Original Article

Co-expression of VEGF-C and survivin predicts poor prognosis in esophageal squamous cell carcinoma

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Background: Lymphatic metastasis is one of the main factors affecting prognosis in esophageal squamous cell carcinoma (ESCC). Vascular endothelial growth factor-C (VEGF-C) is an important factor that promotes lymphangiogenesis. Survivin also plays a significant role in lymphatic invasion. However, the role and mechanism of their co-expression are still unclear in ESCC. The purpose of this study was to investigate whether the co-expression of VEGF-C and survivin could be a potential marker to predict patient prognosis and survival in ESCC.

Methods: The levels of VEGF-C, vascular endothelial growth factor receptor 3 (VEGFR-3), survivin, and Ki-67 were determined by immunohistochemistry (IHC) in 97 ESCC patient tumors. The correlations of co-expression of VEGF-C and survivin with pathological features and survival results were also assessed.

Results: High VEGF-C expression was observed in 64.9% of the patients and significantly correlated with T stage (P=0.024), node status (P=0.038), and lymph node metastasis (P=0.015). High survivin expression was significantly associated with T stage (P=0.013), N stage (P=0.016), lymph node metastasis (P=0.005), and differentiation (P=0.044) in 67.0% of the patients. Co-expression of VEGF-C and survivin (V+S+) was significantly associated with T stage (P<0.001), N stage (P=0.015), lymph node metastasis (P=0.003), differentiation (P=0.0045), and Ki-67 levels (P=0.024). High expression of VEGF-C or survivin was associated significantly with worse disease-free survival (DFS) and overall survival (OS) (P<0.05). Moreover, the V+S+ group had a worse DFS (P<0.001) and OS (P=0.001) than any other group (i.e., V–S–, V+S–, V–S+). Furthermore, multivariate DFS analyses (95% CI: 1.147–2.220, P=0.006) and multivariate OS analyses (95% CI: 1.080–2.193, P=0.017) revealed that co-expression of VEGF-C and survivin was an independent prognostic factor in ESCC patients.

Conclusions: Co-expression of VEGF-C and survivin was predictive of poor prognosis in ESCC. Combined detection of VEGF-C and survivin could represent a feasible and effective marker to predict the prognosis and survival of ESCC patients.

Keywords: Vascular endothelial growth factor-C (VEGF-C); survivin; esophageal squamous cell carcinoma (ESCC); lymph node metastasis; prognosis

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Introduction

Esophageal cancer (EC) is the eighth most common malignant tumor and the sixth most leading cause of cancer death worldwide (1,2). China has the highest morbidity and mortality from EC in the world, particularly from esophageal squamous cell carcinoma (ESCC) (1,3). The average number of new EC cases is about 223,000 per year, with 150,000 deaths (1). There are radiotherapy, chemotherapy and surgery, the main treatment for EC (4). Even with surgery, the 5-year survival rate is low, mainly due to recurrence and metastasis (5). For the locally advanced ESCC, the clinical trial NEOCRTEC5010 demonstrated that neoadjuvant chemoradiotherapy improves survival over surgery alone (6). Growing evidence has indicated that prognostic biomarkers, including carbohydrate antigen 72-4 (CA72-4), cytokeratin 19 fragment antigen 21-1 (Cyfra21-1) and carbohydrate antigen 19-9 (CA19-9) were reported to be crucial in diagnosis and prognosis in ESCC (7). Lymphatic involvement is the main route of EC metastasis and one of the prognostic factors in ESCC (8). Identifying a factor that can be used for early detection of lymph node metastasis to predict the prognosis of ESCC patients is essential. Some studies have indicated that vascular endothelial growth factor-C (VEGF-C) and survivin, a member of the inhibitor of apoptosis protein (IAP) family, may be involved in lymphatic metastasis.

VEGF-C is a stimulator of lymphatic endothelial cells. Multiple studies have demonstrated a relationship between VEGF-C, its receptors [vascular endothelial growth factor receptor 3 (VEGFR-3)], and micro-lymphatic vessel density (MLVD) (8), suggesting that VEGF-C and VEGFR-3 are important factors in inducing lymphangiogenesis (9,10) and promoting cancer metastasis (11,12). Survivin can promote cell proliferation and inhibit cell apoptosis (13). It is a key factor in tumor angiogenesis, metastasis (14,15), and progression (16). Studies in oral squamous cell carcinoma and breast, esophageal, and gastric cancer have demonstrated a significant relationship between survivin expression and lymphatic metastasis (17-19) and identified survivin as a prognostic factor. Furthermore, the expression of both VEGF-C and survivin has been positively correlated with lymph node invasion (20). Zhang *et al.* (21) demonstrated that HIF-1α, survivin and VEGF were positive correlation in EC, suggesting that they may play a synergistic role in the occurrence and development of EC. However, studies on the relationship between VEGF-C and survivin and their combined role in determining the prognosis and survival of ESCC patients are scarce. The current study aimed to analyze the potential link between the co-expression of VEGF-C and survivin and clinicopathological features, particularly the influence of VEGF-C and survivin co-expression on the prognosis of ESCC patients. This study also investigated whether the analysis VEGF-C and survivin co-expression could be used as a feasible and effective marker to predict the prognosis and survival of ESCC patients. We present the following article in accordance with the REMARK reporting checklist (available at http://dx.doi.org/10.21037/tcr-20-2498).

