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Original Research Article

Vasculogenic mimicry and p53 expression in esophageal squamous cell carcinoma patients of Kazakh ethnicity, the association between vasculogenic mimicry and p53 expression and its influence on prognosis

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ABSTRACT

Background: Esophageal cancer (EC), particularly esophageal squamous cell carcinoma (ESCC), is prevalent in Xinjiang, with poor prognosis and notable ethnic differences. Kazakhs are the most affected group. The p53 gene mutation is linked to tumor malignancy and metastasis in EC, though its prognostic role is debated. Vasculogenic mimicry (VM), a non-classical angiogenesis model, contributes to tumor progression and correlates with poor outcomes. This study investigates the relationship between VM and p53 expression in Kazakh ESCC patients to understand their clinical and prognostic significance.
Methods: This retrospective cross-sectional study analyzed 76 Kazakh ESCC patients to assess VM and p53 expression using histological staining and immunohistochemistry between December 2010 and November 2015. Clinicopathological features, survival outcomes, and the correlation between VM and p53 were evaluated using Spearman correlation, Kaplan-Meier curves, and Cox regression analysis. Ethical approval and informed consent were obtained.
Results: VM was identified in 42.1% of Kazakh ESCC tissues, associated with advanced stages, lymph node metastasis, and poor differentiation ($p<0.001$). Kaplan-Meier analysis revealed that VM presence significantly reduced survival time ($p<0.05$). Mutant p53 expression correlated with tumor differentiation but not survival. Spearman analysis showed a significant association between VM and p53 positivity. VM and lymph node metastasis were independent prognostic factors, underscoring VM's critical role in ESCC progression.
Conclusions: VM is a marker of poor prognosis in Kazakh EC, correlating with advanced stages and higher metastasis rates. The association between VM and p53 mutations highlights their combined role in tumor progression and potential as therapeutic targets.

Keywords: Esophageal cancer, Esophageal squamous cell carcinoma, Kazakh ethnicity, VM, p53, Prognosis

INTRODUCTION

Esophageal cancer is the sixth most common cause of cancer death worldwide and is therefore a major global health challenge. Esophageal cancer (EC) is a common malignant tumor of the digestive tract; >500,000 people succumb to EC every year; likewise, >500,000 people die from EC.[1] The two major subtypes of esophageal cancer are esophageal squamous cell carcinoma (OSCC) and adenocarcinoma (OAC), which are epidemiologically and biologically distinct.[2] Half of the world's new cases occur in China every year, and most of them are squamous cell carcinomas.[3-5] Esophageal cancer is a malignant tumor with a high incidence in Xinjiang, and its incidence shows obvious regional and ethnic differences.[6] Patients of the Kazakh ethnicity are the most common ethnic group. The

incidence of EC in high- and low-risk areas can differ by 500 times. The prognosis of EC is poor, particularly for advanced EC, for which the 5-year survival rate is <20%.[7,8]

The p53 gene mutation plays an important role in the occurrence and development of numerous different types of tumors, including EC.[9,10] Although the biological significance of the P53 gene mutation is indisputable, its clinical significance in treating EC, particularly as a prognostic biomarker, remains controversial. Previous studies have demonstrated that p53 is highly expressed in EC tissues and is associated with tumor malignancy.[11,12] In addition, studies have suggested that the overexpression of mutant p53 is closely associated with the occurrence of EC and may also promote the metastasis of cancer cells.[13]

Invasion and metastasis of malignant tumors are the primary causes of death. Vasculogenic mimicry (VM) is an endothelial-independent tumor angiogenesis model that is completely different from the classical tumor angiogenesis pathway in that the tumor cells and extracellular matrix mimic the pipeline-like structure of angiogenesis in the body.[14] VM is a complement to the classical angiogenesis pathway and provides a new way to inhibit the growth and spread of tumors. VM reportedly occurs in esophageal squamous cell carcinoma (ESCC), and the prognosis of patients with VM is poor; however, few reports on VM and the p53 gene are available.

In the present study, a morphological analysis combined with the immunohistochemical excision method was used to assess the existence and distribution of VM in Kazakh patients with EC to estimate the association between mutant p53 expression and clinical significance and to provide an experimental basis for predicting the metastasis and prognosis of patients with EC.

METHODS

Study design and population

This retrospective cross-sectional study included a total of 76 Kazakh patients with ESCC who underwent thoracic surgery at the First Affiliated Hospital of Xinjiang Medical University between December 2010 and November 2015 were enrolled in the present study.

