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Original Article

Evaluation of tumor metastasis-associated markers for molecular classification in patients with esophageal squamous cell carcinoma

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Abstract: This study aims to ascertain the relationship of tumor metastasis-associated markers cyclin D1, connective tissue growth factor (CTGF) and vascular endothelial growth factor (VEGF) with the clinicopathologic features and prognosis of patients with esophageal squamous cell carcinoma (ESCC), and to investigate their value in ESCC molecular classification. The expression of cyclin D1, CTGF and VEGF in 100 specimens from patients and 20 from normal esophageal mucosa were detected by immunohistochemistry. The relationship of their expression with prognosis of the patients with ESCC was evaluated by Cox regression model and Kaplan-Meier survival curve analysis. High levels of expression of cyclin D1, CTGF, and VEGF were observed in 61 (61%), 53 (53%), 49 (49%) cases, respectively. Univariate survival analysis indicated that the levels of expression of cyclin D1, CTGF and VEGF were associated with survival (all *P*-value < 0.05). Multivariate analysis indicated that cyclin D1 and VEGF were independent prognostic factors affecting the three-year survival rate of patients ($P = 0.001$, 0.017, respectively). Furthermore, high level expression of cyclin D1, CTGF and VEGF in stage I patients was found associated with poor three-year survival rate (all *P*-value < 0.05). The prognosis probably was favorable for patients with low expression of cyclin D1 even in stage III, or VEGF even in stage IV. Tumor metastasis-associated markers such as cyclin D1 and VEGF may be independent prognostic factors affecting survival rate of postoperative ESCC patients. It is possible to judge prognosis better and tailor treatments to each individual patient when these markers were applied to ESCC molecular classification.

Keywords: Esophageal squamous cell carcinoma, cyclin D1, CTGF, VEGF, molecular classification

Introduction

Due to its high invasiveness, easy metastasis and resistance to treatment, esophageal cancer has worse prognosis than other gastrointestinal tumors. Although TNM stage is still routinely used to judge prognosis of esophageal carcinoma in the clinical application, it is often observed that the patients with in the same TNM stage may have different outcome of prognosis. Therefore, to make more reliable prognosis of esophageal carcinoma, in addition to using the method of clinical pathologic staging, behavior of certain inherent biological factors of this carcinoma are also worth exploring. Many studies have shown that the changes of some molecular markers can aid in effectively predicting the prognosis of a variety of tumors, including esophageal cancer [1-4]. Research work showed that cyclin D1, connective tissue growth factor (CTGF) and vascular endothelial growth factor (VEGF), all of which have extensive biological activities, are closely related to the metastasis of esophageal carcinoma [5-7]. China is a high incidence area for esophageal cancer, and the pathological type of the majority of esophageal cancer is ESCC. This research was to investigate the relationship between the expression levels of cyclin D1, CTGF and VEGF in tissues of ESCC, their clinicopathological characteristics, and survival rate of the patients with ESCC at different TNM stage. A potential value of these markers for molecular classification in patients with ESCC was also examined.

Materials and methods

Patients and follow-up evaluation

Surgically removed tumors embedded in paraffin wax blocks from 100 esophagus cancer cases were retrieved from Department of Thoracic Surgery, People's Hospital of Taizhou (Taizhou Medical School, Jiangsu and Nantong University). The cases were treated between January 2009 and June 2010. A follow-up had been obtained, clinical data were available and ESCC was confirmed by pathology for all the cases selected. Mean age at surgery was 62.3±7.6 years (range 40 to 78), and 78 patients were male and 22 were female. The grade of tumor differentiation was decided by the classification of the World Health Organization [8]. In the evaluation of histological degree of differentiation, 32 cases were well differentiated, 48 cases moderately differentiated, and 20 cases poorly differentiated. Staging was decided by the American Joint Committee on Cancer TNM classification [9]. There were 45 patients with lymph node metastases, with the rest of the cases negative. 23 cases were Stage I, 34 cases were Stage II, 30 cases were Stage III, and 13 cases were Stage IV. To be selected for this retrospective study, all specimens were obtained from patients who had neither received chemotherapy nor radiotherapy prior to surgical resection. Clinical follow-up data, including overall survival and disease-specific survival, were available for all the patients. Survival rates were calculated from the date of surgery until death or the last follow-up. The last follow-up date was June 31, 2013. The median follow-up period of surviving patients was 27 months (range, 4~48 months). During the follow-up period, 43 (43%) patients were alive with no evidence of disease, it was censored. 57 died of the disease, and none died of the other diseases.