Methods

Patients

A total of 97 patients at the Shantou University Medical College Cancer Hospital were enrolled in this study from May 2014 to April 2016. Inclusion criteria was defined as: patients with ESCC without surgical treatment and distant metastasis. Exclusion criteria was those who did not meet the above inclusion criteria. There were 78 males and 19 females, ranging from 43 to 75 years of age, with a mean age of 59.28 years and a median age of 60 years. According to the infiltration depth, eight patients had pT1 tumors, nine had pT2 tumors, 79 had pT3 tumors, and one patient had a pT4 tumor. Tumor metastasis to the lymph nodes was detected in 56 cases (57.7%). Among the 97 cases, 38 were stage N1, 14 were stage N2, four were stage N3, and the remaining 41 cases were stage N0. In total, there were 33 cases with high differentiation, 54 with medium differentiation, and ten with low differentiation. The median follow-up time was 37.6 (range, 6.9 to 60) months. The time from the date of definitive surgery to the date of death due to any cause or the date of the last follow-up was defined as overall survival (OS).

Experimental reagents

The VEGF-C and VEGFR-3 polyclonal antibodies were purchased from Beijing Zhongshang Biotechnology Co., Ltd. (Beijing, China). The survivin and Ki-67 polyclonal antibodies, ready-to-use immunohistochemistry (IHC) MaxVision^{TM} secondary antibody, and 3,3'-diaminobenzidine (DAB) kit were purchased from Fuzhou Maixin Biotechnology Development Co., Ltd. (Fuzhou, China).

IHC

All samples were fixed in 10% neutral buffered formalin

for 8 to 48 h, followed by dehydration with alcohol and xylene. The dehydrated samples were embedded in paraffin. Immunohistochemical staining was performed using the MaxVision two-step method. Briefly, tissues were cut into 4-μm sections. The sections were baked at 65 ℃ for 1 h, deparaffinized with xylene, and rehydrated with an ethanol gradient. The sections were incubated with 0.3% H_2O_2 for 10 min to block endogenous peroxidases, followed by antigen retrieval (VEGF-C, survivin, and Ki-67 with pH 6.0, 0.01 M citrate buffer, high-pressure repair; VEGFR-3 with pH 9.0 EDTA, high-temperature repair). After allowing the samples to cool at room temperature, they were washed three times with phosphate-buffered saline (PBS) for 3 min. The sections were incubated with primary antibody for 1 h at 37 ℃ and secondary antibody at 37 ℃ for 15 min. Finally, the sections were stained for 5 min with DAB and counterstained with hematoxylin for 1 min. The slides were sealed with neutral gum. Negative controls were incubated with PBS buffer instead of the primary antibodies.

IHC analysis

Stained slides were analyzed by two pathologists in a blinded fashion. Positive cellular staining was brownish-yellow. Negative staining was defined as no significant difference in color intensity from that of the background. Staining was visualized by light microscopy. For analysis, the strongest stained area was selected under low power (50×), and then ten visual fields were observed under high power (400×). The staining for 100 cells was scored for each patient sample.

VEGF-C, VEGFR-3, and survivin staining were scored using a semi-quantitative scoring system, as previously described (22-24). Briefly, scoring was performed according to the intensity of specific staining: 0, no staining; 1, weak staining; 2, intermediate staining; 3, strong staining. In addition, the percentage of positive cells within the total cells counted was semi-quantitatively scored as follows: 0, negative; 1, 1% to 10% positive; 2, 11% to 50% positive; 3, >50%. Finally, the intensity and percentage scores were multiplied to yield the immunohistochemical score. An immunohistochemical score of ≥3 was considered positive. Specifically, the final immunohistochemical scores were defined as follows: 3 to 4, weakly positive (+); 5 to 7, moderately positive (++); 8 to 9, strongly positive (+++). Ki-67 staining was analyzed as the percentage of Ki-67-positive cells in the total tumor cells.

Statistical analysis

All data were analyzed using SPSS 19.0 statistical software. The measurement data are presented as the mean ± standard deviation ($\bar{x}$ ± SD). The counting data are expressed as the rate using Pearson's χ^2 test. Spearman used for correlation analysis. Survival curves were assessed using the Kaplan-Meier method, and the data were compared using the log-rank test. Univariate and multivariate analyses used to determine the impact of the variables on patient survival. Two-sided P values <0.05 were considered statistically significant. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by medical ethics committee of the Cancer Hospital of Shantou University Medical College (No. 2019039). The informed consent is not required for this study.