Inclusion criteria

Required histopathological confirmation of ESCC, availability of clinical data, and no prior cancer-related treatments (e.g., radiotherapy or chemotherapy) before surgery. Paraffin-embedded cancerous tissues and normal adjacent tissues (5 cm from the tumor) were obtained from the Department of Pathology.

Clinicopathological and survival data

Patient clinicopathological data, including tumor histology, stage (AJCC 7th edition), lymph node metastasis, and degree of tumor differentiation, were collected. Follow-up data were available for 54 patients, with survival time defined as the period from surgery to death or the last follow-up.

Experimental reagent use of p53 is associated with poor prognosis in patients with ESCC

Mouse anti-human CD34 monoclonal antibody (Dako; Agilent Technologies, Inc.), mouse anti-human p53 monoclonal antibody (Sigma Aldrich; Merck KGaA), an incision kit and a PAS staining kit were purchased from Beijing Zhongshan Jinqian Biotechnology Co., Ltd. CD34-PAS double staining combined with hematoxylin and eosin staining was used to detect VM patterns in 76 patients with ESCC and 20 patients with adjacent normal tissue.

CD34-PAS double staining

Paraffin tissue sections were routinely dewaxed and hydrated to remove endogenous peroxidase. The Envision two-step method was used following antigen retrieval by heating. Under the microscope, the color reaction was stopped by washing after the endothelial cells were stained, after which PAS staining was performed. After this, hematoxylin counterstaining and neutral resin sealing were performed.

p53 immunohistochemical staining

The two-step method was used to determine the immunohistochemical results. A positive control was used for the positive tissue sections, and PBS was used as a negative control as an alternative to the primary antibody.

Criteria for the existence of VM

Tumor cells were confirmed by hematoxylin and eosin (HE) staining of cells enclosed with duct-like structures. CD34 staining revealed negative red blood cells located in the duct structure, and PAS-positive basement membrane-like structures were observed between red blood cells and tumor cells.

Immunohistochemical criteria

Primary lesions (>10%) had obvious nuclear or brown staining. The basal cell layer of some mucosa (>10%) with characteristic p53 staining was considered positive, and limited weak staining of basal cells was considered negative.

Statistical analysis

All the data were analyzed using statistical package for the social sciences (SPSS) 17.0 statistical software. $P<0.05$ was considered to indicate a statistically significant difference.

Associations

Relationships between VM, p53 expression, and clinicopathological features were analyzed using chi-square tests and Spearman correlation analysis.

Survival analysis

Kaplan-Meier (K-M) curves and log-rank tests were used to compare survival rates between patients with and without VM.

Prognostic factors

Cox regression analysis was employed to identify independent factors affecting prognosis, including VM and lymph node metastasis.

RESULTS

Morphological observation

VM was observed in 42.1% (32/76) of the Kazakh EC tissues. Microscopically, the duct-like structure was surrounded by tumor cells, and red blood cells were observed in some ducts (Figure 1a). A layer of PAS-positive basement membrane-like structure was observed on the inner wall of the lumen without CD34-positive vascular endothelial cells (Figure 1b).

Associations between VM and p53 expression and patient clinicopathological characteristics

The associations between VM and p53 expression and between VM and the assessed clinicopathological features are presented in Table 1. The incidence of VM was significantly associated with clinical stage (p=0.006), T classification (p=0.005), lymph node metastasis (p=0.001) and degree of tumor differentiation (p<0.001). p53 was stained brown in the nucleus, with a percentage of 61.8% (47/76) positive cells. The expression of p53 was associated with the degree of tumor differentiation (p=0.001), and there were no significant associations between p53 expression and clinical stage, T stage or lymph node metastasis (p>0.05).

Associations between VM and p53 expression and postoperative survival time

The mean survival time was 29.796±2.880 months (20.410±2.527 for patients with VM; 36.406±4.078 for patients without VM), and the median survival time was 25 months (patients with VM 18 months; patients with no VM for 31 months). K–M survival curve analysis revealed that the survival rate of patients with EC with VM was significantly lower than that of patients without VM (log rank test, c^2=7.803; p<0.05). However, there was no significant correlation between the expression of p53 and survival (log rank result, c^2=0.447; p=0.504) (Figure 2). Cox univariate regression analysis revealed that VM and lymph node metastasis were independent risk factors affecting the prognosis of patients with EC (p<0.05) (Table 2).

