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Original Article

Angiopoietin-like protein 3 is an indicator of prognosis in esophageal cancer patients

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Abstract: Angiopoietin-like protein 3 (ANGPTL3) plays an important role in angiogenesis. This study aimed to examine the protein expression of ANGPTL3, and to evaluate its clinical significance in esophageal cancer. ANGPTL3 expression was detected using immunohistochemistry in 98 pairs of esophageal cancer and adjacent non-cancerous tissues. The expression levels of ANGPTL3 in esophageal cancer tissues were significantly higher than those in adjacent noncancerous tissues ($P < 0.05$). No association was observed between ANGPTL3 expression and clinical features ($P > 0.05$). Although ANGPTL3-negative patients had longer survival time than ANGPTL3-positive patients, the difference did not reach statistical significance ($P = 0.090$). Stratified analysis of ANGPTL3 expression according to clinical features revealed that there was significant association between ANGPTL3 expression and overall survival among patients aged 65 years or younger, female, or with lymph node metastasis ($P < 0.05$). When after adjusted for clinical features, the association remained significant only in patients aged 65 years or younger ($P = 0.021$). Taken together, our findings provide preliminary evidence of association of ANGPTL3 expression with the prognosis of subgroups of patients with esophageal cancer.

Keywords: Esophageal cancer, angiopoietin-like protein 3, angiogenesis, prognosis

Introduction

Esophageal cancer is one of the most common cancer, with 455,800 new cases and 400,200 deaths estimated to have occurred in 2012 worldwide [1]. In China, esophageal cancer is the sixth most common cancer and the fourth most common cause of cancer mortality [2]. Despite significant progress in detection and therapy, the prognosis of esophageal cancer remains poor, with the 5-year-survival rate of ~25% [3]. Invasion and metastasis are the leading reason for the resultant mortality of patients with esophageal cancer [4]. Tumor angiogenesis is closely related to the invasion and metastasis of esophageal cancer.

Most precancerous lesions are lack of neovascularization, whereas solid tumors cannot grow beyond a size of 2 mm in the absence of neovascularization. Neovascularization is necessary for the growth and metastatic spread of solid tumor. To satisfy the requirements of tumor growth, cancer cells regulate angiogenesis through a variety of mechanisms. Therefore, studies on cancer therapy have focused on the mechanisms by which early angiogenesis is inhibited and tumor blood vessels are disrupted. Any intervention in existing blood vessels and undergoing angiogenesis in cancer tissues can block the pathway leading to the formation of new vessels, which can effectively inhibit tumor growth and induces a conversion of cancer cells to a dormant state [5, 6]. Anti-angiogenic drugs such as bevacizumab and sorafenib are commonly used for cancer treatment.

Similar to vascular endothelial growth factor-A, angiopoietin-like protein 3 (ANGPTL3) can induce neovascularization [7] and promote cancer growth and invasion [8-12]. However,

