Characterization of the expression and clinical features of epidermal growth factor receptor and vascular endothelial growth factor receptor-2 in esophageal carcinoma

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Abstract. The present study aimed to understand the expression characteristics of epidermal growth factor receptor (EGFR) and vascular endothelial growth factor receptor-2 (VEGFR-2) in individuals of Uygur, Han and Kazak ethnicity with esophageal carcinoma in Xinjiang (China) and their interrelation analysis, and to investigate the expression differences in these genes between esophageal carcinoma and pericarcinoma tissue samples, and between the three ethnic groups. The expression levels of EGFR and VEGFR-2 from 119 pairs of esophageal carcinoma tissue and corresponding pericarcinoma tissue from Uygur, Han and Kazak patients with esophageal carcinoma were detected by immunohistochemistry following surgical resection, and an additional five carcinoma *in situ* specimens were also tested. The relative expression was analyzed among the ethnic groups and clinicopathological parameters. The positive rate of EGFR in esophageal carcinoma tissue from patients of Uygur, Han and Kazak heritage was 70.73, 68.42 and 67.5%, respectively. For VEGFR-2 the positive rate was 73.17, 68.42 and 67.5%, respectively. No significant difference was detected in their expression between the three ethnic groups ($P>0.05$); however, EGFR and VEGFR-2 overexpression were correlated with lymph node metastasis ($P<0.05$). VEGF expression was also correlated with the expression of VEGFR-2 in esophageal carcinoma tissues. EGFR was positive in carcinoma *in situ* samples, while VEGFR-2 was negative. The overexpression of EGFR is therefore an early event and may have a significant role in the progression of esophageal carcinoma pathogenesis. EGFR overexpression may correlate with the expression of VEGFR-2 in esophageal cancer. These results may aid the early diagnosis of esophageal cancer, and the development of individual target treatment in the future.

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Introduction

A unique characteristic in the epidemiology of esophageal carcinoma is the significant differences in incidence rate which exist between regions and ethnic groups (1,2). The worldwide incidence and morbidity of esophageal carcinoma is greatest in China compared with other countries (3). Xinjiang is a residential province of China, populated by multiple ethnic groups, and is one of the areas associated with high incidence of esophageal carcinoma (4). Current treatments available for esophageal carcinoma result in poor prognosis (1). The majority of patients with esophageal carcinoma are diagnosed in the progressive or resection-resistant stage and novel treatment strategies are urgently required. Research has focused on understanding the characteristics of the progression and transformation of esophageal carcinoma at the genetic and molecular level.

Research has indicated that abnormal activation of the kinase activity of epidermal growth factor receptor (EGFR) (5,6) is significant in the occurrence and development of esophageal carcinoma. Domestic (Chinese) and foreign studies have identified an overexpression of EGFR protein in esophageal carcinoma (7,8). This phenomenon is correlated with tumor cell proliferation, invasion, metastasis, vascular growth and inhibition of cell apoptosis, and is associated with poor prognosis (7,9). Reports of the expression rate of EGFR in esophageal cancer tissues are vary significantly, ranging from 29 to 99% (10,11).

In the process of tumor growth, overexpression of vascular endothelial growth factor (VEGF) and VEGF receptor (VEGFR) disrupt the balance between angiogenesis inducers and angiogenesis inhibiting factors, promoting tumor angiogenesis (12,13). VEGFR-2 distribution in the endothelial cells has a critical role in the process of tumor angiogenesis. The main function of VEGFR-2 is to mediate VEGF-dependent proliferation of vascular endothelial cells and chemotaxis of endothelial cells, as well as enhance vascular permeability (12,13). VEGFR-2 is therefore the main functional receptor of VEGF (12).

VEGFR is a protein similar to EGFR in terms of their roles in cancer occurrence and development, therefore evaluating the expression of VEGFR and tumor angiogenesis-associated VEGFR tyrosine kinase activity may identify a potential target for anti-angiogenesis in tumor therapy. Previous studies have found that the overexpression of EGFR and VEGFR-2 is closely associated with the invasion and metastasis of a variety of solid tumors (8,13). To date, comprehensive domestic research on these two proteins in the invasion and metastasis of esophageal cancer has been rarely reported. The present study detected the expression levels of esophageal carcinoma-associated proteins EGFR and VEGFR-2 in three ethnic populations (Uygur, Han and Kazak) in Xinjiang, in order to elucidate the differences and correlations between the expression levels of these proteins and the occurrence and development of esophageal cancer. The results may reveal potential novel anti-tumor treatments for esophageal carcinoma at the molecular level, and may aid the elucidation of differences in esophageal cancer between the ethnic groups evaluated.