Results

Correlation between the expression of VEGF-C, VEGFR-3 and survivin and clinicopathological features of ESCC

To investigate whether VEGF-C, VEGFR-3, survivin, and Ki-67 were expressed in ESCC, we performed IHC on tumors from patients with ESCC. We observed that VEGF-C, VEGFR-3, and survivin were mainly located in the cytoplasm of cancer cells and interstitial cells that were surrounding the cancer nest. The staining was brownish-yellow, granular, and focal or diffusely distributed in the marginal part of the cancer nest. Survivin was occasionally present in the nucleus, whereas Ki-67 was specifically localized in the nucleus. Representative IHC images of VEGF-C, VEGFR-3, survivin were presented in *Figure 1*. The positive rates of VEGF-C, VEGFR-3, and survivin expression were 64.9% (63/97), 51.5% (50/97), and 67.0% (65/97), respectively. As shown in *Table 1*, positive VEGF-C expression was associated with advanced T stage (70.0% *vs.* 41.2%, P=0.024), more advanced N stage (100.0% *vs.* 85.7% *vs.* 68.4% *vs.* 48.8%, P=0.038), and lymph node metastasis (75.0% *vs.* 51.2%, P=0.015). However, there were no statistical differences between male and female groups, <60- and ≥60-year-old age groups, perineural invasion negative and positive groups, or well-differentiation and poor differentiation groups (P>0.05).

The correlations between survivin and clinicopathological characteristics shown in *Table 1*. Positive survivin expression was associated with advanced T stage (72.5% *vs.* 41.2%, P=0.013), more advanced node stage