Immunohistochemical reagents and methods

The following antibodies were used in the study: rabbit monoclonal antibody antihuman cyclin D1 (Maixin. Biotechnology Co., LTD, Fuzhou, China) with the final diluted concentration of 1:100; mouse monoclonal antibody antihuman VEGF (Maixin. Biotechnology Co., LTD, Fuzhou, China) with the final diluted concentration of 1:50; mouse monoclonal antibody antihuman CTGF (Santa Cruz. LTD, America) with the final diluted concentration of 1:100, and PV-9000 test kit (Zhongshan Golden bridge Biotechnology Co., LTD, Beijing, China). The specimens were cut into 4-5 mm thick sections, mounted onto slides, deparaffinized with xylene, and rehydrated with graded concentrations of ethanol. After endogenous peroxidase activity was blocked by incubating with 3% hydrogen peroxide (H_2O_2) in deionized water for 10 min, the slides were washed three times with TBS buffer (10 mmol/L Tris-HCl, 100 mmol/L NaCl, pH 7.5) for 2 min. Before application of the primary antibody, an antigen retrieval technique was used (10 mmol/L sodium citrate solution, pH 6.0 in a rice cooker, at 640 W for 30 min). After three washes with TBS, an aliquot of 100 μL of primary antibody was then applied to each section and incubated at 4°C overnight. After washing three times with TBS and following the directions in the kit manual, the agent one and the agent two (included in the kit) were applied respectively for 20 min at RT. Finally, the sections were washed three times with TBS, and the immunoreactions were visualized with 0.0067% diaminobenzidine as the substrate with 0.03% H_2O_2 in 100 mmol/L Tris-HCl buffer for 3 min. The sections were lightly counterstained in Haris hematoxylin solution for microscopic examination.

Immunohistochemical analysis for cyclin D1, CTGF and VEGF

The immunostained specimens were analyzed by two independent pathologists. The staining of cyclin D1 (brown reaction product) was confined to the nuclei of the cells. This molecular marker was observed in 1000 cells at least at high power (×400), and the percent of positive cells was used to evaluate the expression condition of cyclin D1: 0: -; 1%-10%: +; 11%~50%: ++; ≥ 51%: +++. It was considered high expression when the nuclear staining was above ++ for cyclin D1 [10]. Cytoplasm staining (brown reaction production) was regarded as a positive result for CTGF and VEGF. The expression of CTGF and VEGF were scored based on intensity and the rate of the positive cells. The intensity was graded as follows: 0, negative; 1, weak; 2, moderate; 3, strong. The rate of positive cells was defined as: 0, < 5%; 1, 5% to 25%; 2, 26% to 50%; 3, 51% to 75%; 4, > 75%. The final score was calculated by multiplying the inten-

