Original Article
The expression of EGFL7 and E-Cadherin in human adenocarcinoma of esophagogastric junction and their clinical significance

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Abstract: EGFL7 and E-Cadherin are abnormal expression in many malignant tumors, and involved in occurring and progressing of malignant tumors. The aim of this study is to investigate the expression of EGFL7 and E-Cadherin in human adenocarcinoma of esophagogastric junction (AEG) and explore their clinical and pathological significance. The expression of EGFL7 and E-Cadherin protein were detected in 146 cases of human AEG and 21 cases of tumor-adjacent tissues by immunohistochemical method. Our results demonstrate that the positive rate of EGFL7 was 63.7% in human AEG which was higher than that in tumor-adjacent tissues (19.0%), <0.001. High levels of EGFL7 protein were significantly correlated with tumor TNM classification and lymphatic metastasis and distant metastasis (P=0.002, P=0.021 and P=0.029, respectively). However, EGFL7 protein expression was not associated with gender, histopathological types and Siewert classification (P=0.925, P=0.938, P=0.369, respectively). The positive expression level of E-Cadherin was 41.8% in AEG, which was lower than that in tumor-adjacent tissues (85.7%), P<0.001. Low levels of E-Cadherin protein were significantly related with tumor histopathological types, TNM classification, Lymphaic metastasis and distant metastasis (P=0.008, P=0.014, P=0.019 and P=0.023, respectively). However, E-Cadherin protein expression was not associated with gender and Siewert classification (P=0.867, P=0.776, respectively). EGFL7 protein was negatively correlated with E-Cadherin protein (r=-0.429, P<0.001). Patients with higher EGLF7 or lower E-Cadherin expression had shorter overall survival time, while patients with lower EGFL7 or higher E-Cadherin expression had better survival time. In conclusion, expression of EGLF7 and E-Cadherin are markedly related with tumor TNM classification and lymphatic metastasis and distant metastasis of AEG. E-Cadherin is markedly related with tumor histopathological types of AEG. EGLF7 is negatively related with the expression of E-Cadherin. To detect EGLF7 and E-Cadherin may be helpful to evaluate prognosis and infiltrative capability of AEG.

Keywords: Adenocarcinoma of esophagogastric junction, immunohistochemistry, EGLF7, E-Cadherin, protein, survival

Introduction

Adenocarcinoma of the esophagogastric junction (AEG) is one of the most common malignant tumors of digestive system. The study indicated that compared with the declining of distal gastric adenocarcinoma, the incidence of AEG is rising rapidly in the world [1, 2]. The most effective treatment of AEG is radical resection. However, tumors are usually detected at the advanced stage due to atypical early symp-

toms. Therefore, some patients miss the golden chance of surgery, or the effect of the operation is not satisfactory. Obviously, the overall survival rate is not satisfactory [3]. It is well received that early diagnosis and early treatment are important for improving the prognosis of the patients with tumor [4]. Therefore, it is very important for the diagnosis and treatment of AEG to find some non-invasive tumor markers, which can effectively monitor tumor development. Thus, AEG can be early diagnosed and

Table 1. Expressions of EGL7 and E-Cadherin in human adenocarcinoma of esophagogastric junction and tumor-adjacent tissues

Tissue type	EGL7		E-Cadherin	
	Negative (-)	Positive (+)	Negative (-)	Positive (+)
AEG tissue	53 (36.3)	93 (63.7)	85 (58.2)	61 (41.8)
tumor-adjacent tissue	17 (81.0)	4 (19.0)	3 (14.3)	18 (85.7)
χ^2	15.034		14.216	
P value	<0.001		<0.001	

treated, leading to improving the operation effect and increasing the survival rate of patients.

The EGFL7 gene, also known as vascular endothelial-statin (VE-statin), which is overexpressed in many kinds of malignant tumors (such as liver cancer, prostate cancer, glioma and colon cancer), and it is closely related to the invasion and metastasis of malignant tumor [5-9]. Recent studies show that EGFL7 can regulate the adhesion of endothelial cell, inhibit the migration of vascular smooth muscle cells, thus delaying the maturation of blood vessels [10, 11]. Because of the EGFL7's highly up-regulation, the degree of neonatal blood vessel maturation is comparatively low, the permeability of the tube wall is high for the lack of smooth muscle cells in the wound, so the cancer cells can easily pass through the wall of blood vessels, causing tumor invasion and metastasis [10, 11].