Materials and methods

Clinical characteristics. A total of 119 pairs of esophageal carcinoma and corresponding pericarcinoma tissue were collected between February 2011 and December 2012 from patients with esophageal carcinoma following surgical treatment at the First Affiliated Hospital of Xinjiang Medical University (Urumqi, China). All samples were dewaxed twice with xylene (Tianjin Yong Sheng Chemical Co., Ltd., Tianjin, China) for 15 min after collection and stored at the Xinjiang Esophageal Cancer Research Institute/Medical Research Center of Xinjiang Medical University (Urumqi, China). Of these 119 samples, 41 cases were Uygur, 38 cases were Han and 40 cases were Kazak. A total of 80 cases were male, and 39 cases were female. The age of patients ranged from 38 to 79 years, with a median age of 60 years. An additional five carcinoma *in situ* samples obtained via biopsy during preoperative endoscopy (n=3) or postoperatively (n=2) between February 2011 and August 2013 were acquired from the Xinjiang Esophageal Cancer Research Institute/Medical Research Center of Xinjiang Medical University for the analysis of the expression levels of EGFR and VEGFR-2 in the early stages of esophageal carcinoma. The samples were staged according to the seventh edition of the TNM staging criteria (14), classified based on the 2010 World Health Organization histological tumor classification standard (14) and divided by differentiation degree. Pathological type distribution included 117 cases of squamous cell carcinoma and 2 cases of adenocarcinoma. The degree of cellular differentiation was high in 38 cases, medium in 43 cases medium and low in 38 cases. Tumor T stage classification identified 12 cases as T1, 34 cases as T2, 59 cases as T3 and 14 cases as T4. N stage classification revealed 59 cases of N0, 49 cases of N1 and 11 cases of N2. Postoperative pathological pTNM staging indicated 9 cases of Ia, 21 cases of Ib, 30 cases of IIa, 13 cases of IIb, 28 cases of IIIa, 8 cases of IIIb and 10 cases of IIIc, plus an additional 5 cases of carcinoma *in situ*. Specimen collection was approved by the Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University. All the patients and their families provided informed consent following an explanation of the significance of the study.

Immunohistochemistry. The streptavidin peroxidase immunohistochemical method was used for immunostaining. Rabbit polyclonal anti-EGFR and anti-VEGFR-2 antibodies were purchased from Abcam (Cambridge, MA, USA). Immunostaining was performed as follows: Samples were first conventionally dewaxed and hydrated using Triton X-100 (Shanghai Solarbio Co., Shanghai, China). Subsequently, to eliminate endogenous peroxidase activity, 3% hydrogen peroxide was added and the mixture was incubated for 10 min at room temperature. Antigens were retrieved by high pressure heating in a microwave (NN-GF352M; Panasonic Corporation, Osaka, Japan) at 96˚C for 10 min. Samples were blocked in 10% normal goat serum (Shanghai Solarbio Co.) at 37˚C for 30 min, and then incubated with polyclonal rabbit anti-mouse EGFR (1:100 dilution; cat. no. ab2430; Abcam) or polyclonal rabbit anti-mouse VEGFR-2 (1:50 dilution; cat. no. ab2349; Abcam) antibody at 4˚C overnight, followed by 3 washes with phosphate-buffered saline (PBS; Abcam) for 5 min. The biotinylated goat anti-rabbit IgG secondary antibody (cat. no. SP-9000; ZSGB-BIO, Beijing, China) was added and the mixture was incubated at 37˚C for 30 min. Subsequently, the samples were washed 3 times with PBS, colorized with diaminobenzidine (Shanghai Solarbio Co.), rinsed with distilled water, stained with hematoxylin (Shanghai Solarbio Co.) and finally sealed using the Histostain-Plus Kit (ZSGB-BIO). PBS (0.01 mol/l) was used as a negative control.