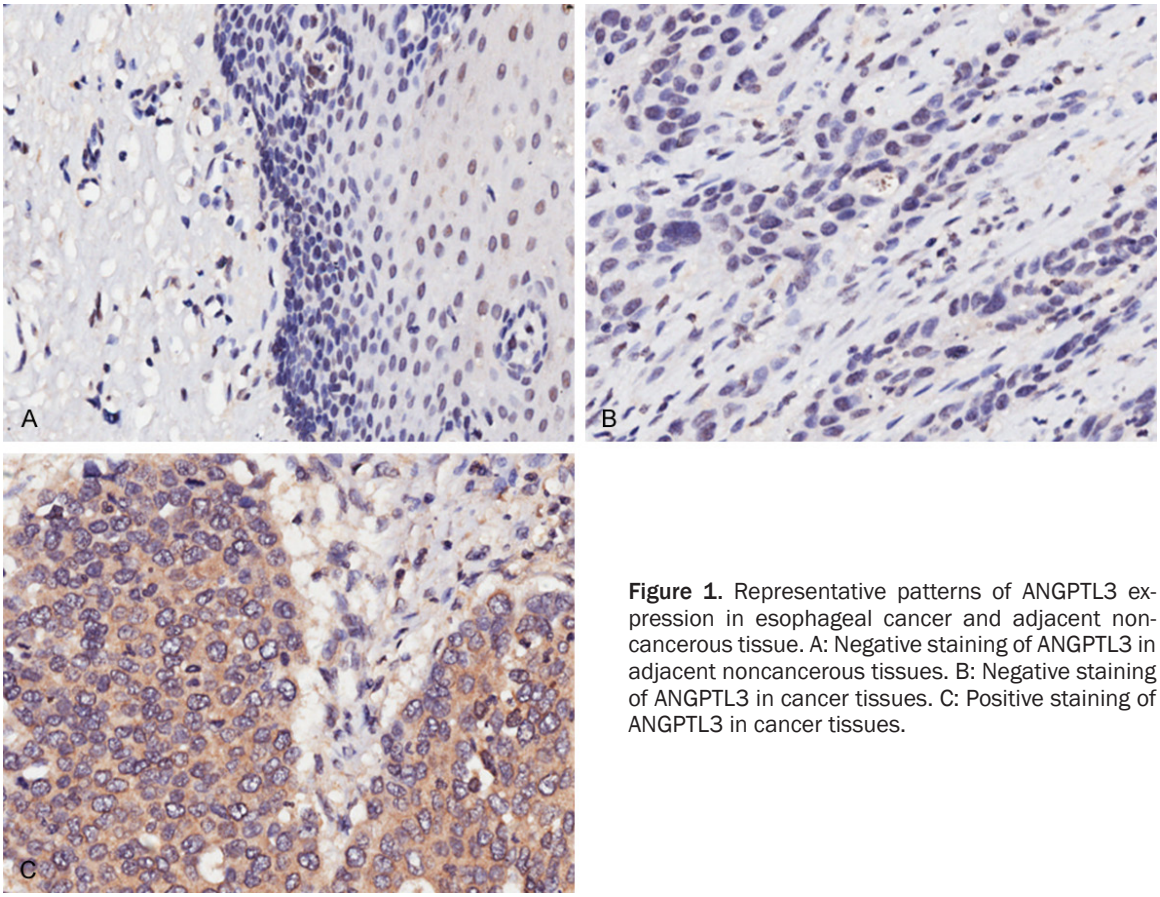

Figure 1. Representative patterns of ANGPTL3 expression in esophageal cancer and adjacent noncancerous tissue. A: Negative staining of ANGPTL3 in adjacent noncancerous tissues. B: Negative staining of ANGPTL3 in cancer tissues. C: Positive staining of ANGPTL3 in cancer tissues.

Table 1. Association of ANGPTL3 expression with clinicopathologic parameters

Clinical features	ANGPTL3		*P* value
	Positive	Negative	
Age, years			
≤ 65	30	17	0.140
> 65	26	25	
Sex			
Male	40	31	0.489
Female	16	11	
Histologic grade			
1+2	41	29	0.409
3	15	13	
Tumor size			
< 5 cm	23	20	0.389
≥ 5 cm	27	19	
LNM			
Positive	30	21	0.490
Negative	26	20	
TNM stage			
I+II	26	21	0.338
III+IV	29	18	

the role of ANGPTL3 in esophageal cancer remains unknown. In this study, we investigated ANGPTL3 expression in esophageal cancer tissues and evaluated its effect on the survival of esophageal cancer patients. The results may provide the theoretical basis of the clinical diagnosis, treatment, and prognosis of esophageal cancer.