Pinte S found that EGFL7 could promote tumor metastasis by down regulating the expression of cell adhesion molecules on the surface of endothelial cells, which caused the tumor cells to escape the immune system [12]. Epithelial cadherin (E-cadherin) is a calcium dependent intercellular adhesion molecule that can mediate cell connection, maintain normal epithelial cell morphology and tissue structural integrity [13, 14]. Down-regulation or loss of E-cadherin reduced cell-cell adhesion, which enhanced the tumor cells' potential for metastatic dissemination [15, 16].

AEG was divided into three types (type I, type II and type III) according to the anatomical features and the location of the tumor center, and the incidence rate of type I was the lowest among them [17, 18]. Type II/III was different from type I in etiology, origin, tumor biology, ect. These make type II/III AEG considered as an independent cancer compared with type I [18, 19].

In this study, we used immunohistochemical method to assay the expression of the EGFL7 and E-cadherin in 146 cases of AEG (Type II/III), which was aimed at exploring their clinical and pathological significance. They may be helpful to increase the detection rate of AEG and develop a practical diagnostic and monitoring prognosis tool for it.

Materials and methods

Patients

The study protocol was approved by the ethics committee of the First Affiliated Hospital of Soochow University, and all tissue samples were collected from patients with appropriate informed consent. 146 patients underwent surgery between March 2010 and March 2015. 21 cases of tumor-adjacent tissues were taken from the control group. Each patient with detailed clinical data and operation record. Among the 146 AEG samples, 104 were from male patients, and the rest 42 were from female patients of average age 60.3 (rang from 38 to 85) years old. 67 patients have lymphatic metastasis (45.9%) and 24 patients had distant metastasis (16.4%), Among the 24 distant metastasis samples, 17 patients have abdominal metastasis and 9 patients have Liver metastasis. Sections were divided into well-moderately (97 cases) and poorly (49 cases) according to histopathological types. Sections were divided into T1/T2 (58 cases) and T3/T4 (90 cases) according to the TNM classification system proposed by American Joint Committee on Cancer (AJCC) in 2010 by two expert pathologists [20]. 65 patients were divided into type II and 81 patients were divided into type III according to Siewert classification [17]. AEG patients in the experimental group were shown in **Tables 1** and **2**. All sections were confirmed as human AEG by pathologists. Endoscopy or CT scan was performed once at 6-month intervals after surgery. They were followed up for 3 to 60 months via telephone. None of these patients received pre-operative chemotherapy or radiotherapy.

Table 2. Analysis of EGL7 and E-Cadherin expression and related factors

Related Factor	n	EGL7 expression		χ^2	P value	E-Cadherin expression		χ^2	P value
		Negative (-)	Positive (+)			Negative (-)	Positive (+)		
Gender									
Male	104	38 (36.5)	66 (63.5)	0.009	0.925	61 (58.7)	43 (41.3)	0.028	0.867
Female	42	15 (35.7)	27 (64.3)			24 (57.1)	18 (42.9)		
Histopathological types									
Well-moderately	97	35 (36.1)	62 (63.9)	0.006	0.938	49 (50.5)	48 (49.5)	7.052	0.008
Poorly	49	18 (36.7)	31 (63.3)			36 (73.5)	13 (26.5)		
TNM classification									
T1/T2	58	30 (51.7)	28 (48.3)	9.896	0.002	26 (45.6)	31 (54.4)	6.108	0.014
T3/T4	88	23 (26.1)	65 (73.9)			59 (66.3)	30 (33.7)		
Siewert classification									
Type II	65	21 (32.3)	44 (67.7)	0.808	0.369	37 (56.9)	2 (43.1)	0.081	0.776
Type III	81	32 (39.5)	49 (60.5)			48 (59.3)	33 (40.7)		
Lymphatic metastasis									
No	79	41 (51.9)	38 (48.1)	5.371	0.021	39 (49.4)	40 (50.6)	5.546	0.019
Yes	67	22 (32.8)	45 (67.2)			46 (68.7)	21 (31.3)		
Distant metastasis									
No	122	49 (40.2)	73 (59.8)	4.789	0.029	66 (54.1)	56 (45.9)	5.181	0.023
Yes	24	4 (16.7)	20 (83.3)			19 (79.2)	5 (20.8)		

