with high differentiation, TNM stage and lymph node metastasis. HPV infection is a good prognostic indicator of esophageal squamous cell carcinoma. At the same time, our study showed that the increased expression levels of PIK3CA and PIK3CB were related to the occurrence of ESCC. The imbalance of PI3K/Akt signaling pathway is closely related to HPV infection and prognosis. In ESCC, PIK3CA and p53 are markers of poor prognosis. The

combined detection of PIK3CA and p16 is of great significance for the prognosis evaluation and treatment optimization of esophageal squamous cell carcinoma.

Abbreviations

EC: Esophageal cancer, ESCC: esophageal squamous cell carcinoma, HPV: human papilloma virus, PI3K: phosphatidylinositol-3-kinase, PCR: Polymerase Chain Reaction, mRNA: messenger ribonucleic acid, OS: Overall survival.

Declarations

Authors' contributions

Feifei Wen and Shuhua Wu conceived the idea of the study, Xiaoyang Xu, Ningjie Guo, Zhenzhen Jia and Lizhen Lu performed the research, Feifei Wen, Shuhua Wu, Yangyang Li, Shuang He and Zhongze Cui analysed the data, Feifei Wen and Shuhua Wu interpreted the results, Feifei Wen wrote the paper, all authors discussed the results and revised the manuscript.

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Availability of data and materials

All the generated data in this review are included in the manuscript.

Ethics approval and consent to participate

The experimental protocol was established, according to the ethical guidelines of the Helsinki Declaration and was approved by the Human Ethics Committee of Binzhou Medical University Hospital. Written informed consent was obtained from individual or guardian participants.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

Figure 1

Immunohistochemical staining of p16, PIK3CA, PIK3CB and p53 protein expressions in esophageal squamous cell carcinomas tissues. High-power view (Original magnification $\times 200$) shows strong staining for p16 in the cytoplasm and nucleus of cancer cells (A), PIK3CA and PIK3CB in the cytoplasm of cancer cells (B, C), p53 in the nucleus of cancer cells (D), .

Figure 2

Western-blotting and Real-time-PCR analysis showed that the protein expression of p16, PIK3CA, PIK3CB and p53 in human esophageal squamous cell carcinomas tissues in comparison with non-tumor tissues have more upregulated expression.

Figure 3

Kaplan–Meier survival curves for p16 (A), PIK3CA (B), PIK3CB (C) and p53 (D) expression in ESCC. In Kaplan–Meier analysis, high p16 expression correlated with a longer OS in ESCC patients than the corresponding controls. High PIK3CA and p53 expressions correlated with a shorter OS in ESCC patients than the corresponding controls.