Vasculogenic mimicry in esophageal squamous cell carcinoma from kazakh patients: association with P53 expression and prognosis implications

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Abstract:

Objective: To investigate vasculogenic mimicry (VM) in Kazakh patients with esophageal squamous cell carcinoma (ESCC), as well as its association with p53 expression and its influence on prognosis. Methods: 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. 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. Results: VM was observed in 42.1% (32/76) of the Kazakh EC tissues. Both VM incidence and P53 expression were significantly associated with the degree of tumor differentiation ($P < 0.001$). The survival rate of patients with EC with VM was significantly lower than that of patients without VM (log rank test, $\chi^2 = 7.803$; $P < 0.05$). However, there was no significant correlation between the expression of p53 and survival (log rank test, $\chi^2 = 0.447$; $P = 0.504$). Conclusion: Thepresent study demonstrated that VM occurs in the EC of Kazakh

individuals and coexists with endothelium-dependent blood vessels. There is a synergistic effect between angiogenesis mimicry and mutant p53 in the malignant progression of EC, which is associated with the poor prognosis of patients with EC.

Key words: ESCC; Kazakh ethnicity; VM; p53; Prognosis

Introduction

Esophageal cancer (EC) is the sixth leading cause of cancer-related death worldwide and is, therefore, a major global health challenge. As a common malignant tumor of the digestive tract, more than 500 000 people succumb to EC every year [1]. The two major sub-types of EC are ESCC and adenocarcinoma (EAC), which are epidemiologically and biologically distinct [2]. Half of the world's new cases occur in China every year, and most of them are ESCC [3-5]. EC 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].

Thep53 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. VM is an endothelial-independent tumor angiogenesis modelthat 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 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 immunohistochemicalexcision 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

Patients

A total of 76 Kazakh patients with ESCC who underwent thoracic surgeryat the First Affiliated Hospital of Xinjiang Medical University between December 2010 and November 2015 were enrolled in the present study. Paraffin-embedded cancerous and normal adjacent tissues (5 cm away from the tumor) from patients with ESCC were collected from the Department of Pathology. The tumor histology, lymph node metastasis status and disease stage (referenced AJCC 7th edition) data were available for all the patients with ESCC. Patients had not received radiotherapy, chemotherapy or other cancer- related treatments prior to surgery. A total of 54 patients had complete follow-up data. The survival time of patients ranged from surgery to death or the last follow-up. Informed written consent was obtained from all patients enrolled in the present study, and the study was approved by the Ethical Committee of the First Affiliated Hospital of Xinjiang Medical University.

Experimental reagents

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 Jinqiao 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 asa 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-likestructures were observed between red blood cells and tumor cells.

Immunohistochemical criteria

Primarylesions ($>10\%$) 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

Allthe data were analyzed using SPSS 17.0 statistical software. Technical data were tested using the χ^2 test. Correlations were assessed using Spearman grade correlation analysis, and survival analysis was performed via the Kaplan – Meier method and log-rank test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Morphological observation

VM was observed in 42.1% (32/76) of the Kazakh ESCC 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).

A) HE staining showed that the canal-like structure (VM) was surrounded by cancer cells, and red blood cells (arrows) could be observed. B) CD34 – PAS double staining showed PAS-positive VM (arrow) surrounding CD34-positive vessels.

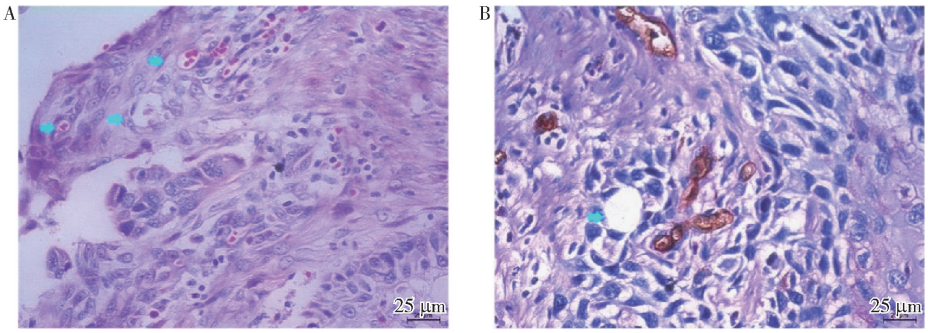

Fig. 1 Morphological observation of VM in ESCC tissues from Kazakh patients

Associations between VM and p53 expression and patient clinicopathological characteristics

Theassociations 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 ($P>0.05$).