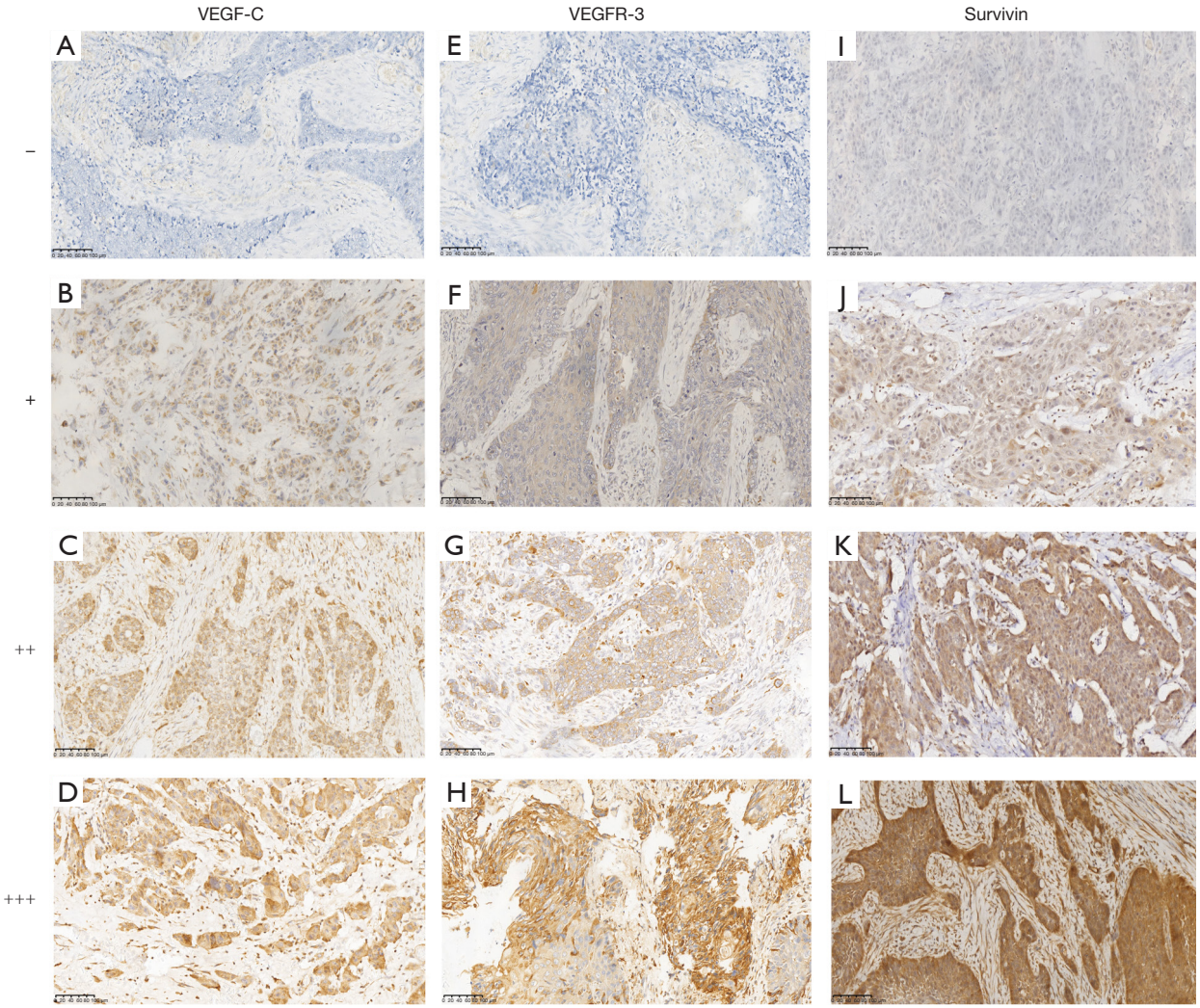

Figure 1 Representative images of immunohistochemical staining for VEGF-C, VEGFR-3, survivin and Ki-67 in ESCC tumors (magnification: 200×). (A) VEGF-C negative expression; (B) VEGF-C positive staining (+), (C) (++), (D) (+++); (E) VEGFR-3 negative expression; (F) VEGFR-3 positive staining (+), (G) (++), (H) (+++); (I) survivin negative expression; (J) survivin positive staining (+), (K) (++), (L) (+++). VEGF-C, vascular endothelial growth factor-C; VEGFR-3, vascular endothelial growth factor receptor 3; ESCC, esophageal squamous cell carcinoma.

(100.0% *vs.* 64.3% *vs.* 81.6% *vs.* 51.2%, P=0.016), lymph node metastasis (78.6% *vs.* 51.2%, P=0.005), and worse differentiation (100.0% *vs.* 66.7% *vs.* 57.6%, P=0.044). There were no differences between the male and female groups, <60- and ≥60-year-old age groups, or perineural invasion-negative and -positive groups (P>0.05). Positive VEGFR-3 expression was associated with more advanced lymph node status (75.0% *vs.* 78.6% *vs.* 57.9% *vs.* 34.1%, P=0.014) and lymph node metastasis (64.3% *vs.* 34.1%, P=0.003). However, there were no statistical differences between the male and female groups, <60- and ≥60-year-old age groups, P ≤ T2 and P > T2 groups, perineural invasion-negative and -positive groups, or well differentiation and poor differentiation groups (P>0.05).

Association of co-expression of VEGF-C and survivin with pathological characteristics of ESCC

As shown in *Table 2*, survivin expression was positively correlated with VEGF-C (r_s=0.280, P=0.006) and VEGFR-3 (r_s=0.286, P=0.005) in the 97 ESCC tissues. Based on the expression patterns of VEGF-C and survivin, the enrolled

Table 1 Correlation of VEGF-C, VEGFR-3 and survivin expression with clinicopathological characteristics in 97 ESCC patients

Features	N	VEGF-C positive				VEGFR-3 positive				Survivin positive			
		Case	%	χ^2	P	Case	%	χ^2	P	Case	%	χ^2	P
Gender													
Male	78	51	65.4	0.033	0.855	40	51.3	0.011	0.916	54	69.2	0.888	0.346
Female	19	12	63.2			10	52.6			11	57.9		
Age (y)													
<60	47	30	63.8	0.500	0.823	26	55.3	0.520	0.471	29	61.7	1.162	0.281
≥60	50	33	66.0			24	48.0			36	72.0		
T stage													
≤T2	17	7	41.2	5.117	0.024	7	41.2	0.887	0.346	7	41.2	6.223	0.013
>T2	80	56	70.0			43	53.8			58	72.5		
N stage													
N0	41	20	48.8	8.406	0.038	14	34.1	10.558	0.014	21	51.2	10.289	0.016
N1	38	26	68.4			22	57.9			31	81.6		
N2	14	12	85.7			11	78.6			9	64.3		
N3	4	4	100.0			3	75.0			4	100.0		
Lymph node metastasis													
Negative	41	21	51.2	5.880	0.015	14	34.1	8.609	0.003	21	51.2	8.010	0.005
Positive	56	42	75.0			36	64.3			44	78.6		
Perineural invasion													
Negative	87	57	65.5	0.120	0.729	45	51.7	0.011	0.918	56	64.4	2.666	0.103
Positive	10	6	60.0			5	50.0			9	90.0		
Differentiation													
Low	10	8	80.0	1.114	0.573	6	60.0	0.412	0.814	10	100.0	6.225	0.044
Medium	54	34	63.0			28	51.9			36	66.7		
High	33	21	63.6			16	48.5			19	57.6		

Statistically significant (P<0.05) values are in bold. VEGF-C, vascular endothelial growth factor-C; VEGFR-3, vascular endothelial growth factor receptor 3; ESCC, esophageal squamous cell carcinoma.