Assessment of results. Two pathologists observed the sections and performed double-blinded diagnoses. The dyeing area intensity score and positive cell area ratio scoring methods (10,12) were adopted to compare cellular differences in EGFR and VEGFR-2 protein expression. Positive EGFR staining was localized in the cell membrane, and cell membrane staining with yellow, brown or deeper brown particles were regarded as positive cells. According to the degree of cell positive staining (antigen content), the samples were divided into: 0 (no coloring), 1 point (yellow), 2 points (yellow-brown) or 3 points (brown). Each section was observed under a light microscope (DM3000; Leica Microsystems Ltd., Wetzlar, Germany) in five randomized high-power fields (magnification, x20), and the percentage of positive cells was counted and scored as follows: 0 (0%), 1 (1-25%), 2 (26-50%), 3 (51-75%) and 4 (>75%). The product of the cell coloring intensity score and positive area ratio score provided the final score points: 0 (-), 1-2 (+), 3-4 (++), >4 points (+++). Low expression was indicated by (-) and (+), while (++) and (+++) indicated high (positive) expression. Identical scoring methods were used for VEGFR-2 and EGFR.

Statistical analysis. Data were analyzed using SPSS 17 software (SPSS, Inc., Chicago, IL, USA). The expression levels of EGFR and VEGFR-2 protein are presented as percentages. Protein expression levels between groups were compared using the χ^2 test. Multiple independent samples were compared using multiple independent samples non-parametric tests. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Comparison of clinicopathological characteristics of patients with esophageal carcinoma of varying ethnicity. According to

Table I. Protein expression levels of EGFR in Uygur, Han and Kazak esophageal carcinoma tissues.

		EGFR expression					
Ethnicity	n	-	+	++	+++	Positive rate, %	P-value
Uygur	41	3	9	13	16	92.68	P>0.05
Han	38	4	8	9	17	89.47	
Kazak	40	2	11	15	12	95.00	

(-) and (+) indicate low expression, while (++) and (+++) indicate high (positive) expression. EGFR, epidermal growth factor receptor.

Table II. Protein expression levels of VEGFR-2 in Uygur, Han and Kazak esophageal carcinoma tissues.

		VEGFR-2 expression					
Ethnicity	n	-	+	++	+++	Positive rate, %	P-value
Uygur	41	8	3	21	9	80.49	P>0.05
Han	38	8	4	17	9	78.95	
Kazak	40	7	6	22	5	82.50	

(-) and (+) indicate low expression, while (++) and (+++) indicate high (positive) expression. VEGFR-2, vascular endothelial growth factor receptor-2.