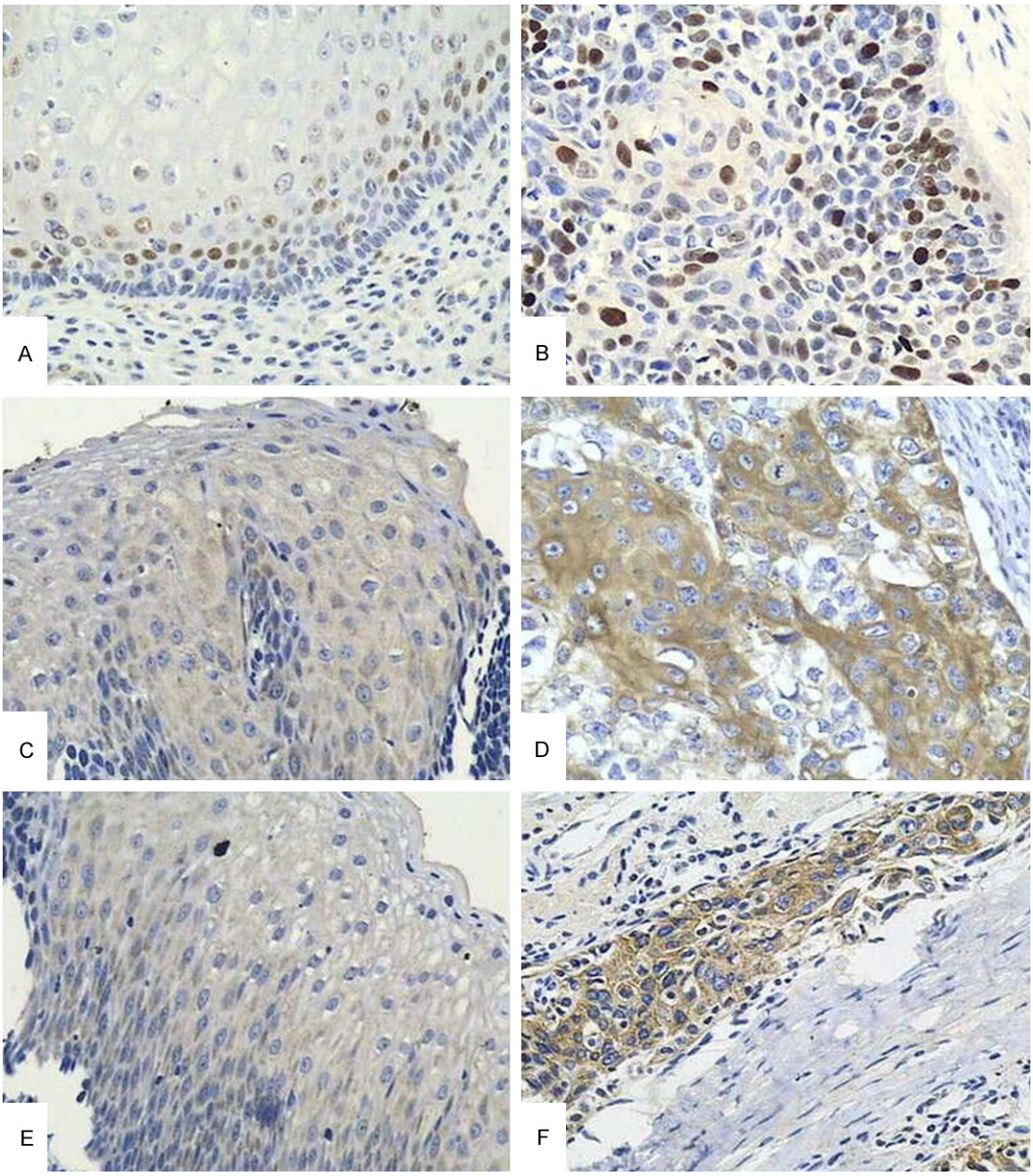

Figure 1. A. Low expression of cyclin D1 in para-carcinoma tissue, nucleus staining (IHC×200); B. High expression of cyclin D1 in tumor tissue, nucleus staining (IHC×200). C. Low expression of CTGF in para-carcinoma tissue, cytoplasm staining (IHC×200); D. High expression of CTGF in tumor tissue, cytoplasm staining (IHC×200). E. Low expression of VEGF in para-carcinoma tissue, cytoplasm staining (IHC×200); F. High expression of VEGF in tumor tissue, cytoplasm staining (IHC×200).

sity grade and the rate of positive cells producing a total range of 0 to 12. For statistical analyses, scores of 0 to 4 were considered-, scores of 5 to 8 were considered +, scores of 9 to 12 were considered ++. ++ was defined as overexpression [6].