Materials and methods

Patients

A total of 98 patients with histologically confirmed esophageal squamous cell carcinoma (ESCC) were recruited from Taizhou People's Hospital during May 2007 through July 2008. All patients had no history of other cancer. Tumor specimens and normal esophageal tissues were obtained before patients received any anti-cancer therapy. The mean age was 65.2±9.4 years (range 48-82 years). Of the 98 patients, 71 (72.4%) were male and 27 (27.6%) were female. All patients provided written informed consent before participating in this

Table 2. Stratification analyses of ANGPTL3 expression associated with overall survival of patients with esophageal cancer

Variables	Univariate		Multivariate	
	HR (95% CI)	*P* value	HR (95% CI)*	*P* value
Age, years				
≤ 65	2.169 (1.083-4.343)	0.029	3.121 (1.188-8.200)	0.021
> 65	1.104 (0.689-2.701)	0.757	1.230 (0.642-2.357)	0.532
Sex				
Male	1.108 90.668-1.838)	0.691	1.193 (0.671-2.122)	0.548
Female	3.388 (1.085-10.579)	0.036	4.000 (0.866-18.481)	0.076
Histologic grade				
1+2	1.497 (0.863-2.595)	0.151	1.403 (0.739-2.666)	0.301
3	1.360 (0.595-3.111)	0.466	1.236 (0.510-2.997)	0.640
Tumor size				
< 5 cm	1.476 (0.722-3.019)	0.286	1.115 (0.504-2.464)	0.788
≥ 5 cm	1.298 (0.673-2.500)	0.436	1.657 (0.821-3.345)	0.159
LNM				
Positive	1.987 (1.073-3.678)	0.029	1.882 (0.956-3.702)	0.067
Negative	1.239 (0.610-2.515)	0.553	1.119 (0.507-2.471)	0.780
TNM stage				
I+II	1.236 (0.604-2.526)	0.562	1.113 (0.504-2.458)	0.791
III+IV	1.782 (0.951-3.338)	0.071	1.899 (0.964-3.740)	0.064

*Adjusted for age, sex, histologic grade, tumor size, LNM, and TNM stage, as appropriate.

research according to the study protocol approved by the Ethical Committee of Taizhou People's Hospital.

Immunohistochemistry (IHC)

Surgical specimens were sampled conventionally and fixed with 10% neutral-buffered formalin and embedded in paraffin. The paraffin-embedded specimens were then sliced to a thickness of 4 μm. Afterward, IHC was performed in accordance with the instruction manual to detect the ANGPTL3 expression in human ESCC and paracarcinoma tissues. High-pressure repair was also conducted to repair antigens. A PBS buffer solution was used as a negative control, and a known positive specimen was used as a positive control. DAB staining was also performed. The nuclei were stained with Mayer's hematoxylin. An ANGPTL3-positive cell exhibited brown granular staining in the cytoplasm, and coloration is significantly brighter than the background or the cell is colored but the background is not. Negative indicated that no positive tumor cells were found.

Statistical analyses

The primary endpoint of the study was overall survival. Survival time was calculated from the date of diagnosis to the date of death due to any cause or the date of last follow-up. Quantitative data were analyzed using *T*-test, while qualitative data were analyzed using χ^2 test. The cumulative cause-specific survival rate was estimated by using the Kaplan-Meier method, and difference in overall survival between subgroups were compared by log-rank test. Univariate and multivariate analysis were carried out using the Cox proportional hazards model. All statistical tests were two sided and a *P* value < 0.05 was considered statistically significant. All statistical analyses were carried out with SPSS version 19.0 for Windows (IBM SPSS Inc., Chicago, IL, USA).

Results

ANGPTL3 is upregulated in esophageal cancer tissues

In this study, ANGPTL3 expression was detected in 98 pairs of esophageal cancer and paracarcinoma tissues using IHC. ANGPTL3 was expressed strongly in esophageal cancer tissues. Of 98 cases, 56 have positive immunohistochemical expression of ANGPTL3. By contrast, ANGPTL3 was rarely expressed in paracarcinoma tissues. The typical IHC results of esophageal and paracarcinoma tissues were shown in **Figure 1**. The expression levels of ANGPTL3 in esophageal cancer tissues were significantly higher than those in paracarcinoma tissues (P < 0.001). However, ANGPTL3 expression was not significantly associated with sex, age, tumor size, histological grade, lymph node metastasis (LNM), TNM staging, and other clinical indexes (P > 0.05, **Table 1**).