Immunohistochemical (IHC) analysis

Immunohistochemical analysis was performed similarly as previously described [21]. The tumor tissues and tumor-adjacent tissues from AEG patients were fixed in 4% paraformaldehyde and were embedded in paraffin, and then were sectioned for IHC analysis.

Specifically, each section needed to be deparaffinized, dehydrated and subjected to antigen retrieval. The slides filled with 0.01 mmol/L citrate buffer (pH 6.0) and were placed in a microwave oven for 15 mins. Then 3% hydrogen peroxide solution was used to block endogenous peroxidase activity for 10 mins at room temperature. After being washed with PBS, 3 times × 5 mins, slides were incubated for 1 h at room temperature with primary antibodies mouse anti-human-EFGL7 (1:100; Maixin Biotechnology Co. Ltd; Fuzhou, China) and mouse anti-human-E-Cadherin (1:100; Wuhan Boster Biological Technology, Ltd; Wuhan, China). Sections were washed in PBS 3 times ×5 mins, followed by further incubation with anti-mouse secondary antibody (Wuhan Boster Biological Technology, Ltd; Wuhan, China) for 15 mins at room temperature. 100 µL DAB solution (Wuhan Boster Biological Technology, Ltd; Wuhan, China) was dropped to each section (protect from light), then observed under the microscope. The slides were stained with hematoxylin. Each section was dehydrated with the alcohol, and processed transparently with xylene. PBS instead of primary antibodies was as negative controls.

Evaluation of AEG-1 and Cyclin D1 staining

The sections were evaluated by the sum of two parts: (1) Percentage of positive cells: 0 (0-10% positive cells), 1 (10-40% positive cells), 2 (2=40-70% positive cells) and 3 (≥70% positive cells). (2) staining intensity (0=negative, 1=light yellow, 2=yellow brown, 3=brown). In the study, the EGFL7 and E-Cadherin expression is defined as positive (+, high expression) when score is more than or equal to 2, and negative (-, low expression) when the score is less than 2 [21].

Statistical analysis

Data are presented as mean ± standard deviation (S.D). Statistical analyses were performed with SASS v.9.2 and GraphPad Prism version 5.0 (GraphPad Software, San Diego, CA, USA). We compared the differences of enumeration data among different groups using χ^2 test (**Tables 1** and **2**). The relationship between EGFL7 and E-Cadherin expression was evaluat-

Table 3. COX analyses of different clinicopathological variables and *EGFL7 and E-Cadherin* expression status as predictors for overall survival in adenocarcinoma of esophagogastric junction

Variable	Hazard Ratio	95% Hazard Ratio Confidence Limits		p value
Gender (male vs. female)	0.983	0.498	1.937	0.9596
Histopathological types (well-moderately vs. poorly)	1.257	0.639	2.471	0.5076
TNM Classification (T1/T2 vs. T3/T4)	4.878	1.636	14.543	0.0045
Siewert classification (type II vs. type III)	1.024	0.525	1.996	0.9447
Lymphatic metastasis (yes vs. no)	6.884	3.052	15.52	<.0001
Distant metastasis (yes vs. no)	3.193	1.611	6.329	0.0009
EGFL7 (positive vs. negative)	4.832	1.703	13.709	0.0031
E-Cadherin (positive vs. negative)	0.423	0.191	0.938	0.0342

Table 4. Correlations between EGFL7 and E-Cadherin expression in adenocarcinoma of esophagogastric junction tissue

E-Cadherin	EGFL7 +	EGFL7 -	Contingency coefficien (r)	χ^2	P value
+	24	37	-0.429	26.876	<0.001
-	69	16			

ed using Pearson χ^2 test (**Table 4**). Overall survival rates were calculated with Kaplan-Meier method and log-rank tests (**Figure 2**). Cox proportional hazard model was used for multivariate analysis and to determine the 95% confidence interval (**Table 3**).