Statistical analysis

The comparison of high level of cyclin D1, CTGF and VEGF protein expression in the tumor tissues and para-carcinoma tissues of ESCC patients, the relationship between cyclin D1,

Table 1. The Comparison of high level of cyclin D1, CTGF and VEGF protein expression in the tumor tissues and para-carcinoma tissue of ESCC patients (%)

	n	Cyclin D1	CTGF	VEGF
Tumor tissue	100	61 (61)	53 (53)	49 (49)
Para-carcinoma	20	0 (0)	3 (15)	3 (15)
Tissue				
χ^2		24.814	9.670	7.846
P		0.000	0.002	0.005

CTGF and VEGF expression and TNM staging, and the relationship between cyclin D1, CTGF, VEGF expression in different TNM stages and prognosis were performed using the χ^2 test. Survival was assessed by Kaplan-Meier analysis with log-rank score for determining statistical significance. Relative risk was evaluated by the multivariate Cox proportional hazards model, The results were quantified by calculating hazard ratios with 95% confidence intervals (95% CI). In all tests, $P < 0.05$ was used to determine statistical significance. All statistical tests were performed with SPSS 17.0 System (SPSS, Chicago, IL, USA).

Results

Expression pattern of biologic molecular markers in ESCC

The positive staining (pale brown) of cyclin D1 is located in cell nucleus, but the positive staining (pale brown) of CTGF and VEGF is located in cell cytoplasm. The positive expression rate of cyclin D1 in ESCC was 61% (61/100). The high-expression rate of CTGF and VEGF were 53% (53/100), 49% (49/100), respectively. Their expression characteristics are showed in **Figure 1A-F**. The expression of cyclin D1, CTGF and VEGF in ESCC were significantly high, in contrast with the expression levels of these markers in para-carcinoma tissues, and the difference had statistical significance ($P < 0.05$; **Table 1**).

Univariate analysis of clinic-pathological characteristics and biologic molecules

Kaplan-Meier survival analysis was used to evaluate the association between expression of cyclin D1, CTGF and VEGF and the overall survival of the patients studied. The overall survival rate of all the 100 patients was 43%. Results of the statistical analysis demonstrated that no difference in the survival rate was observed between different ages, sexes and differentiation. However, with lymph node involvement, systemic dissemination, and TNM stage considered, the patients in N0, M0, and I-II stage had a better survival rate than those in N1, M1, and III-IV (all *P*-value < 0.05; **Table 2**). As far as the biological markers are concerned, the survival rate was better in the group of low cyclin D1, CTGF and VEGF expression than in the group of high-expression (all *P*-value < 0.05; **Table 2**). Result of the statistical analysis demonstrated that overexpression of cyclin D1 was associated with decreased overall survival. The patients without cyclin D1 overexpression had a 3-year survival of 71.8%, whereas those patients with cyclin D1 overexpressing had a much lower 3-year survival rate of 24.6% ($P = 0.000$, **Figure 2A**; **Table 2**). Those patients with low CTGF expression had a considerably better 3-year survival rate of 57.4% than those with high CTGF expression (a 3-year survival rate of 30.2%; $P = 0.001$; **Figure 2B**; **Table 2**). The finding for VEGF was similar to that for CTGF, i.e. the patients with low VEGF expression enjoyed a significantly higher 3-year survival rate of 56.9% that those with low VEGF expression (a low survival rate of only 28.6%; **Figure 2C**, $P = 0.001$, **Table 2**).

Multivariate analysis of clinic-pathological characteristics and biologic molecular markers

We performed multivariate analysis to assess the independent predictive value of T stage and TNM stage, cyclin D1, CTGF, VEGF, lymph node involvement and systemic dissemination on univariate analysis. Among all of the above factors, only cyclin D1, VEGF, lymph node involvement and systemic dissemination were independent prognostic factors ($P = 0.001$, 95% CI = 1.587-6.473; $P = 0.017$, 95% CI = 1.142-3.879; $P = 0.042$, 95% CI = 1.034-6.673, $P = 0.003$, 95% CI = 1.505-7.794, respectively; **Table 3**).

The relationship between cyclin D1, CTGF, VEGF expression and TNM staging

When comparison was made for the expression levels of cyclin D1, CTGF and VEGF in different TNM staging of ESCC, the results indicated that the expression level of cyclin D1 in TNM I and IV stage had obvious difference, i.e. cyclin D1 expression level in TNM IV stage (84.6%) was

Table 2. Univariate analysis of clinic-pathological characteristics and biologic molecular markers in patients with ESCC