Table 1 Associations between VM and TP53 expression and pathological characteristics

Factors	n	VM		χ^2	P	TP53		χ^2	P
		Positive(%)	Negative(%)			Positive(%)	Negative(%)		
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)		
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)		

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), andthe 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 ESCC with VM was significantly lower than that of patients without VM (log rank test, χ^2 = 7.803; P < 0.05). However, there was no significant correlation between the expression of p53 and survival (log rank result, χ^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 ESCC (P < 0.05) (Table 2).

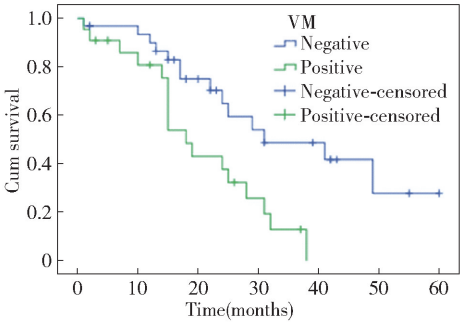

Fig. 2 Survival analysis of patients with VM in ESCC evaluated using Kaplan – Meier curves

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

Variable	Univariate analysis	
	HR (95% CI)	P value
N stage (negative vs. 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 vs. medium to low differentiation)	1.983 (0.864 – 4.552)	0.106
VM level (negative vs. positive)	2.731 (1.293 – 5.770)	0.008*
TP53 expression (negative vs. positive)	0.781 (0.372 – 1.637)	0.512

* The differencewas statistically significant (P < 0.05).

Association between VM and p53 expression

The VM and p53 stainingresults 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.

A) CD34 – PAS double staining confirmed the presence of VM (arrows) in ESCC. B). IHC staining showed multiple VMs (arrows) in TP53- positive ESCC tissues.

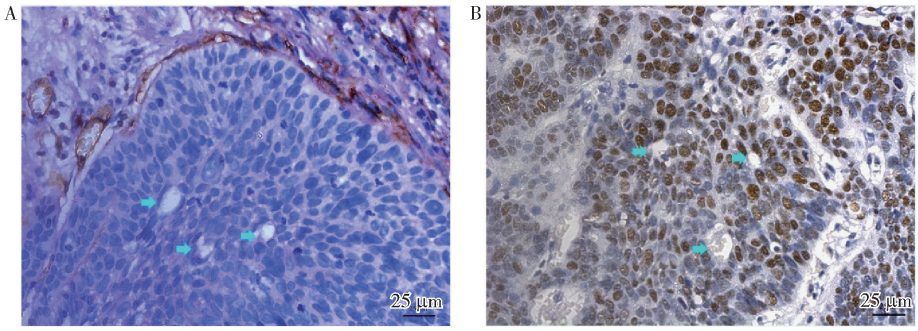

Fig. 3 VM and TP 53 expression in Kazakh ESCC tissues

Table 3 Relationships between VM and TP53 expression in esophageal cancer

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

Discussion

VM was first reported by Manioti[14] in a study on melanoma using a novel tumor microcirculation model that operates independent of traditional angiogenesis pathways and does not rely on endothelial cells. This PAS-positive reticular duct is different from angiogenesis and the tumor mesenchymal response. VM has been reported in a variety of highly malignant tumors [15-21], such as malignant melanoma, breast cancer, ovarian cancer, liver cancer, lung cancer and glioblastoma. Most studies suggest that the presence of VM is associated with a poor prognosis.