Results

Relationship of EGFL7 and E-Cadherin expression and clinicopathological parameters

As is shown in **Figure 1**, EGFL7 expressions were indicated by yellow or brown in cytoplasm, and positive (**Figure 1A**), and negative (**Figure 1B**). E-Cadherin expressions were indicated by yellow or brown in cytomembrane, and positive (**Figure 1C**), and negative (**Figure 1D**).

As was shown in **Tables 1** and **2**, the positive rate of EGFL7 was 63.7% in AEG tissues which was higher than that in tumor-adjacent tissues (19.0%), <0.001. High levels of EGFL7 protein were significantly related to tumor TNM classification and lymphatic metastasis and distant metastasis (P=0.002, P=0.021 and P=0.029, respectively). However, EGFL7 protein expression was not associated with gender, histopathological types and Siewert classification (P= 0.925, P=0.938, P=0.369, respectively). The positive rate of E-Cadherin was 41.8% in AEG tissues, which was lower than that in tumor-

adjacent tissues (85.7%), P<0.001. Low levels of E-Cadherin protein were significantly related to tumor histopathological types, TNM classification, Lymphatic metastasis and distant metastasis (P=0.008, P=0.014, P=0.019 and P=0.023, respectively). However, E-Cadherin protein expression was not associated with gender and Siewert classification (P=0.867, P=0.776, respectively).

Correlations between expressions of EGFL7 and E-Cadherin and survival

The correlations were shown in **Figure 2**. Kaplan-Meier survival curves of AEG patients based on EGFL7 or E-Cadherin expression. Patients with high EGFL7 expression showed significantly worse survival compared to those patients with low expression (P<0.001, log-rank test) (**Figure 2A**). Patients with low E-Cadherin expression showed significantly worse survival compared to those patients with high E-Cadherin expression (P<0.001, log-rank test) (B). Cox multivariate analysis showed that TNM classification, lymphatic metastasis and distant metastasis as well as EGFL7 expression levels were negatively correlated with AEG survival, and positively correlated with E-Cadherin expression levels, suggesting that high levels of EGFL7 and low levels of E-Cadherin are relative risk factors for prognosis (**Table 3**).

Correlations between EGFL7 and E-Cadherin expression in AEG tissue and clinicopathological parameters