Factor	*n*	Survival rate (%)	Mean survival time (days)	X^2	*P*
Age (years)					
≥ 60	62	40.3	32	1.017	0.313
< 60	38	47.4	35		
Sex					
Male	78	39.7	32	1.614	0.204
Female	22	54.5	37		
Differentiation					
Well	32	53.1	37	1.553	0.460
Moderate	48	39.6	31		
Poor	20	35.0	28		
T stage					
T1-2	37	54.1	37	3.790	0.052
T3-4	63	36.5	31		
N stage					
N0	55	54.5	38	11.524	0.001
N1	45	28.9	27		
M stage					
M0	86	46.5	35	10.259	0.001
M1	14	21.4	20		
TNM stage					
I-II	56	50.0	37	5.507	0.019
III-IV	44	34.1	28		
Cyclin D1					
Low expression	39	71.8	41	22.606	0.000
High expression	61	24.6	28		
CTGF					
Low expression	47	57.4	39	10.319	0.001
High expression	53	30.2	28		
VEGF					
Low expression	51	56.9	38	10.933	0.001
High expression	49	28.6	28		

higher than in the TNM I stage (43.5%, P = 0.016, **Table 4**); With the ESCC TNM staging increased, the expression level of VEGF also increased, i.e. the expression level of VEGF in TNM staging of I and III, I and IV, II and IV, III and IV all have significant difference (P = 0.014, 0.000, 0.001, 0.034, respectively; **Table 4**). The expression level of CTGF had no significant difference with different TNM staging (**Table 4**).

The relationship between cyclin D1, CTGF, VEGF expression in TNM staging and survival

Statistically significant correlation was found between ESCC TNM staging and the expression of cyclin D1, CTGF and VEGF. In TNM I, II and III stage of ESCC, the 3-year survival rates of patients with low-expression of cyclin D1 were 84.6%, 66.7% and 66.7%, while patients with overexpression had lower 3-year survival rates of 30.0%, 31.6% and 23.8% (P = 0.008, 0.042, 0.026, respectively, **Table 5**). With regard to CTGF, the 3-year survival rate of patients with CTGF overexpression in I and II were inferior to that of patients in I and II with low-expression (36.4% vs. 83.3%; P = 0.021; 29.4% vs. 64.7% P = 0.039, respectively; **Table 5**). The finding for VEGF was that the VEGF overexpression of ESCC in TNM I and IV stage was associated negatively with the 3-year survival rate (P = 0.022, 0.015, respectively; **Table 5**).

Discussion

Due to the complexity of the biological behavior of esophageal cancer, patients with ESCC present variability and heterogeneity the clinical process. Great progress has been made in the treatment of esophageal cancer including options of surgical operation, radiotherapy and chemotherapy, but there is still a lot of controversy in selection of treatment mode and the curative effect is still unsatisfactory. With the development of molecular targeted therapies in the successful practice of some cancer treatment, the molecular markers perform an increasingly important role in the diagnosis, prognosis and molecular classification of ESCC. Researchers have so far gained preliminary results in the study of molecular classification of ESCC [11]. In our previous study in 2012, our data demonstrated that cyclin A, COX-2 may be promising candidates in molecular classification of ESCC [1]. In this study, we chose the metastasis related markers cyclin D1, CTGF and VEGF to assess their value in ESCC molecular classification.

One of the essences of malignant tumor is beyond of cell cycle regulation. The tumor cells go through proliferation and division without restrictions. Cyclin D1, a member of the cyclin family, is a cell cycle positive regulatory factor. Studies confirmed that cyclin D1 protein plays