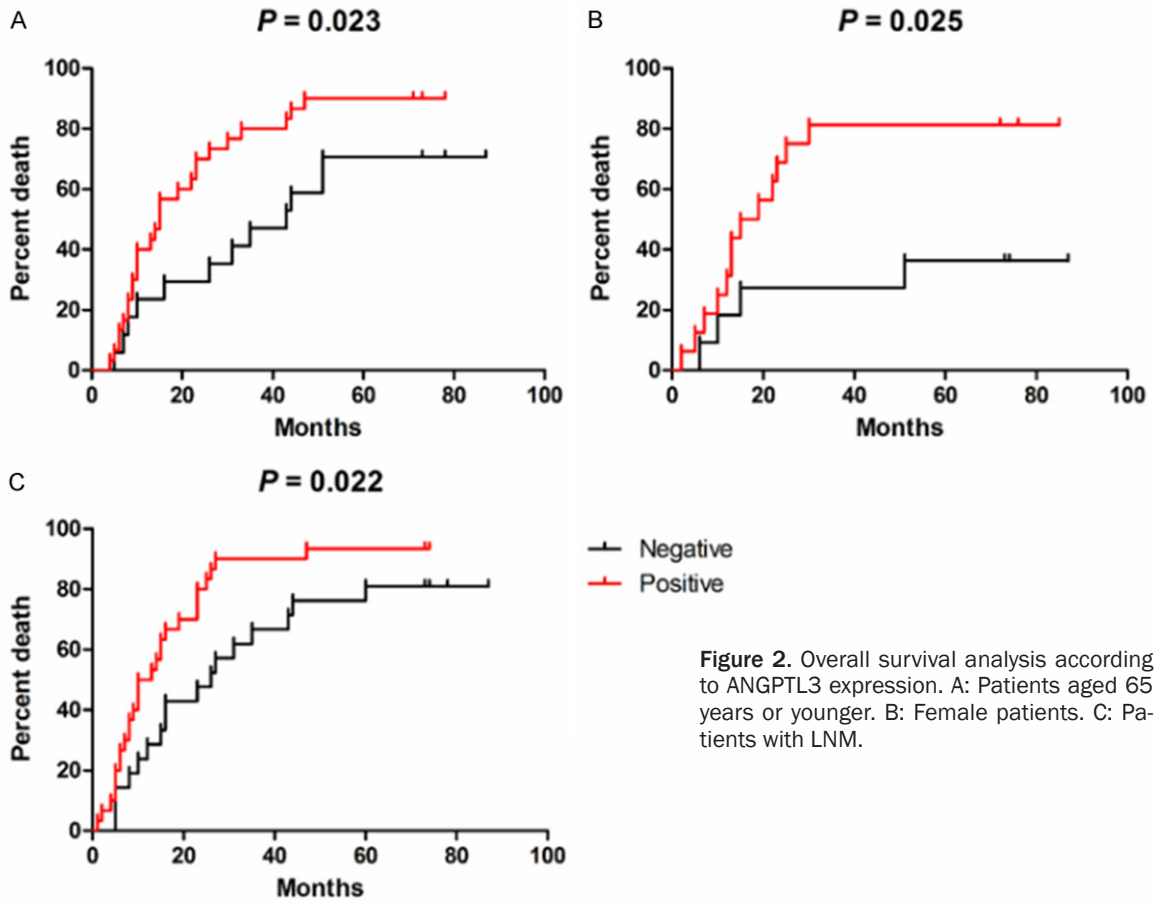

Figure 2. Overall survival analysis according to ANGPTL3 expression. A: Patients aged 65 years or younger. B: Female patients. C: Patients with LNM.

Association of ANGPTL3 expression with overall survival of esophageal cancer patients

In this study, 98 esophageal cancer patients were followed up for 87 months. Of these 98 patients, 78 died and 20 survived. The median survival time was 19.9 months. The five-year-survival rate of the patients with ESCC was 18.9%. Of the 56 ANGPTL3-positive patients, 47 died (83.9%). The five-year survival rate of ANGPTL3-positive patients was 16.1%. Of 42 ANGPTL3-negative patients, 31 died (73.8%). The five-year survival rate of ANGPTL3-negative patients was 26.2%. Although ANGPTL3-negative patients survived longer than ANGPTL3-positive patients, this difference was not statistically significant (P = 0.090).