patients were classified into four groups: VEGF-C (–)/survivin (–), (V–S–); VEGF-C (+)/survivin (–), (V+S–); VEGF-C (–)/survivin (+), (V–S+); VEGF-C (+)/survivin (+), (V+S+). We found that the frequency of V+S+ was higher in advanced T stage (P<0.001). As shown in *Table 3*, there was a significant association of the V+S+ group with lymph node involvement. Patients with N3 lymph node metastasis had a significant higher proportion of V+S+ (100%) than N0–2 patients (29.3%/52.6%/57.1%) (r_s=0.336, P=0.001). Co-expression of VEGF-C and survivin was associated with a lower level of differentiation (r_s=–0.204, P=0.0045). However, there were no statistical differences between the V+S+ and other groups in terms of gender, age, or perineural invasion (P>0.05). As shown in *Table 4*, the V+S+ group had significantly higher Ki-67 levels (proliferation index) compared to the other groups (V–S–/V+S–/V–S+) (r_s=0.230, P=0.024).

Table 2 Correlation of survivin with VEGF-C and VEGFR-3 expression in 97 ESCC patients

Biomarkers	N	Survivin				r_s	P
		–	+	++	+++		
VEGF-C							
–	34	13	20	1	0	0.280	0.006
+	39	13	18	7	1		
++	23	6	7	9	1		
+++	1	0	0	1	0		
VEGFR-3							
–	47	20	21	5	1	0.286	0.005
+	40	12	19	9	0		
++	9	0	4	4	1		
+++	1	0	1	0	0		

Statistically significant (P<0.05) values are in bold. VEGF-C, vascular endothelial growth factor-C; VEGFR-3, vascular endothelial growth factor receptor 3; ESCC, esophageal squamous cell carcinoma.

Table 3 Correlations of different expression status of VEGF-C and survivin with clinicopathological characteristics in 97 ESCC patients

Features	V–S– (n=13)		V+S– (n=19)		V–S+ (n=21)		V+S+ (n=44)		χ^2	P
	Case	%	Case	%	Case	%	Case	%		
Gender										
Male	10	12.8	15	19.2	17	21.8	37	47.4	0.775	0.855
Female	3	15.8	5	26.3	4	21.1	7	36.8		
Age (y)										
<60	7	14.9	11	23.4	10	21.3	19	40.4	0.325	0.723
≥60	6	12.0	8	16.0	11	22.0	25	50.0		
T stage										
≤T2	8	47.1	2	11.8	2	11.8	5	29.4	20.152	<0.001
>T2	5	6.3	17	21.3	19	23.8	39	48.8		
N stage										
N0	11	26.8	9	22.0	9	22.0	12	29.3	20.515	0.015
N1	1	2.6	6	15.8	11	28.9	20	52.6	0.336*	0.001#
N2	1	7.1	4	28.6	1	7.1	8	57.1		
N3	0	0.0	0	0.0	0	0.0	4	100.0		
Lymph node metastasis										
Negative	11	26.8	9	22.0	9	22.0	12	29.3	13.814	0.003
Positive	2	3.6	10	17.9	12	21.4	32	57.1		

Table 3 (*continued*)

Table 3 (*continued*)

Features	V–S– (n=13)		V+S– (n=19)		V–S+ (n=21)		V+S+ (n=44)		χ^2	P
	Case	%	Case	%	Case	%	Case	%		
Perineural invasion										
Negative	13	14.9	18	20.7	17	19.5	39	44.8	3.805	0.283
Positive	0	0.0	1	10.0	4	40.0	5	45.4		
Differentiation										
Low	0	0.0	0	0.0	2	20.0	8	80.0	7.589	0.270
Medium	7	13.0	11	20.4	13	24.1	23	42.6	–0.204*	0.045#
High	6	18.2	8	24.2	6	18.2	13	39.4		

Statistically significant (P<0.05) values are in bold. *, using spearman test, r_s value; #, using spearman test, P value. VEGF-C, vascular endothelial growth factor-C; ESCC, esophageal squamous cell carcinoma; V–S–, VEGF-C (–)/survivin (–); V+S–, VEGF-C (+)/survivin (–); V–S+, VEGF-C (–)/survivin (+); V+S+, VEGF-C (+)/survivin (+).

Table 4 Correlation between the expression of VEGF-C, survivin and Ki-67 in 97 ESCC patients

Factors	N	Ki-67 (%), mean ± SD	r_s	P
VEGF-C				
Negative	34	53.67±23.68	0.162	0.112
Positive	63	61.90±18.54		
Survivin				
Negative	32	54.53±19.19	0.187	0.066
Positive	65	61.23±21.26		
Case				
V–S–	13	50.00±17.91	0.230	0.024
V+S–	19	57.63±19.89		
V–S+	21	55.95±26.82		
V+S+	44	63.75±17.85		

Statistically significant (P<0.05) values are in bold. VEGF-C, vascular endothelial growth factor-C; ESCC, esophageal squamous cell carcinoma; SD, standard deviation; V–S–, VEGF-C (–)/survivin (–); V+S–, VEGF-C (+)/survivin (–); V–S+, VEGF-C (–)/survivin (+); V+S+, VEGF-C (+)/survivin (+).