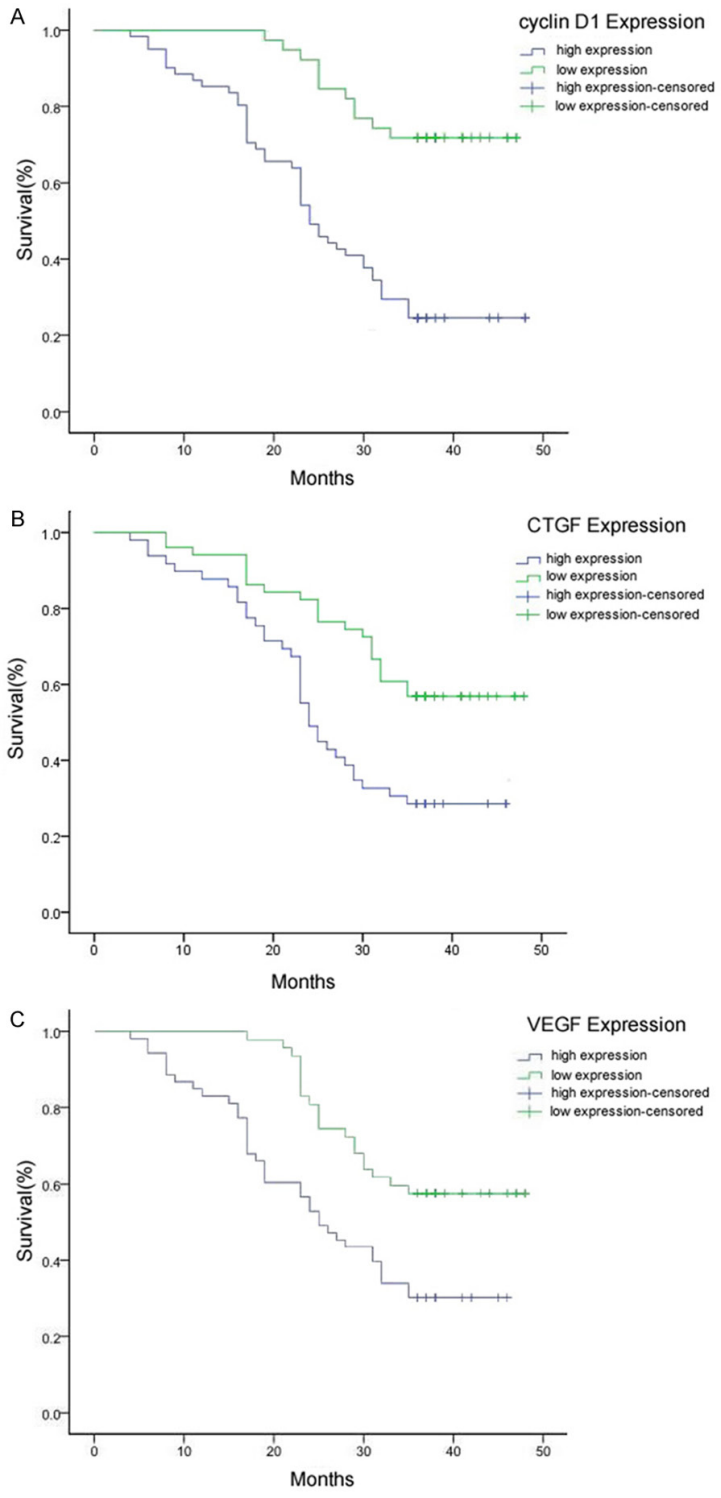

Figure 2. A. Overall survival curve according to cyclin D1 expression (P = 0.000). B. Overall survival curve according to CTGF expression (P = 0.001). C. Overall survival curve according to VEGF expression (P = 0.001).

an important role during the key rate-limiting point G1→S phase transition in the cell cycle. Therefore, detection of cyclin D1 expression level may provide a good reference value for evaluating cell proliferation, and is a reliable indicator for evaluating the occurrence and development of malignant tumor. It is well known that cyclin D1 is a new oncogene. At present, cyclin D1 amplification and excessive expression have been found in a variety of tumors. With the deepening of research on cyclin D1, researchers found that the expression of cyclin D1 not only correlated with tumor occurrence, development and metastasis, but also correlated with tumor prognosis [12-14]. We found that 61% of the patients with ESCC had high expression of cyclin D1, which had a short survival compared with the patients with low expression. This result is consistent with Zhao's report [5]. Thus, cyclin D1 overexpression was positively correlated with poor prognosis. The result of multivariate analysis are in agreement with Wang's data [15], namely the high expression of cyclin D1 may be an independent risk factor of prognosis in patients with ESCC. Further analysis showed that the expression level of cyclin D1 was different in different TNM staging: the high expression rate of cyclin D1 in IV stage (84.6%) were significantly higher than that of in I stage (43.5%, P = 0.016). It also shown that in stage I, II and III, patients with high expression of cyclin D1 have poorer prognosis than those with low expression of cyclin D1. Roy believed that the level of cyclin D1 was in correspondence with the TNM stage, and the high expression of cyclin D1 was closely related with bad prognosis in breast cancer [4], which is consistent with our study in patients with ESCC. These results suggested that the expression of cyclin D1 was correlated with TNM staging, and can better be used for molecular classification of ESCC.