ESCC is a digestive tract tumor with a poor prognosis. The present study showed that 32 of the 76 Kazakh patients with ESCC had VM. Patients with VM have a greater degree of malignancy, 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 oftumors, 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]. Wang et al performed a systematic retrospective 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]. In the present study, there was no significant association between the p53-positive expression rate and survival time in Kazakh patients with ESCC, but this association was significant for the degree of tumor differentiation, which is one of the characteristics associated with poor prognosis.

The P53 gene can regulate angiogenesis, but the exact mechanisminvolved remains unknown. Izawa et al reported that stem cell-like cancer cells resistant to p53-induced apoptosis formed VM faster than did control cells [25]. In the present study, 61.5% of the Kazakh ESCCs 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. The association between mutant p53 expression and VM in ESCC has not yet been reported in the literature. The results of the present study suggest that the biological behavior of the P53 gene and VM in ESCC provide an experimental basis for targeted antiangiogenic therapy.

In conclusion, the results of the present study-

demonstrated that VM occurs in the EC of Kazakh individuals and coexists with endothelium-dependent blood vessels. There is a synergistic effect between angiogenesis mimicry and mutant p53 in the malignant progression of EC, which is associated with the poor prognosis of patients with EC.

Acknowledgments

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Conflicts of interest

Allthe authors declare no conflicts of interest regarding this study.

Specific author contributions

Julaiti Ainiwaerdesigned the study and wrote the main text of the manuscript. LUYEYE analyzed and interpreted the data and performed the statistical analysis. Madiniyet Niyaz provided the study materials and revised the manuscript. Zheng Shutao and LUYEYE performed the experiments. Zhang Liwei and Ilyar Sheyhidin revised the article for important intellectual content.

Ethics approval and consent to participate

All the experiments were approved by the Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University, and written informed consent was obtained from all the participants.

References

[1] Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018 Nov;68(6): 394 – 424.

[2] Kamangar F DGAWF. Patterns of cancer incidence, mortality, and prevalence across five continents: defining priorities to reduce cancer disparities in different geographic regions of the world. Journal of Clinical Oncology . 2005 Sep 21;May 10;24(14): 2137 – 50.

[3] Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. CA Cancer J Clin. 2014 Jan;64(1):9 – 29.

[4] Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, et al. Cancer statistics in China, 2015. CA Cancer J Clin. 2016 Mar;66(2):115 – 32.

[5] Zeng H, Zheng R, Zhang S, Zuo T, Xia C, Zou X, et al. Esophageal cancer statistics in China, 2011: Estimates based on 177 cancer registries. Thorac Cancer. 2016 Mar 1;7(2): 232 – 7.

[6] Awut I, Niyaz M, Huizhong X, Biekemitoufu H, Yan HZ, Zhu Z, et al. Genetic diagnosis of patients with esophageal cancer using FISH. Oncol Lett. 2010 Sep;1(5):809 – 13.

[7] Arnal MJD, Arenas ? F, Arbeloa ? L. Esophageal cancer: Risk factors, screening and endoscopic treatment in Western and Eastern countries. Vol. 21, World Journal of Gastroenterology. WJG Press; 2015. p. 7933 – 43.

[8] Hassanipour S, Mohammadian-Hafshejani A, Ghoncheh M, Salehiniya H. The incidence and mortality of esophageal cancer and its relationship with development in the world. Biomedical Research and Therapy. 2017 Sep 20;4(9):1607.

[9] Greenblatt MS, Bennett WP, Hollstein M, Harris2 CC. Mutations in the p53 Tumor Suppressor Gene: Clues to Cancer Etiology and Molecular Pathogenesis [Internet]. Vol. 54, CANCERRESEARCH. 1994.

[10] Song Y, Li L, Ou Y, Gao Z, Li E, Li X, et al. Identification of genomic alterations in esophageal squamous cell cancer. Nature. 2014;508(7498):91 – 5.