Co-expression of VEGF-C and survivin predicts worse prognosis in ESCC

The follow-up period for all patients of the study ranged from 6.9 to 60 months, with a median duration of 37.6 months. Of the 97 ESCC patients, 42 patients died during the follow-up period. The remaining 55 patients were followed until July 2020. Factors, including patient gender, age, N stage, T stage, lymph node metastasis, perineural invasion, differentiation, VEGF-C, VEGFR-3 and survivin expression, and VEGF-C and survivin co-expression groups (V–S–/V+S–/V–S+/V+S+), were subjected to univariate and multivariate analyses for disease-free survival (DFS) and OS. The survival analysis is presented in *Tables 5*,*6* and *Figure 2*. Univariate analysis showed that six variables (greater N stage, lymph node metastasis-positive, VEGF-C-positive, VEGFR-3-positive, survivin-positive, and the V+S+ group) had a worse prognosis. Gender, age, T stage, perineural invasion, and differentiation were not correlated with DFS or OS. DFS was significantly worse in the VEGF-C-

Table 5 Univariate and multivariate DFS analyses in 97 ESCC patients

Prognostic factors	Univariate analyses			Multivariate analyses		
	OR	95% CI	P	OR	95% CI	P
Gender						
Male/female	0.960	0.480–1.921	0.908			
Age (y)						
<60/≥60	0.897	0.515–1.562	0.701			
T stage						
≤T2/>T2	1.791	0.763–4.204	0.181			
N stage						
N0/N1/N2/N3	1.989	1.478–2.678	<0.001	1.707	1.254–2.324	0.001
Lymph node metastasis						
Negative/positive	2.566	1.382–4.764	0.003			
Perineural invasion						
Negative/positive	1.683	0.755–3.750	0.203			
Differentiation						
Low/medium/high	0.794	0.516–1.222	0.294			
VEGF-C						
Negative/positive	2.382	1.243–4.567	0.009			
VEGFR-3						
Negative/positive	1.778	1.009–3.134	0.047			
Survivin						
Negative/positive	2.675	1.336–5.356	0.005			
Case						
V+S+/V+S–/V–S+/V–S–	1.822	1.323–2.509	<0.001	1.596	1.147–2.220	0.006

Statistically significant (P<0.05) values are in bold. DFS, disease-free survival; ESCC, esophageal squamous cell carcinoma; VEGF-C, vascular endothelial growth factor-C; VEGFR-3, vascular endothelial growth factor receptor 3; V–S–, VEGF-C (–)/survivin (–); V+S–, VEGF-C (+)/survivin (–); V–S+, VEGF-C (–)/survivin (+); V+S+, VEGF-C (+)/survivin (+).

positive patients compared to VEGF-C-negative patients (P=0.007, *Figure 2A*). Similarly, survival was significantly worse for the survivin-positive patients compared to the survivin-negative patients (P=0.004, *Figure 2B*). The V+S+ group had a worse prognosis than the other groups (V–S–/V+S–/V–S+) (P<0.001, *Figure 2C*). As for the OS, OS was significantly worse in the VEGF-C-positive patients compared to VEGF-C-negative patients (P=0.037, *Figure 2D*). Similarly, survival was significantly worse for the survivin-positive patients compared to the survivin-negative patients (P=0.009, *Figure 2E*). The V+S+ group had a worse prognosis than the other groups (V–S–/V+S–/V–S+) (P=0.001, *Figure 2F*). Most importantly, the multivariate DFS and OS analysis demonstrated that N stage, co-expression of VEGF-C and survivin were independent prognostic factors for ESCC (DFS: 95% CI: 1.254 to 2.324, P=0.001; 95% CI: 1.147 to 2.220, P=0.006; OS: 95% CI: 1.142 to 2.205, P=0.006; 95% CI: 1.080 to 2.193, P=0.017) (*Tables 5*,*6*).

Discussion

Tumor spread and metastasis are the leading causes of treatment failure. Lymphatic metastasis is one of the primary

Table 6 Univariate and multivariate OS analyses in 97 ESCC patients

Prognostic factors	Univariate analyses			Multivariate analyses		
	OR	95% CI	P	OR	95% CI	P
Gender						
Male/female	0.850	0.394–1.833	0.172			
Age (y)						
<60/≥60	0.915	0.503–1.665	0.772			
T stage						
≤T2/>T2	1.788	0.704–4.544	0.222			
N stage						
N0/N1/N2/N3	1.836	1.341–2.513	<0.001	1.587	1.142–2.205	0.006
Lymph node metastasis						
Negative/positive	2.261	1.161–4.406	0.016			
Perineural invasion						
Negative/positive	1.421	0.599–3.373	0.426			
Differentiation						
Low/medium/high	0.777	0.489–1.234	0.284			
VEGF-C						
Negative/positive	2.046	1.030–4.063	0.041			
VEGFR-3						
Negative/positive	1.933	1.041–3.588	0.037			
Survivin						
Negative/positive	2.721	1.261–5.870	0.011			
Case						
V+S+/V+S–/V–S+/V–S–	1.746	1.240–2.459	0.001	1.539	1.080–2.193	0.017