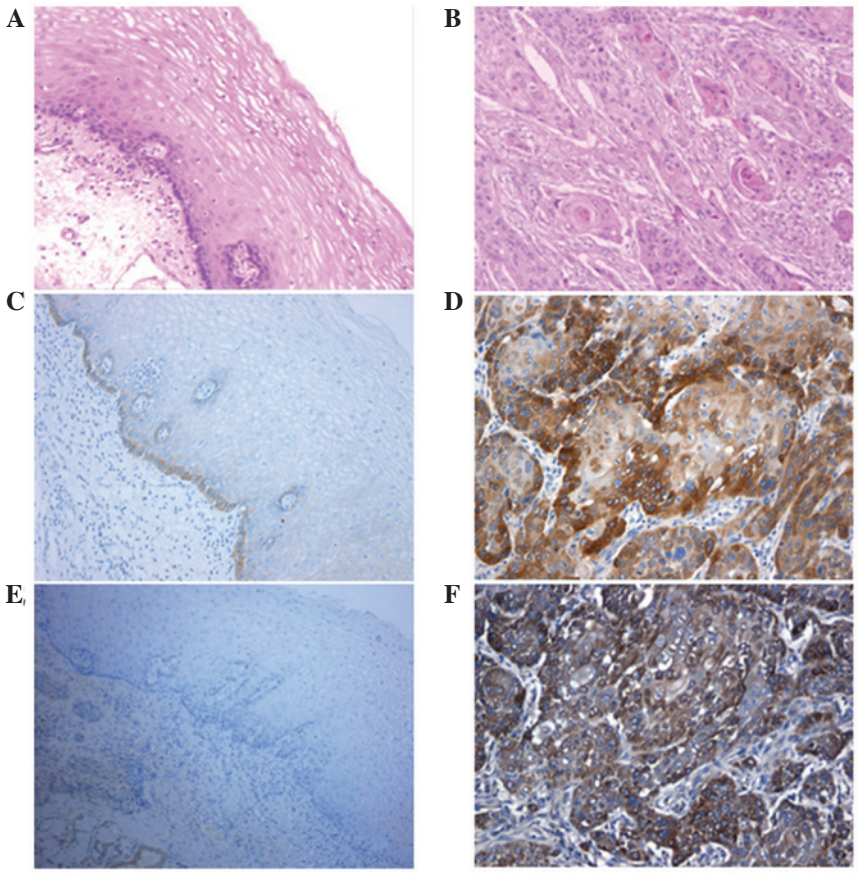

Figure 1. Expression levels of EGFR and VEGFR-2 in normal and cancer tissues from the Kazak ethnic group. (A) Normal esophageal epithelium and (B) highly differentiated squamous cell carcinoma (hematoxylin and eosin staining; magnification, x20). (C) Low level staining of EGFR in normal esophageal mucosal epithelial basal cells and (D) positive expression of EGFR was observed in the membrane and cytoplasm of cancer tissue cells. (E) Normal esophageal mucosal epithelial cells were negative for the expression of VEGFR-2. (F) Positive expression of VEGFR-2 was observed in the interstitial vascular epithelium and carcinoma cytoplasm of esophageal carcinoma tissue samples. Immunohistochemical streptavidin peroxidase-conjugated method (magnification, x20). EGFR, epidermal growth factor receptor; VEGFR-2, vascular endothelial growth factor receptor-2.

Table III. Correlation between expression levels of EGFR and VEGFR-2 and various clinicopathological factors in Uygur patients with esophageal carcinoma.

A, EGFR expression

		EGFR expression				
Clinicopathological factor	n	High	Low	Positive, %	P-value	χ^2
Gender					0.7851	
Male	28	20	8	71.43		
Female	13	8	5	61.54		
Age, years					0.7404	
<60	24	17	7	70.83		
≥60	17	12	5	70.59		
Tumor size, cm					0.7625	
<4	22	16	6	72.73		
≥4	19	13	6	68.62		
Tumor differentiation					>0.05	1.29
High	12	8	4	66.66		
Medium	16	11	5	68.75		
Low	13	11	2	84.61		
Lymph node metastasis					0.0004	
Yes	23	20	3	86.96		
No	18	6	12	33.33		

B, VEGFR-2 expression

		VEGFR-2 expression				
Clinicopathological factor	n	High	Low	Positive, %	P-value	χ^2
Gender					0.7969	
Male	28	22	6	78.57		
Female	13	9	4	69.23		
Age, years					0.9652	
<60	24	18	6	75.00		
≥60	17	12	5	70.58		
Tumor size, cm					0.945	
<4	22	16	6	72.72		
≥4	19	14	5	73.68		
Tumor differentiation					>0.05	0.37
High	12	8	4	66.66		
Medium	16	11	5	68.75		
Low	13	10	3	76.92		
Lymph node metastasis					0.0003	
Yes	23	21	2	91.30		
No	18	7	11	38.88		

EGFR, epithelial growth factor receptor; VEGFR-2, vascular endothelial growth factor receptor-2.

the ethnic composition of the Xinjiang area, the 119 cases of esophageal cancer were divided into 3 groups: Uygur, Han and Kazak. The percentage of patients <60 years old in the three groups was 58% in Uygur, 26% in Han and 47% in Kazak; however, these differences in age were not determined to be statistically significant (P>0.05).

Table IV. Correlation between expression levels of EGFR and VEGFR-2 with various clinicopathological factors in Han patients with esophageal carcinoma.