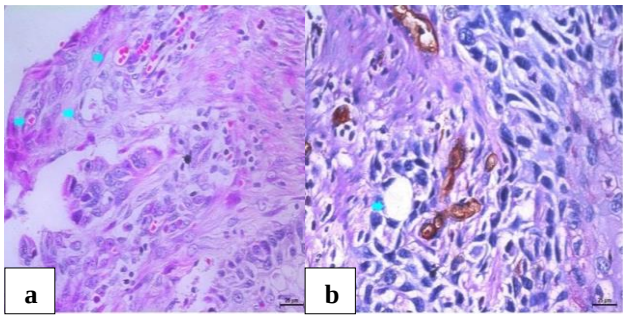

Figure 1: Morphological observation of VM in Kazakh ESCC tissues, (a) A) HE staining showing that the canal-like structure (VM) was surrounded by cancer cells, and red blood cells (arrows) could be observed; and (b) CD34 -PAS double staining showed PAS - positive VM (arrow) surrounding CD34-positive vessels.

Table 1: Relationships between VM and TP53 expression and pathological characteristics.

Parameters	N	VM				TP53			
		Positive (%)	Negative (%)	χ^2	P	Positive (%)	Negative (%)	χ^2	P
Clinical stage				10.241	0.006*			2.503	0.286
I	13	3 (23.1)	10 (76.9)			9 (69.2)	4 (30.8)		
II	54	21 (38.9)	33 (61.1)			29 (53.7)	25 (46.3)		
III	9	8 (88.9)	1 (11.1)			7 (77.8)	2 (22.2)		
T stage				8.523	0.005*			0.357	0.642
T1-T2	41	11 (26.8)	30 (73.2)			23 (56.1)	18 (43.9)		
T3	35	21 (60.0)	14 (40.0)			22 (62.9)	13 (37.1)		
Lymph node metastasis				11.009	0.001*			0.005	0.592
None	61	20 (32.8)	41 (67.2)			36 (59.0)	25(41.0)		
Have	15	12 (80.0)	3 (20.0)			9 (60.0)	6 (40.0)		

Continued.

Parameters	N	VM		χ^2	P	TP53		χ^2	P
		Positive (%)	Negative (%)			Positive (%)	Negative (%)		
Differentiation				19.116	<0.001*			11.048	0.001*
High-moderate differentiation	59	17 (28.8)	42 (71.2)			29 (49.2)	30 (50.8)		
Poorly differentiated	17	15 (88.2)	2 (11.8)			16 (94.1)	1 (5.9)		

*The difference was statistically significant (p<0.05)

Table 2: Cox analysis of prognostic factors in patients with esophageal cancer.

Variables	Univariate analysis	
	HR (95% CI)	P value
N stage (negative versus positive)	2.738 (1.279-5.861)	0.009*
T stage (T1-T2 vs. T3-T4)	1.922 (0.938-3.936)	0.074
Degree of differentiation (high differentiation versus medium to low differentiation)	1.983 (0.864-4.552)	0.106
VM level (negative versus positive)	2.731 (1.293-5.770)	0.008*
TP 53 expression (negative versus positive)	0.781 (0.372-1.637)	0.512

*The difference was statistically significant (p<0.05)

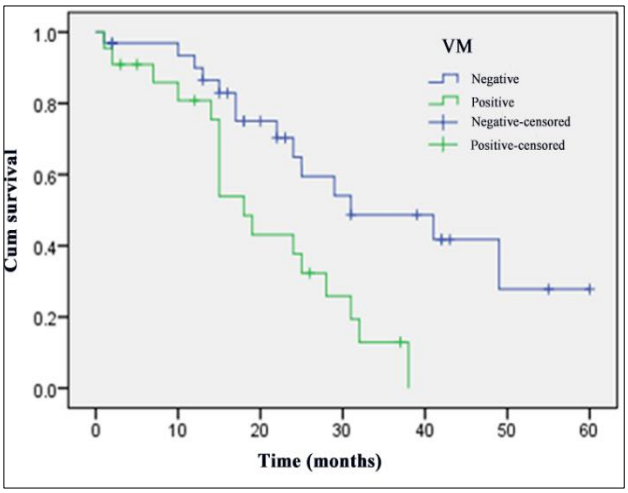

Figure 2: Survival analysis of patients with VM in ESCC evaluated using Kaplan–Meier curves.