Statistically significant (P<0.05) values are in bold. OS, overall survival; ESCC, esophageal squamous cell carcinoma; VEGF-C, vascular endothelial growth factor-C; VEGFR-3, vascular endothelial growth factor receptor 3; V–S–, VEGF-C (–)/survivin (–); V+S–, VEGF-C (+)/survivin (–); V–S+, VEGF-C (–)/survivin (+); V+S+, VEGF-C (+)/survivin (+).

means of metastasis and a major factor in the prognosis of patients with ESCC. VEGF-C is a member of the VEGF family and well known for its involvement in the selective proliferation of lymphatic vessels during oncogenesis and progression (25,26). VEGF-C enhances cell proliferation, growth, and invasion, and promotes angiogenesis, tumor metastasis, and progression by activating the tyrosine kinase receptor VEGFR-3 (27). The VEGF-C/VEGFR3 signaling pathway promotes cell migration and invasion, thereby inducing tumor metastasis. High VEGF-C expression levels are associated with shorter survival time in breast (28) and lung (29,30) cancer, and ESCC (31). Melanoma patients with lymph node involvement have high VEGF-C expression (32). In addition, VEGFR-3 serum levels are significantly higher in ESCC patients than in healthy donors, suggesting that VEGFR-3 may be a valuable diagnostic marker for ESCC (33). In the current study, we evaluated the VEGF-C and VEGFR-3 expression levels in 97 ESCC patient samples by IHC and found that 64.9% of the samples were VEGF-C-positive, and 51.5% were VEGFR-3-positive. These results were consistent with previous studies (34). We found that VEGF-C was associated with T and N stage but not gender, age, perineural invasion, or differentiation. In addition,