A, EGFR expression

Clinicopathological factor	n	EGFR expression		Positive, %	P-value	χ^2
		High	Low			
Gender					0.435	
Male	24	18	6	75.00		
Female	14	8	6	57.14		
Age, years					0.1889	
<60	10	9	1	90.00		
≥60	28	17	11	60.71		
Tumor size, cm					0.2391	
<4	20	12	8	60.00		
≥4	18	14	4	77.77		
Tumor differentiation					>0.05	2.88
High	11	9	3	81.81		
Medium	13	7	6	53.84		
Low	14	11	3	78.57		
Lymph node metastasis					0.001	
Yes	19	16	3	84.21		
No	19	6	13	31.57		

B, VEGFR-2 expression

Clinicopathological factor	n	VEGFR-2 expression		Positive, %	P-value	χ^2
		High	Low			
Gender					0.1655	
Male	24	20	4	83.33		
Female	14	8	6	57.14		
Age, years					0.1889	
<60	10	9	1	90.00		
≥60	28	17	11	60.71		
Tumor size, cm					0.9139	
<4	20	13	7	65.00		
≥4	18	12	6	66.66		
Tumor differentiation					>0.05	2.88
High	11	9	3	81.81		
Medium	13	7	6	53.84		
Low	14	11	3	78.57		
Lymph node metastasis					0.0202	
Yes	19	15	4	78.94		
No	19	8	11	42.10		

EGFR, epithelial growth factor receptor; VEGFR-2, vascular endothelial growth factor receptor-2.

EGFR and VEGFR-2 are not differentially expressed between ethnic groups. Only base levels of staining were observed in the epithelial basal cells of normal esophageal mucosa epithelium (distance from the tumor margin, >5 cm) (Fig. 1A and B), and therefore basal cells were considered negative for EGFR expression (Fig. 1C). Hematoxylin-eosin staining indicated that EGFR

Table V. Correlation between expression levels of EGFR and VEGFR-2 with various clinicopathological factors in Kazak patients with esophageal carcinoma.

A, EGFR expression

		EGFR expression				
Clinicopathological factor	n	High	Low	Positive, %	P-value	χ^2
Gender					0.498	
Male	28	21	7	75.00		
Female	12	7	5	58.33		
Age, years					0.9058	
<60	19	13	6	68.42		
≥60	21	14	7	66.66		
Tumor size, cm					0.9781	
<4	26	17	9	65.38		
≥4	14	10	4	71.42		
Tumor differentiation					>0.05	1.72
High	15	10	5	66.67		
Medium	14	7	6	57.14		
Low	11	9	2	81.82		
Lymph node metastasis					0.0131	
Yes	17	14	3	82.35		
No	23	10	13	43.47		

B, VEGFR-2 expression

		VEGFR-2 expression				
Clinicopathological factor	n	High	Low	Positive, %	P-value	χ^2
Gender					0.2385	
Male	28	21	7	75.00		
Female	12	6	6	50.00		
Age, years					0.577	
<60	19	12	7	63.16		
≥60	21	15	6	71.43		
Tumor size, cm					0.5013	
<4	26	19	7	73.08		
≥4	14	8	6	57.14		
Tumor differentiation					>0.05	0.21
High	15	10	5	66.67		
Medium	14	9	5	64.29		
Low	11	8	3	72.73		
Lymph node metastasis					0.0189	
Yes	17	13	4	76.47		
No	23	9	14	39.13		

EGFR, epithelial growth factor receptor; VEGFR-2, vascular endothelial growth factor receptor-2.

localized at the cell membrane and cytoplasm of esophageal cancer cells (Fig. 1D). EGFR expression in esophageal carcinoma of the three groups was as follows: Uygur, 3 cases negative, 7 cases weakly positive, 15 cases positive and 16 cases strongly positive; Han, 4 cases negative, 7 cases weakly positive, 9 cases positive and 18 cases strongly positive; and Kazak, 3 cases