Table 3. Multivariate Cox model analysis of clinic-pathological characteristics and biologic molecular in patients with ESCC

	B	SE	Wald	Sig.	Exp (B)	95.0% CI for Exp (B)	
						Lower	Upper
T (T1-2 vs T3-4)	0.614	0.380	2.609	0.106	1.847	0.877	3.889
Lymph node inv-olvement (N1)	0.966	0.476	4.124	0.042	2.627	1.034	6.673
Systemic diss-emination (M1)	1.231	0.420	8.609	0.003	3.425	1.505	7.794
TNM (I-II VS III-IV)	-1.101	0.571	3.717	0.054	0.332	0.108	1.019
Cyclin D1 (positive)	1.165	0.359	10.553	0.001	3.205	1.587	6.473
CTGF (positive)	0.503	0.309	2.640	0.104	1.653	0.901	3.031
VEGF (positive)	0.744	0.312	5.684	0.017	2.104	1.142	3.879

Table 4. The relationship between the expression of cyclin D1, CTGF, VEGF and TNM staging

TNM staging	*n*	Positive (over) expression rate (%)	χ^2		*P*	
Cyclin D1						
I	10 (23)	43.5	0.845[a]	3.772[b]	0.358[a]	0.052[b]
II	19 (34)	55.9	5.783[c]	1.355[d]	0.016[c]	0.244[d]
III	21 (30)	70.0	3.363[e]	1.018[f]	0.067[e]	0.313[f]
IV	11 (13)	84.6				
CTGF						
I	11 (23)	47.8	0.026[a]	0.158[b]	0.872[a]	0.691[b]
II	17 (34)	50.0	1.514[c]	0.710[d]	0.214[c]	0.790[d]
III	16 (30)	53.3	1.407[e]	0.942[f]	0.236[e]	0.332[f]
IV	9 (13)	69.2				
VEGF						
I	6 (23)	26.1	0.911[a]	6.043[b]	0.340[a]	0.014[b]
II	13 (34)	38.2	14.569[c]	3.023[d]	0.000[c]	0.082[d]
III	18 (30)	60.0	11.044[e]	4.488[f]	0.001[e]	0.034[f]
IV	12 (13)	92.3				

a: I to II; b: I to III; c: I to IV; d: II to III; e: II to IV; f: III to IV.

It should be noted that, in this study, the prognosis was still poor for ESCC patients with high expression of cyclin D1 even in stage I, and the prognosis may be good for ESCC patients with low expression of cyclin D1 even in stage III. This may explain why patients in the same TNM stage often have different clinical outcomes. This discrepancy suggests that cancer does not always have biological malignancy potential in proportion to cancer development. Some patients may require multimodal therapy despite early TNM stage. Therefore, a prognostic assessment system that combines TNM and molecular biological classification may help clinicians tailor treatment to each individual patient.

CTGF, which belongs to the CCN family, named CCN2, is a downstream mediator of transforming growth factor-β (TGF-β). CTGF, when widely expressed in a variety of human tissues and organs, could stimulate mitosis, adhesion, extracellular matrix production, growth and migration. Dysregulation of CTGF has been shown in a number of cancers, such as hepatocellular carcinoma [16] and gastric cancer [17]. However, CTGF appears to have different or even opposite functions in different cancers. For example, in lung and colon cancer, high expression of CTGF was favorable for survival. It is suggested that CTGF may behave as a secreted tumor suppressor protein in the normal lung, and its expression is suppressed in many NSCLCs [18]. Lower CTGF level in colorectal cancer (CRC) patients is associated with higher peritoneal recurrence rate after surgery. Inducing CTGF expression in cancer cells resulted in decreased incidence of peritoneal carcinomatosis and increased rate of mice survival [19]. Previous studies showed that CTGF was overexpressed in ESCC and involved in the tumorigenicity of ESCC cells [20]. Our results confirmed up-regulation of CTGF in ESCC. We also found that CTGF was overexpressed in ESCC and participated in the regulation of proliferation and invasiveness. High CTGF expression in ESCC tissues was negatively correlated with prognosis, which was identical with the observation in Xie's study [6]. Cox multivariate analysis showed that CTGF may be not inde-