There is a negative correlation between EGFL7 and E-Cadherin expressions in AEG tissue (r= -0.429, P<0.001), as is shown in **Table 4**.
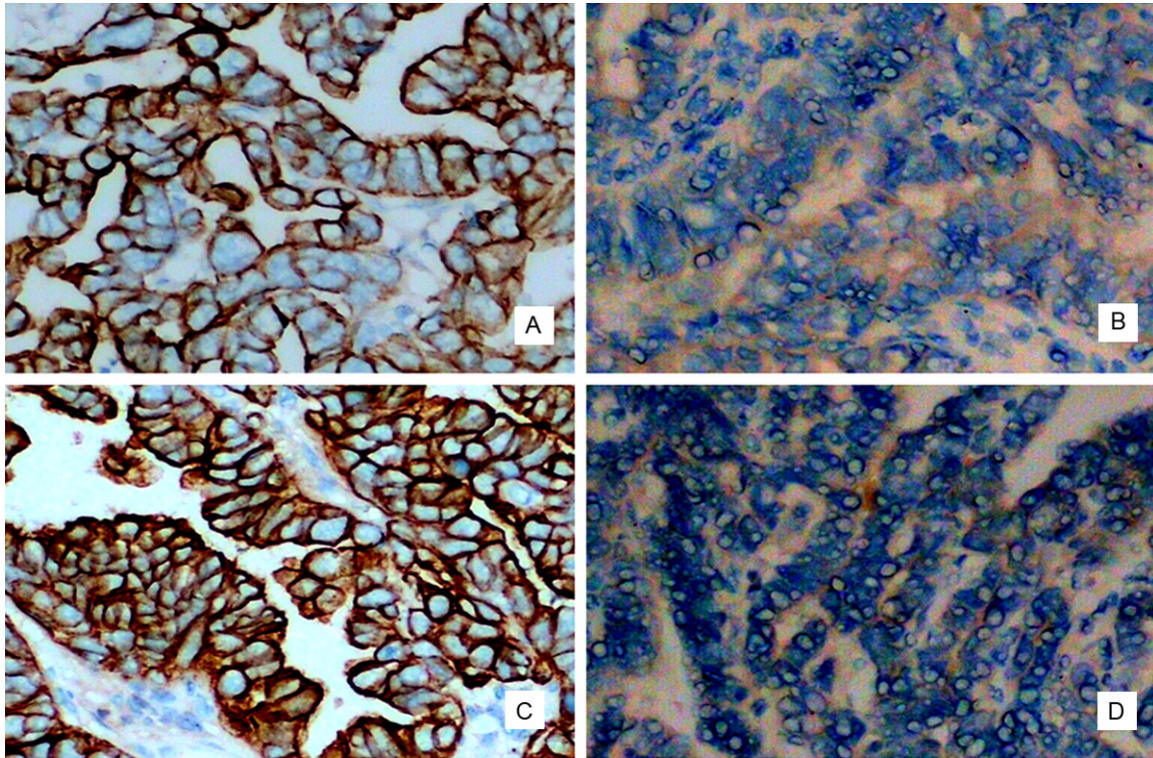
Figure 1. EGFL7 were higher expressed in AEG tissues and its cytoplasm was stained brown (200×) (A). EGFL7 were lower expressed in AEG tissues and tumor cells were not stained (200×) (B). E-Cadherin were higher expressed in AEG tissues and its cytomembrane was stained brown (200×) (C). E-Cadherin were lower expressed in AEG tissues and tumor cells were not stained (200×) (D).
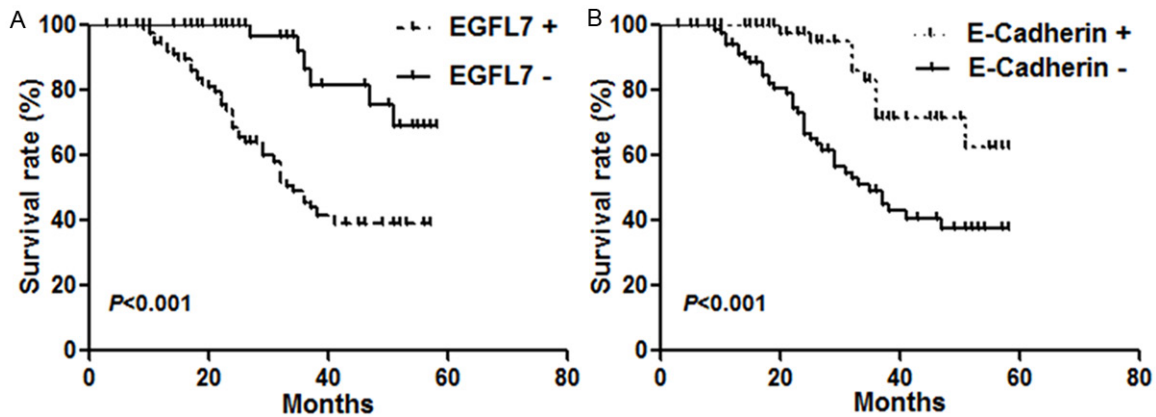

Figure 2. Kaplan-Meier survival curves of AEG patients based on EGFL7 expression (A) or E-Cadherin expression (B).