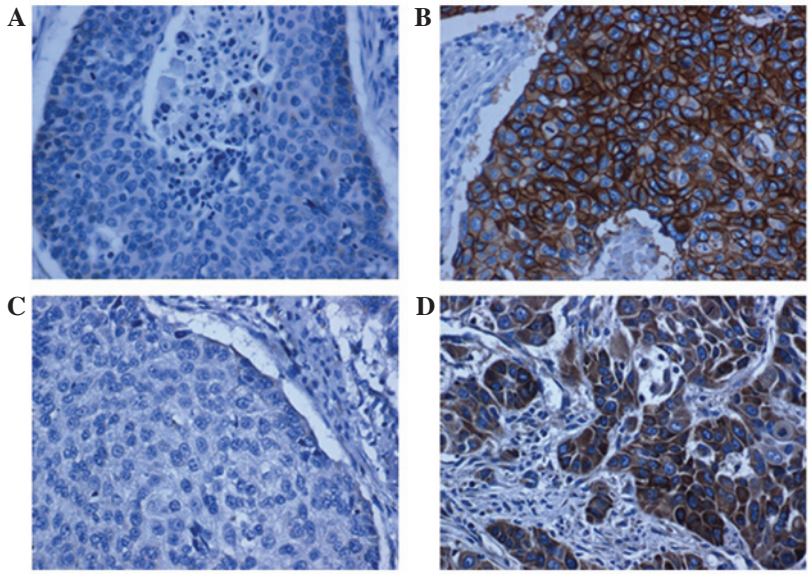

Figure 2. Detection of the expression of EGFR and VEGFR-2 by immunohistochemistry. (A) Negative and (B) strong positive expression of EGFR protein. (C) Negative and (D) strong positive expression of VEGFR-2 protein. Immunohistochemical streptavidin peroxidase-conjugated method; magnification, x40. EGFR, epidermal growth factor receptor; VEGFR-2, vascular endothelial growth factor receptor-2.

negative, 7 cases weakly positive, 14 cases positive, and 16 cases strongly positive. The normal esophageal mucosa epithelium was negative for VEGFR-2 (Fig. 1E), while VEGFR-2-positive staining was identified to be at the cytoplasm and vascular endothelial cells of esophageal carcinoma samples (Fig. 1F). VEGFR-2 expression in esophageal carcinoma of the three ethnic groups were as follows: Uygur, 6 cases negative, 4 cases weakly positive, 22 cases positive and 9 cases strongly positive; Han, 6 cases negative, 5 cases weakly positive, 19 cases positive and 8 cases strongly positive; and Kazak, 6 cases negative, 7 cases weakly positive, 21 cases positive and 6 cases strongly positive.

Statistical analysis indicated no significant differences in the protein expression of EGFR and VEGFR-2 among the three ethnic groups of patients (Tables I and II; $P>0.05$).

Association between expression of EGFR and VEGFR-2 and clinicopathological factors. No significant differences were detected in the EGFR- or VEGFR-2-positive rate of esophageal carcinoma tissues according to age, gender, tumor size or tumor differentiation of the Uygur, Han and Kazak groups (Tables III-V; $P>0.05$). However, a significant difference was identified in lymph node metastasis between the three groups ($P<0.05$). In addition, although statistically insignificant, EGFR expression was strongly positive (+++) in low and medially differentiated esophageal carcinoma, and moderate (++) or weakly positive (+) in highly differentiated cancer tissues.

Association between EGFR and VEGFR-2 expression. A correlation study was performed on the protein expression of EGFR and VEGFR-2 in 119 esophageal cancer specimens from the Uygur, Han and Kazak ethnic groups. Low expression was defined as (-) and (+), while (++) and (+++) were considered to indicate positive expression (Fig. 2). Statistical analysis revealed a significant correlation between the expression of EGFR and VEGFR-2 in all three groups (Table VI; $P<0.05$).

EGFR and VEGFR-2 expression in carcinoma in situ samples. Carcinoma *in situ* samples were stained with hematoxylin and eosin (Fig. 3A). EGFR expression was positive in 5 cases of carcinoma *in situ* (Fig. 3B), while VEGFR-2 expression was negative (Fig. 3C). These results indicate that EGFR expression may be an early event of esophageal cancer.