Table 5. The relationship between the survival and the expression of cyclin D1, CTGF, VEGF in different TNM staging

TNM staging	Expression	*n*	3-year survival (%)	χ^2	*P*
Cyclin D1					
I	Low	13	84.6	7.078	0.008
	High	10	30.0		
II	Low	15	66.7	4.142	0.042
	High	19	31.6		
III	Low	9	66.7	4.983	0.026
	High	21	23.8		
IV	Low	2	50	2.176	0.140
	High	11	9.1		
CTGF					
I	Low	12	83.3	5.316	0.021
	High	11	36.4		
II	Low	17	64.7	4.250	0.039
	High	17	29.4		
III	Low	14	35.7	0.010	0.919
	High	16	37.5		
IV	Low	4	25.0	0.410	0.522
	High	9	11.1		
VEGF					
I	Low	17	70.6	5.247	0.022
	High	6	16.7		
II	Low	21	52.4	0.624	0.429
	High	13	38.5		
III	Low	12	50	0.833	0.361
	High	18	33.3		
IV	Low	1	100	5.958	0.015
	High	12	8.3		

pendent prognostic factor in patients with ESCC. We observed the relationship between the expression of CTGF and TNM staging, and discovered that the more expression of CTGF there was in patients with ESCC, the later TNM stage the patient was in ESCC. This research result was consistent with that of Deng's data [20]. In addition, in stage I and II of ESCC, this study suggests that patients with high expression of CTGF had obviously poor prognosis than those with low expression of CTGF. Therefore, this finding also suggested that CTGF may be a potential molecule for molecular classification in patients with ESCC.

Experimental and clinical studies have found that if there is no angiogenesis, primary tumor growth does not exceed 1 to 3 mm^3. With the increase of the tumor mass, the tumor itself will establish a new vascular network to supply the tumor body nutrition, which is necessary for tumor growth and metastasis. VEGF is currently known as tumor angiogenesis factor which has the strongest effect and the most specificity. VEGF is one of the decisive factors in tumor angiogenesis. It could promote endothelial cell division, migration and proliferation, and could induce the formation of microvessel, increase microvessel density. The involvement of VEGF in cellular proliferation and invasiveness has been reported in a variety of cancers including ESCC [21, 22].

It was reported that VEGF expression had an obvious relationship with the poor prognosis of ESCC [7], and was a significant prognostic factors [3]. In our study, the retrospective analysis of a large amount of ESCC samples further proved that VEGF was over-expressed in ESCC and revealed that over-expression of VEGF was related to poor survival. Cox multivariate analysis showed that VEGF expression level may be independent prognostic factors in patients with ESCC. Therefore, the expression level of VEGF may have a prognostic value in ESCC. With the ESCC TNM staging changed from early to late, the expression level of VEGF also increased. Chen et al also obtained the same results on ESCC, and found that overexpression of VEGF occurred in a significant proportion of ESCC samples that were of a high tumor grade and metastatic [7]. Further analysis suggested that in I and IV stage of ESCC, the three-year survival rate in patients with high expression of VEGF was significantly lower than that in patients with low expression.

In conclusion, our study suggested that tumor metastasis related factors cyclin D1, CTGF and VEGF all have higher expression rate in ESCC tissues, moreover, cyclin D1 and VEGF are likely to be independent prognostic factor in patients with ESCC. The high expression of above three factors all showed that the prognosis was poor in stage I of ESCC, in another word, if their expression levels are low, the prognosis was good. This better reminds us that the molecular markers can be used in ESCC molecular subtyping and individualized treatment, that is,

patients in stage I of ESCC, if high expression of these molecules, postoperative multidisciplinary treatment may lead to better prognosis. These result also imply that the ESCC has heterogeneity and may be one of molecular mechanisms in some patients what may require multimodal therapy despite TNM stage I.

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Disclosure of conflict of interest

None.

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