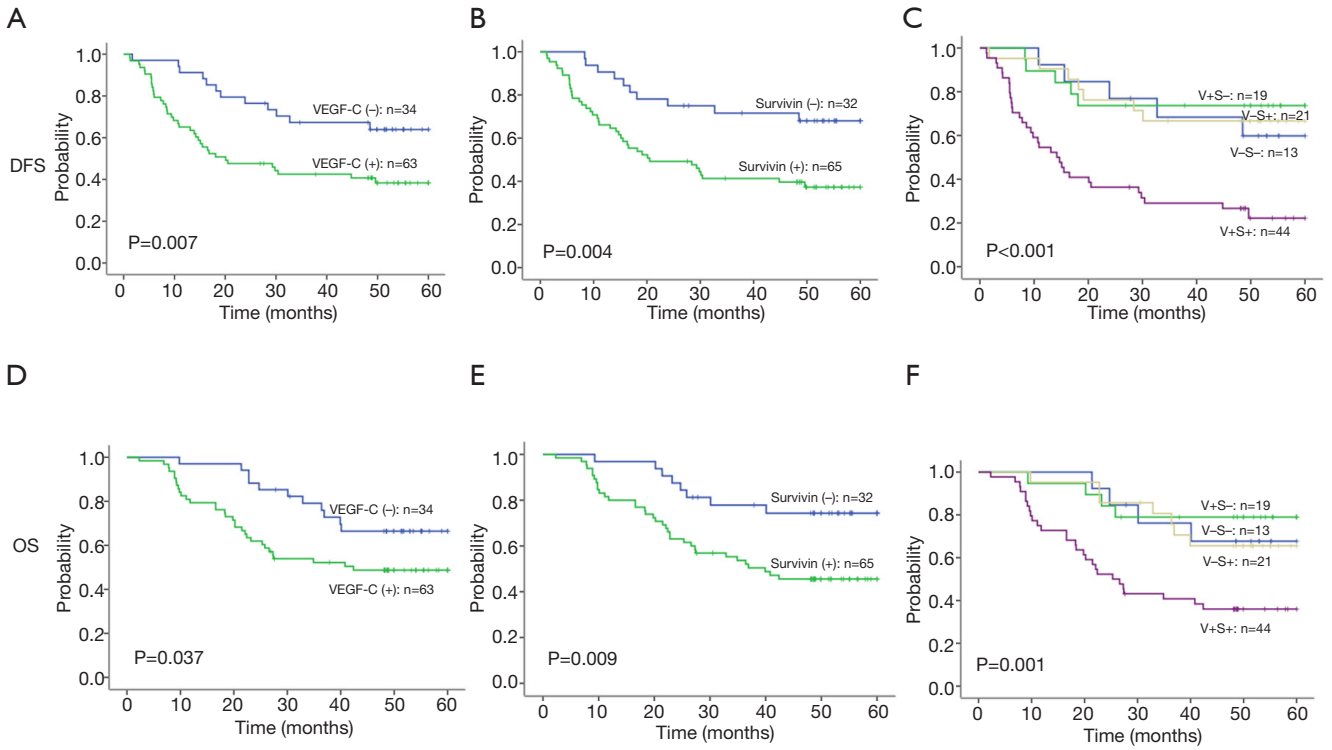

Figure 2 DFS and OS curves of VEGF-C and survivin expression in ESCC patients. (A) Over-expression of VEGF-C predicts worse DFS in patients with ESCC; (B) over-expression of survivin, predicts worse DFS in patients with ESCC; (C) patients with co-expression of VEGC-C and survivin (V+S+) have worst DFS compared to patients with other groups; (D) over-expression of VEGF-C predicts worse OS in patients with ESCC; (E) over-expression of survivin, predicts worse OS in patients with ESCC; (F) patients with co-expression of VEGC-C and survivin (V+S+) have worst OS compared to patients with other groups. DFS, disease-free survival; OS, overall survival; VEGF-C, vascular endothelial growth factor-C; ESCC, esophageal squamous cell carcinoma; V+S–, VEGF-C (+)/survivin (–); V–S+, VEGF-C (–)/survivin (+); V–S–, VEGF-C (–)/survivin (–); V+S+, VEGF-C (+)/survivin (+).

our results showed that the positive rate of VEGF-C expression in the negative lymph node metastasis group was significantly lower than that in the positive group.

Survivin is a member of the IAP family that is a significant regulator of cell proliferation and apoptosis (35,36). Survivin inhibits caspase-3 and caspase-7 activity and the processing of caspase-9, thereby preventing cell apoptosis (37). In this study, we demonstrated that 67.0% of the ESCC tumors were survivin-positive, which was similar to previously reported data (38). Survivin expression was associated with T and N stage, lymph node metastasis, and differentiation but not gender, age, or perineural invasion.

High VEGF-C or survivin expression levels are related to lymph node involvement and positively correlated with each other in papillary thyroid carcinoma (39). In breast cancer, VEGF-C and survivin are highly expressed and also positively correlated (40). The co-expression of VEGF-C and survivin is positively correlated with positive lymph node, while downregulation of survivin can decrease VEGF-C expression and reduce lymphatic metastasis and invasion and is associated with a reduction in breast cancer mortality (20). In gastric cancer cells, expression of survivin and VEGF-C are significantly associated with lymph node metastasis (41). Survivin is considered to be a regulator of VEGF-C in gastric cells, and gastric cancer patients with co-expression of survivin and VEGF-C usually have a poor prognosis. In ESCC, the exact mechanism of co-expression of VEGF-C and survivin has not yet been delineated. In our study, VEGF-C positively correlated with survivin. Furthermore, we found that co-expression of VEGF-C and survivin was associated with advanced T stage, lymph node involvement, more advanced node status, and worse differentiation. It was not statistically related to gender, age, or perineural invasion. More importantly, patients with N3

lymph node metastasis had a significant higher proportion of V+S+ (100%) than N0–2 patients (P=0.001).

Nuclear proliferating antigen (Ki-67), is expressed in all phases of the cell cycle, except G0. It is one of the most reliable indicators of tumor cell proliferation and used to predict tumor invasion and prognosis (42). Our results showed that although VEGF-C and survivin expression levels were not correlated with Ki-67, V+S+ patients (i.e., expressing both VEGF-C and survivin) had a higher positive rate for Ki-67 compared to the other expression groups (V–S–/V+S–/V–S+). Thus, ESCC occurrence may be related to an interaction between VEGF-C and survivin.

In this study, we found that patients with positive VEGF-C or survivin expression had significantly worse DFS and OS than those with negative expression. These results are consistent with previous studies that showed that overexpression of VEGF-C, VEGFR-3, or survivin was associated with poor prognosis (8,43-45). In this study, we firstly demonstrated that ESCC patients with overexpression of both VEGF-C and survivin (V+S+ group) had a worse prognosis than those in the other co-expression groups (V–S–/V+S–/V–S+). Furthermore, co-expression of VEGF-C and survivin, but not the expression of the individual genes, was an independent prognostic factor in ESCC patients.

In summary, this study found that VEGF-C and survivin overexpressed in ESCC tissues with lymph node involvement. Overexpression of VEGF-C or survivin may predict poor prognosis. Co-expression of both these factors predicts a worse prognosis in ESCC. In particular, our results identified the co-expression of VEGF-C and survivin as a potential prognostic marker for ESCC. More in-depth studies on the molecular mechanisms underlying the relationship between these two biomarkers and their roles in angiogenesis and metastasis in ESCC are required to fully understand their importance in this disease.

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Footnote

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