Discussion

To date, surgery remains the primary treatment for esophageal carcinoma. Unfortunately, due to the atypical early symptoms of esophageal carcinoma, ~85% patients that seek medical advice have already progressed to an advanced or metastatic stage, which cannot be treated by surgery. Since there is currently no alternative effective treatment available, the prognosis of esophageal carcinoma is poor, with a 5-year survival rate of 8-11%. Though comprehensive treatment and radical chemoradiotherapy have been introduced, esophageal carcinoma survival rates have been minimally altered (1,2). Thus, improving patient prognosis and prolonging survival times has become pivotal to the development of novel treatments for esophageal cancer. Recently, the majority of research has focused on chemotherapy-based treatments with combinative targeted therapy, particularly aimed at the epidermal growth factor and vascular endothelial growth factor receptors. Proto-oncogene EGFR has a critical role in the development of esophageal cancer (7,10,13). The abnormal activation of EGFR promotes tumorigenesis and proliferation through regulating cell signaling transduction, cell proliferation, apoptosis and angiogenesis. Gibault *et al* (15) demonstrated that EGFR overexpression was significantly correlated with vascular invasion ($P=0.023$), local recurrence ($P=0.006$) and a lower survival rate ($P=0.003$). In addition, a further study found that EGFR inhibitors were able to inhibit the overexpression of EGFR and human epidermal growth factor receptor-2, thereby inhibiting the proliferation of esophageal cancer cell lines (16). These

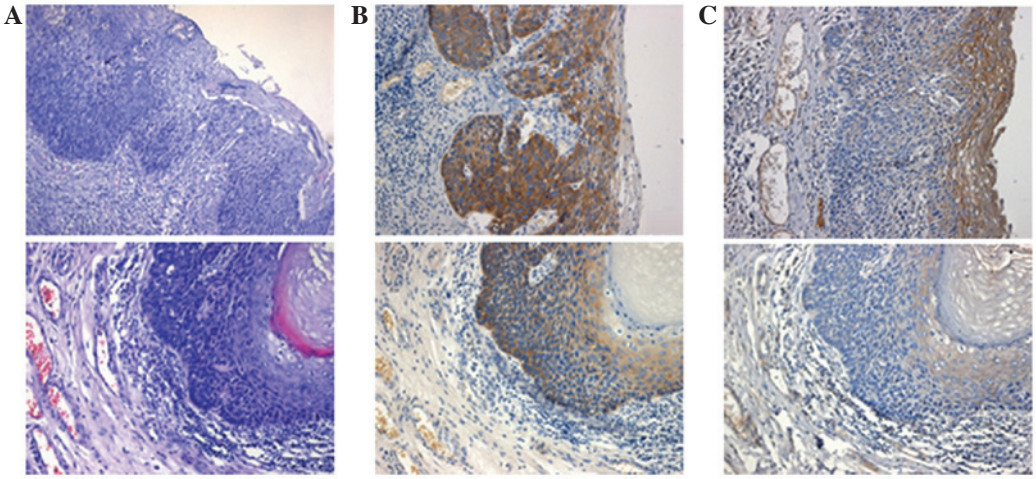

Figure 3. EGFR and VEGFR-2 protein expression in a single representative samples of carcinoma *in situ*. (A) Hematoxylin and eosin staining. (B) Positive expression of EGFR. (C) Negative expression of VEGFR-2. Immunohistochemical streptavidin peroxidase-conjugated method. Upper panel magnification, x40; lower panel magnification, x20. EGFR, epidermal growth factor receptor; VEGFR-2, vascular endothelial growth factor receptor-2.

Table VI. Association between the expression of EGFR and VEGFR-2.

			EGFR		
Ethnicity	n	VEGFR-2	High	Low	P-value
Uygur	41	High	27	4	<0.05
		Low	2	8	
Han	38	High	24	2	<0.05
		Low	2	10	
Kazak	40	High	25	2	<0.05
		Low	2	11	

EGFR, epidermal growth factor receptor; VEGFR-2, vascular endothelial growth factor receptor-2.

results suggest that the expression of EGFR is critical for the determination of targeted therapy for esophageal cancer.

Similarly, another tyrosine kinase receptor, VEGFR-2, is closely associated with tumor angiogenesis (8,12,13). In the tumor tissue, tumor cells and host cells secrete a series of cytokines, generating a favorable microenvironment for angiogenesis; VEGF is one of these cytokines (8,13,17). VEGFR-2 is activated following stimulation with activation signals, for example binding to its ligand VEGF. A series of cascade reactions downstream of the signal transduction are initiated, enhancing gene transcription in the nucleus and promoting the proliferation and angiogenesis of vascular endothelial cells (8,13,16). Zhang *et al* (18) reported a xenograft model of human esophageal adenocarcinoma tissue and mouse tumor through application of neoplastic and non-neoplastic Barrett epithelial cells, confirming the aforementioned hypothesis. However, to the best of our knowledge, there has been no report regarding the correlation between the expression and activation of EGFR and VEGFR-2 in esophageal cancer tissues. To the best of our knowledge, the present study is the first study of EGFR and VEGFR-2 in esophageal carcinoma based on samples from various ethnic groups in Xinjiang.