Discussion

In this study, the positive rate of EGFL7 in AEG was significantly higher than that of the tumor-adjacent tissue, suggesting that EGFL7 may participate in AEG tumorigenesis. However, EGFL7 expression levels were not significantly correlated with hispathological types in AEG patients, maybe due to the relatively small sample size. In addition, the positive rate of EGFL7 had no relationship with gender and Siewert classification. The positive rate of EGFL7 was closely related to the TNM classification, lymphatic metastasis and distant metastasis, suggesting that EGFL7 played a role in the progression of AEG. The research

found that the patients with high EGFL7 expression had short survival time. By contrast, the patients with lower expression had a favorable effect and comparatively long survival time [6, 9, 22, 23].

Therefore, EGFL7 is expected to be an independent tumor prognostic factor. Our follow-up results also showed that the patients with high EGFL7 expression had worse survival time compared with those with low EGFL7 expression. Cox multivariate analysis also suggested that high EGLF7 expression was strongly associated with a higher hazard ratio and unfavorable clinical out comes. With more studies about mechanism of EGFL7 and tumor proliferation, apoptosis, and malignant progress, EGFL7 as the target drugs of gene therapy also gets more and more attention. Making the EGFL7 gene inactive by using RNA interference can reduce the proliferation and the invasion ability of tumor cells [24-26]. In combination with the scholars' researches, EGFL7 protein may be helpful for auxiliary diagnosis of bladder cancer and the judgment of patient prognosis, which may become the new target for tumor gene therapy.

This study found that the positive rate of E-cadherin in AEG tissue cells were significantly lower than that in the tumor-adjacent tissue. E-cadherin expression in poorly was much lower than well-moderately in histopathological stage, and the higher pathologic the stage was, the lower the positive rate was. These suggested that E-cadherin may be a tumor inhibition factor participating in AEG.

Low expression of E-Cadherin is closely related to the TNM classification, lymphatic metastasis and distant metastasis, which indicated that the loss of E-cadherin protein function could lead to the invasion and metastasis of tumor. Studies showed that patients with low E-Cadherin expression had poor prognosis [27, 28]. Our study also showed that patients with low E-cadherin expression showed significantly worse survival compared with those with high E-cadherin expression. Cox multivariate analysis also suggested that low E-cadherin expression was strongly associated with a higher hazard ratio and unfavorable clinical out comes. It may be considered for auxiliary diagnosis of AEG, and judging the prognosis of patients.

Wang YL found that EGFL7 had certain adjustment effect for E-cadherin protein. They found that E-cadherin protein expression increased significantly when the expression of EGFL7 protein was inhibited by using RNA interference [29]. It suggested that EGFL had a negative regulatory effect on E-cadherin protein and it might be related to Epithelial-Mesenchymal Transition (EMT). EMT could promote tumor cell infiltration and tumor metastasis [30, 31]. Luo BH researched that EGFL7 promoted tumor invasion by activating EMT through an EGFR-AKT-Snail signaling pathway in gastric cancer [25]. The research showed that snail, the transcription factor, could down regulate the expression of E-cadherin, which could induce the occurrence of EMT [32].

Our study also found that the expression of EGFL7 was up-regulated, while the expression of E-cadherin protein in AEG was down-regulated, and they were negatively correlated. The specific mechanism that EGFL7 regulate E-cadherin is still unknown. Combined with the literature, we speculate that it may be related to EMT, but its specific mechanism needs to be further studied.

Conclusion

In conclusion, compared with tumor-adjacent tissues, positive expression rates of Egfl7in AEG tissues are significantly higher, while rates of E-cadherin in AEG tissues are significantly lower. EGFL7 and E-cadherin expression were closely related to tumor progression and metastasis in AEG. They are a valuable index to determine the biological behavior of AEG and combined detection of them can be used as an important index to evaluate the prognosis of AEG.

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Disclosure of conflict of interest

None.

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References

[1] Hasegawa S and Yoshikawa T. Adenocarcinoma of the esophagogastric junction: incidence, characteristics, and treatment strategies. Gastric Cancer 2010; 13: 63-73.

[2] Kusano C, Gotoda T, Khor CJ, Katai H, Kato H, Taniguchi H and Shimoda T. Changing trends in the proportion of adenocarcinoma of the esophagogastric junction in a large tertiary referral center in Japan. J Gastroenterol Hepatol 2008; 23: 1662-1665.