[11] Huang K, Chen L, Zhang J, Wu Z, Lan L, Wang L, et al. Elevated p53 expression levels correlate with tumor progression and poor prognosis in patients exhibiting esophageal squamous cell carcinoma. Oncol Lett. 2014;8(4):1441 – 6.

[12] Zhao Z, Wang P, Gao Y, He J. The high expression instead of mutation of p53 is predictive of overall survival in patients with esophageal squamous-cell carcinoma: a meta-analysis. Cancer Med. 2017 Jan 1;6(1):54 – 66.

[13] Muller PAJ, Vousden KH. Mutant p53 in cancer: New functions and therapeutic opportunities. Vol. 25, Cancer Cell. Cell Press; 2014. p. 304 – 17.

[14] Dunleavey JM, Dudley AC. Vascular mimicry: Concepts and implications for anti-angiogenic therapy. Vol. 1, Current Angiogenesis. Bentham Science Publishers BV; 2012. p. 133 – 8.

[15] Kirschmann DA, Seftor EA, Hardy KM, Seftor REB,

Hendrix MJC. Molecular pathways: Vasculogenic mimicry in tumor cells: Diagnostic and therapeutic implications. Vol. 18, Clinical Cancer Research. 2012. p. 2726 – 32.

[16] Liu W, Lv C, Zhang B, Zhou Q, Cao Z. MicroRNA – 27b functions as a new inhibitor of ovarian cancer-mediated vasculogenic mimicry through suppression of VE-cadherin expression. 2017; Available from: http://www.rnajournal.org/cgi/doi/10.1261/rna.059592.

[17] Liu W, Lv C, Zhang B, Zhou Q, Cao Z. MicroRNA – 27b functions as a new inhibitor of ovarian cancer-mediated vasculogenic mimicry through suppression of VE-cadherin expression. 2017;

[18] Yang WY, Tan ZF, Dong DW, Ding Y, Meng H, Zhao Y, et al. Association of aquaporin-1 with tumor migration, invasion and vasculogenic mimicry in glioblastoma multiforme. Mol Med Rep. 2018 Feb 1;17(2):3206 – 11.

[19] Williamson SC, Metcalf RL, Trapani F, Mohan S, Antonello J, Abbott B, et al. Vasculogenic mimicry in small cell lung cancer. Nat Commun. 2016 Nov 9;7.

[20] Chen Q, Lin W, Yin Z, Zou Y, Liang S, Ruan S, et al. Melittin Inhibits Hypoxia-Induced Vasculogenic Mimicry Formation and Epithelial-Mesenchymal Transition through Suppression of HIF – 1 α/Akt Pathway in Liver Cancer. Evidence-based Complementary and Alternative Medicine. 2019;2019.

[21] Luan YY, Liu ZM, Zhong JY, Yao RY, Yu HS. Effect of grape seed proanthocyanidins on tumor vasculogenic mimicry in human triple-negative breast cancer cells. Asian Pacific Journal of Cancer Prevention. 2015; 16(2): 531 – 5.

[22] Cheng TH, Hsu PK, Li AFY, Hung IC, Huang MH, Hsu HS. Correlation of p53, MDM2 and p14ARF protein expression in human esophageal squamous cell carcinoma. J Cancer Res Clin Oncol. 2009;135(11):1577 – 82.

[23] Yasuda M, Kuwano H, Watanabe M, Toh Y, Ohno S, Sugimachi K. p53 Expression in squamous dysplasia associated with carcinoma of the esophagus: Evidence for field carcinogenesis. Br J Cancer. 2000;83(8):1033 – 8.

[24] Wang L, Yu X, Li J, Zhang Z, Hou J, Li F. Prognostic significance of p53 expression in patients with esophageal cancer: A meta-analysis. BMC Cancer. 2016 Jul 1;16(1).

[25] Izawa Y, Kashii-Magaribuchi K, Yoshida K, Nosaka M, Tsuji N, Yamamoto A, et al. Stem-like human breast cancer cells initiate vasculogenic mimicry on Matrigel. Acta Histochem Cytochem, 2018,51(6):173 – 83.