In the present study, the expression levels of EGFR and VEGFR-2 in esophageal carcinoma patients of various ethnicities were detected by immunohistochemistry. The results revealed that adjacent normal esophageal mucosa tissues were negative for EGFR and VEGFR-2 staining, while overexpression of EGFR was detected in 70.73% of Uygur, 68.42% of Han and 67.5% of Kazak esophageal cancer tissue samples and VEGFR-2 was overexpressed in 73.17, 68.42 and 67.5% of the Uygur, Han and Kazak groups, respectively. However, statistical analysis revealed that the EGFR and VEGFR-2 protein expression rate was not significantly different between the three ethnic groups ($P>0.05$). The expression rate of EGFR in esophageal carcinoma detected here was comparable to that observed in the Huizhou Hakka population (19) and the results of a study by Carneiro *et al* (20) (69.81%). Therefore, further studies are required to confirm whether there may be regional differences in esophageal cancer.

Overexpression of EGFR in tumor cells, leading to uncontrolled cell growth and malignant transformation, is associated with the poor prognosis of tumor-associated diseases (9,18). In addition, the overexpression of EGFR in esophageal cancer patients is significantly associated with vascular invasion (21). Based on the comparison of the expression levels of EGFR and VEGFR-2 in the three ethnic groups evaluated, the present study further analyzed the correlation between the expression rate of EGFR, VEGFR-2 and the clinicopathological parameters of patients with esophageal cancer. The results identified no significant differences in the expression rate of EGFR between gender, age or tumor size in esophageal cancer tissues between the three groups ($P>0.05$). However, a significant difference in the expression levels of EGFR was detected between patients with and without lymph node metastasis ($P<0.05$). In addition, although statistically insignificant, EGFR expression was strongly positive (+++) in low and medially differentiated esophageal carcinoma, and moderate (++) or weakly positive (+) in highly differentiated cancer tissues. Thus, EGFR may have a significant role

in the occurrence, development and lymph node metastasis of esophageal cancer in various ethnic groups in Xinjiang, and may potentially be used as an index for the prediction of metastasis and prognosis. Similarly, VEGFR-2 overexpression is associated with tumor progression, lymph node metastasis and poor prognosis (12,13,16). A total of 119 cases of esophageal carcinoma tissue samples from multi-ethnic patients in Xinjiang were used to evaluate the correlation between the expression of EGFR and VEGFR-2, and the results revealed a significant correlation ($P<0.05$). These results demonstrate that EGFR and VEGFR-2 may have a synergistic effect in the development of esophageal carcinoma in various ethnic groups in Xinjiang.

In addition, EGFR expression was positive and VEGFR-2 expression was negative in 5 cases of carcinoma *in situ*. Therefore, it was hypothesized that overexpression of EGFR may be an early event of esophageal cancer and may be used as an early indicator of tumor development. However, further studies with a larger cohort are required to verify this hypothesis.

In 119 cases of esophageal cancer, the proportion of Uygur patients ≤60 years was relatively high, compared with that of the Han and Kazak populations. This may be a result of the dietary habits typical of the Uygur population, which include large quantities of barbecue, hot food and low fresh vegetable intake, as well as the poor sanitary conditions and regional economy in southern Xinjiang (3,4).

In conclusion, the present study found that overexpression of EGFR and VEGFR-2 in Xinjiang Uygur, Han and Kazak patients with esophageal carcinoma was associated with tumor occurrence, development and lymph node metastases, providing an experimental basis for tumor therapy targeting EGFR and VEGFR-2. However, the occurrence and development of esophageal cancer is a gradual and complex process involving numerous factors. Thus further study focusing on the specific mechanisms of activation of the EGFR and VEGFR-2 genes involved in the occurrence, development and metastasis of esophageal carcinoma are required.

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