[3] Mine S, Sano T, Hiki N, Yamada K, Kosuga T, Nunobe S, Shigaki H and Yamaguchi T. Thoracic lymph node involvement in adenocarcinoma of the esophagogastric junction and lower esophageal squamous cell carcinoma relative to the location of the proximal end of the tumor. Ann Surg Oncol 2014; 21: 1596-1601.

[4] Huang L, Xu A, Li T, Han W, Wu S and Wang Y. Detection of perioperative cancer antigen 72-4 in gastric juice pre- and post-distal gastrectomy and its significances. Med Oncol 2013; 30: 651.

[5] Fan C, Yang LY, Wu F, Tao YM, Liu LS, Zhang JF, He YN, Tang LL, Chen GD and Guo L. The expression of Egfl7 in human normal tissues and epithelial tumors. Int J Biol Markers 2013; 28: 71-83.

[6] Li JJ, Yang XM, Wang SH and Tang QL. Prognostic role of epidermal growth factor-like domain 7 protein expression in laryngeal squamous cell carcinoma. J Laryngol Otol 2011; 125: 1152-1157.

[7] Wu F, Yang LY, Li YF, Ou DP, Chen DP and Fan C. Novel role for epidermal growth factor-like domain 7 in metastasis of human hepatocellular carcinoma. Hepatology 2009; 50: 1839-1850.

[8] Musiyenko A, Bitko V and Barik S. Ectopic expression of miR-126*, an intronic product of the vascular endothelial EGF-like 7 gene, regulates prostein translation and invasiveness of prostate cancer LNCaP cells. J Mol Med (Berl) 2008; 86: 313-322.

[9] Huang CH, Li XJ, Zhou YZ, Luo Y, Li C and Yuan XR. Expression and clinical significance of EGFL7 in malignant glioma. J Cancer Res Clin Oncol 2010; 136: 1737-1743.

[10] Soncin F, Mattot V, Lionneton F, Spruyt N, Lepretre F, Begue A and Stehelin D. VE-statin, an endothelial repressor of smooth muscle cell migration. EMBO J 2003; 22: 5700-5711.

[11] Parker LH, Schmidt M, Jin SW, Gray AM, Beis D, Pham T, Frantz G, Palmieri S, Hillan K, Stainier DY, De Sauvage FJ and Ye W. The endothelial-cell-derived secreted factor Egfl7 regulates vascular tube formation. Nature 2004; 428: 754-758.

[12] Pinte S and Soncin F. Egfl7 promotes tumor escape from immunity. Oncoimmunology 2012; 1: 375-376.

[13] Blaschuk OW and Devemy E. Cadherins as novel targets for anti-cancer therapy. Eur J Pharmacol 2009; 625: 195-198.

[14] Borghi N, Sorokina M, Shcherbakova OG, Weis WI, Pruitt BL, Nelson WJ and Dunn AR. E-cadherin is under constitutive actomyosin-generated tension that is increased at cell-cell contacts upon externally applied stretch. Proc Natl Acad Sci U S A 2012; 109: 12568-12573.

[15] Santos-Garcia A, Abad-Hernandez MM, Fonseca-Sanchez E, Julian-Gonzalez R, Galindo-Villardon P, Cruz-Hernandez JJ and Bullon-Sopelana A. E-cadherin, laminin and collagen IV expression in the evolution from dysplasia to oral squamous cell carcinoma. Med Oral Patol Oral Cir Bucal 2006; 11: E100-105.

[16] Beuran M, Negoi I, Paun S, Ion AD, Bleotu C, Negoi RI and Hostiuc S. The epithelial to mesenchymal transition in pancreatic cancer: A systematic review. Pancreatology 2015; 15: 217-225.

[17] Siewert JR, Feith M and Stein HJ. Biologic and clinical variations of adenocarcinoma at the esophago-gastric junction: relevance of a topographic-anatomic subclassification. J Surg Oncol 2005; 90: 139-146; discussion 146.

[18] Huang L and Xu AM. Adenocarcinoma of esophagogastric junction: controversial classification, surgical management, and clinicopathology. Chin J Cancer Res 2014; 26: 226-230.