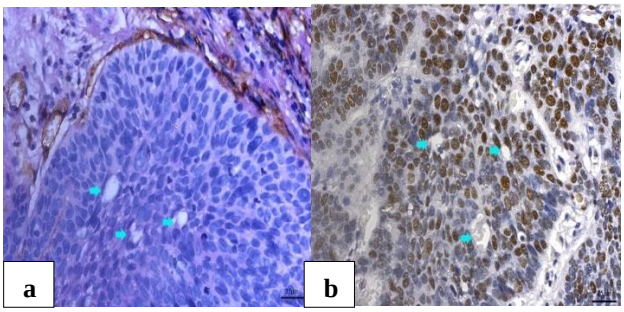

Figure 3: VM and TP 53 expression in Kazakh ESCC tissues, (a) CD34-PAS double staining confirmed the presence of VM (arrows) in ESCC; and (b) IHC staining showed multiple VMs (arrows) in TP53-positive ESCC tissues.

Association between VM and p53 expression

The VM and p53 staining results were divided into two groups: p53-positive patients accounted for 53.3% of the VM-positive patients, and p53-negative patients accounted for 76.7% of the VM-negative patients (Figure 3). Spearman correlation analysis demonstrated that there was a correlation between them (p<0.05) (Table 3). The percentage of p53-positive patients who were VM increased significantly.

Table 3: Relationships between VM and TP53 expression in esophageal cancer.

TP53	VM		r	P
	Positive	Negative		
Positive	24	21	0.274	0.015*
Native	8	23		

*The difference was statistically significant (p<0.05)

DISCUSSION

VM was first identified by Manioti et al in melanoma, a study focusing on melanoma using a classic new tumor microcirculation model with completely different pathways and no dependence on endothelial cells. This PAS-positive reticular duct is different from angiogenesis and the tumor mesenchymal response.[14] Since then, VM has been associated with various highly malignant tumors, including breast, ovarian, and liver cancers, where it correlates with aggressive phenotypes and poor prognosis.[15-21]

Our study corroborates these findings, revealing that 42.1% of Kazakh esophageal carcinoma (EC) cases exhibited VM, associated with advanced stage, a later tumor-node-metastasis stage, a greater lymph node

metastasis rate and a shorter survival time. The importance of VM in tumor biology has been confirmed; that is, without the barrier of endothelial cells, the blood vessels of VM are more conducive to the invasion and metastasis of tumors, leading to poor patient prognosis. Due to the presence of VM, tumor cells obtain a blood supply from pathways other than the classical tumor vasculature, which decreases the therapeutic effect of antiangiogenic agents.

P53 gene mutation is the most common alteration in a number of different types of tumors, including EC, and plays an important regulatory role in the occurrence and development of EC.[10,11] However, there are different views on the association between prognosis and EC.[22,23] A retrospective systematic review study on the value of p53 expression in the prognosis of EC, which has not yet reached a consensus in numerous studies over the past decade, suggesting that the expression of p53 may be a valuable biomarker for predicting the poor prognosis of patients with EC.[24] Our study results also aligned with this review whereby there was significant association between the p53-positive expression rate and the degree of tumor differentiation, which is one of the characteristics associated with poor prognosis in Kazakh patients with EC, however there was no significant association with the survival times.

Interestingly, we observed a significant correlation between VM and p53 mutations, aligning with Izawa et al's findings that stem cell-like cancer cells with p53 mutations, resistant to p53-induced apoptosis accelerate VM formation. This suggests that p53 mutations contribute to VM development by bypassing traditional angiogenic pathways, but the exact mechanism involved remains unknown, further complicating treatment.[25] 61.5% of the Kazakh ECs were p53 mutant proteins, and the percentage of patients with VM harboring the p53 mutant was significantly greater than that of patients without VM ($p<0.05$), which indicated that the p53 gene mutation was associated with VM formation in malignant tumors. Overall, the interplay between VM and p53 mutations in Kazakh EC underscores the need for further investigation into their combined biological mechanisms. The results of the present study suggest that, the biological behavior of the p53 gene and VM in EC provide an experimental basis for targeted therapeutic interventions to improve prognosis in EC cases.

CONCLUSION

This study underscores the dual significance of VM and p53 expression in Kazakh ESCC. VM correlates with advanced tumor stage, lymph node metastasis, and significantly poorer survival outcomes, establishing its role as a robust prognostic marker. While p53 expression showed limited direct impact on survival, however its association with VM suggests a potential contributory role in tumor progression. These findings highlight the need for further exploration of VM and p53 as integrated biomarkers to inform prognosis and therapeutic interventions in ESCC.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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