Stratification analysis based on clinical variants was also performed to evaluate the effect of the ANGPTL3 expression on patient prognosis. Among patients aged 65 years or younger, female cases, or LNM cases, positive ANGPTL3 was associated with shorter survival (P < 0.05, **Table 2**, **Figure 2**). Among patients with other clinical variants, there was no difference in survival time between ANGPTL3-positive and ANGPTL3-negative patients (P > 0.05). After adjusted for clinical variants, ANGPTL3 was an independent prognostic risk factor only in patients aged 65 years or younger [adjusted hazard ratio (HR) = 3.121, 95% CI: 1.188-8.200, P = 0.021].

Discussion

The members of the angiopoietin-like protein family are considered as secretory proteins. Eight members have been identified and named as ANGPTL1 to ANGPTL8. ANGPTLs participate in many physiological and pathophysiological processes. Most members of ANGPTLs are also implicated in the control of many biological processes, including fat and glucose metabolism, inflammation, hematopoiesis, and cancer [13-15]. For instance, ANGPTL1 and ANGPTL4 can inhibit angiogenesis, whileANGPTL3 and ANGPTL6 can induce angiogenesis the effects

of blood vessels [16, 17]. Human ANGPTL3 is a polypeptide composed of 460 amino acids, and consists of a secretory signal peptide, an N-helical coiled structure domain, and a C-end fibrinogen-like domain [13]. Studies on the protein structure and function of ANGPTL3 have revealed that the N-end helical coiled structure domain regulates lipid metabolism [18] and the C-end fibrinogen-like domain is involved in angiogenesis [7]. ANGPTL3 induces endothelial cell adhesion and migration via activation of integrin signaling pathway and promotes angiogenesis [7]. Furthermore, ANGPTL3 facilitates cancer cells proliferation via activation of extracellular-regulated kinase (ERK) signaling pathway and downregulation of cyclin-dependent kinase inhibitor [10], while inhibition of ANGPTL3 expression suppresses proliferation and invasion of cancer cells [9, 10]. Therefore, ANGPTL3 is anattractive therapeutic target for cancer.

ANGPTL3 is upregulated in hepatocellular carcinoma (HCC) [11, 12] and oral cancer [10]. ANGPTL3 expression is closely related to HCC angiogenesis, tumor thrombus formation, and tumor stage [11, 12], indicated that ANGPTL3 is correlated with HCC invasion and metastasis. Therefore, downregulation of ANGPTL3 may aid in the inhibition of angiogenesis, which may extend the survival time of HCC patients. Koyama et al. [10] reported that among patients with pT3 and pT4 oral cancer, ANGPTL3-positive patients had shorter survival time than ANGPTL3-negative patients. In the present study, the ANGPTL3 expression was significantly upregulated in esophageal cancer tissues. Since the blood supply in the esophagus is insufficient, esophageal cancer cells synthesize and secrete a large amount of ANGPTL3 proteins to promote neovascularization and to construct a microenvironment beneficial to cancer cell growth. Furthermore, the survival rate of the ANGPTL3-negative patients was higher than that of the ANGPTL3-positive patients, whereas this difference did not reach statistically significant. Further stratification analyses demonstrated that this effect reached significant only in patients aged 65 years or younger after adjusted for clinical variables. This finding indicates that there exist different pathogenic mechanisms of esophageal cancer at different ages. Therefore, ANGPTL3 may promote esophageal cancer invasion and metastasis, resulting in poor prognosis. ANGPTL3 may be one of the indexes of esophageal cancer prognosis.

In conclusion, our findings provide the first evidence of high expression of ANGPTL3 in esophageal cancer tissues, which is closely correlated with poor survival in patients with esophageal cancer. These results help us better understand the roles of ANGPTL3 in the progression and development of esophageal cancer. Further studies are definitely required to verify these results in large prospective samples and elucidate the precise roles of ANGPTL3 in esophageal cancer.

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Disclosure of conflict of interest

None.

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