[19] Huang L, Xu AM and Peng Q. CD147 and MMP-9 expressions in type II/III adenocarcinoma of esophagogastric junction and their clinicopathological significances. Int J Clin Exp Pathol 2015; 8: 1929-1937.

[20] Edge SB and Compton CC. The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM. Ann Surg Oncol 2010; 17: 1471-1474.

[21] Luo W, Shao C, Li N, Zhang F, Guo S, Duan Z, Zheng Q and He H. Expression of epidermal growth factor-like domain 7 correlates with clinicopathological features of osteosarcoma. Am J Transl Res 2015; 7: 1236-1245.

[22] Zhou L, Li J, Zhao YP, Guo JC, Cui QC, Zhou WX, Zhang TP, Wu WM, You L and Shu H. Prognostic significance of epidermal growth factor-like do-

main 7 in pancreatic cancer. Hepatobiliary Pancreat Dis Int 2014; 13: 523-528.

[23] Oh J, Park SH, Lee TS, Oh HK, Choi JH and Choi YS. High expression of epidermal growth factor-like domain 7 is correlated with poor differentiation and poor prognosis in patients with epithelial ovarian cancer. J Gynecol Oncol 2014; 25: 334-341.

[24] Xu HF, Chen L, Liu XD, Zhan YH, Zhang HH, Li Q and Wu B. Targeting EGFL7 expression through RNA interference suppresses renal cell carcinoma growth by inhibiting angiogenesis. Asian Pac J Cancer Prev 2014; 15: 3045-3050.

[25] Luo BH, Xiong F, Wang JP, Li JH, Zhong M, Liu QL, Luo GQ, Yang XJ, Xiao N, Xie B, Xiao H, Liu RJ, Dong CS, Wang KS and Wen JF. Epidermal growth factor-like domain-containing protein 7 (EGFL7) enhances EGF receptor-AKT signaling, epithelial-mesenchymal transition, and metastasis of gastric cancer cells. PLoS One 2014; 9: e99922.

[26] Wang XX, Yao XB, Qiang ZS and Zhu HL. Attenuation of EGFL7 inhibits human laryngocarcinoma cells growth and invasion. Int J Clin Exp Med 2015; 8: 3141-3155.

[27] Fukata S, Inoue K, Kamada M, Kawada C, Furihata M, Ohtsuki Y and Shuin T. Levels of angiogenesis and expression of angiogenesis-related genes are prognostic for organ-specific metastasis of renal cell carcinoma. Cancer 2005; 103: 931-942.

[28] Yun JA, Kim SH, Hong HK, Yun SH, Kim HC, Chun HK, Cho YB and Lee WY. Loss of E-Cadherin expression is associated with a poor prognosis in stage III colorectal cancer. Oncology 2014; 86: 318-328.

[29] Wang YL, Dong FL, Yang J, Li Z, Zhi QM, Zhao X, Yang Y, Li DC, Shen XC and Zhou J. Suppression of the Epidermal Growth Factor-like Domain 7 and Inhibition of Migration and Epithelial-Mesenchymal Transition in Human Pancreatic Cancer PANC-1 Cells. Asian Pac J Cancer Prev 2015; 16: 4065-4069.

[30] Chaffer CL, Brennan JP, Slavin JL, Blick T, Thompson EW and Williams ED. Mesenchymal-to-epithelial transition facilitates bladder cancer metastasis: role of fibroblast growth factor receptor-2. Cancer Res 2006; 66: 11271-11278.

[31] Lee TK, Poon RT, Yuen AP, Ling MT, Kwok WK, Wang XH, Wong YC, Guan XY, Man K, Chau KL and Fan ST. Twist overexpression correlates with hepatocellular carcinoma metastasis through induction of epithelial-mesenchymal transition. Clin Cancer Res 2006; 12: 5369-5376.

[32] Ray ME, Mehra R, Sandler HM, Daignault S and Shah RB. E-cadherin protein expression predicts prostate cancer salvage radiotherapy outcomes. J Urol 2006; 176: 1409-1414; discussion 1414.