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Original Article

High EGFL6 expression is associated with clinicopathological characteristics in colorectal cancer

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Abstract: To explore the expression levels of EGFL6 in colorectal cancer and the association between EGFL6 and its clinicopathological parameters in patients. Immunohistochemistry assay was used to detect the expression levels of EGFL6 in cancer tissues of 42 colorectal cancer cases and corresponding adjacent normal tissues. The associations between protein expression levels of EGFL6 and the clinicopathological features, such as age, gender, smoking status, drinking status, TNM stage, Tumor T status, lymph node status, distant metastasis, and tumor diameter were also analyzed. We measured the plasma EGFL6 levels of colorectal cancer patients by using a commercial enzyme-linked immunosorbent assay. The positive rate of EGFL6 in cancer tissues was significantly higher than that of cancer-adjacent tissues ($P < 0.05$). Correlation was found between the protein expression of EGFL6 and the TNM stage ($P < 0.05$), the tumor T status ($P < 0.05$), distant metastasis ($P < 0.05$) and tumor diameter ($P < 0.05$). The plasma EGFL6 levels were significantly higher in patients with colorectal cancer than in healthy controls ($P < 0.001$). Moreover, plasma EGFL6 levels were significantly higher in the patients with higher TNM stage ($P = 0.024$), tumor T status ($P = 0.021$), distant metastasis ($P < 0.001$), and tumor diameter ($P = 0.049$). Therefore, these results demonstrated that EGFL6 expression was correlated with the genesis and development of colorectal cancer.

Keywords: Colorectal cancer, EGFL6, ELISA, clinicopathological characteristics

Introduction

Colorectal cancer (CRC) is the third most frequently diagnosed cancer and the fourth most common cause of cancer-related deaths worldwide [1]. Approximately 1.36 million novel CRC cases and 694,000 mortalities are attributed to CRC annually worldwide [2]. Numerous risk factors for CRCinclude age, hereditary components, chronic intestinal inflammation, obesity, excessive intake of alcohol and red meat, smoking and lack of physical exercise [3-5]. Currently, surgery followed by radiotherapy and chemotherapy remains the primary therapeutic strategy for patients with CRC; however, 25%-30% of patients are diagnosed at advanced stages and are unsuitable for surgical resection [6]. Despite the advancement in comprehensive therapy, the long-term survival of CRC patients remains unsatisfactory [7]. The poor therapeutic outcome of patients with CRC is mainly due to local recurrence and distant metastases, particularly liver metastasis [8]. Therefore, a comprehensive understanding of the mechanisms underlying CRC onset and development is particularly urgent for the identification of novel therapeutic strategies for patients with this malignant tumor.

Tumor invasion and metastasis are related to a series of complex processes, including cell adhesion, migration, invasion, angiogenesis, and anchorage-independent growth [9-12]. The epidermal growth factor (EGF) repeat superfamily features a series of conserved cysteines and glycines positioned in a domain of 30 to 40 residues [13]. EGF-like proteins are characterized by their multiple EGF repeats [14]. EGF-like repeat family members are predominantly secreted as cell surface molecules, and are often involved in the regulation of the cell cycle, proliferation, and developmental processes [15, 16]. The binding of EGF-like proteins to their receptors triggers a wide range of biological functions, including proliferation, differentiation, apoptosis, adhesion, and migration. EGF

Table 1. Demographic characteristics and clinical features of colorectal cancer patients

Variable	Colorectal cancer (n = 42)
Age (years)	56.19 ± 6.89
Gender: male (%)	36 (85.7%)
Smoking status	
No	8 (19.0%)
Yes	34 (81.0%)
Drinking status	
No	19 (45.2%)
Yes	23 (54.8%)
EGFL6 (pg/ml)	298.67 ± 94.64
TNM stage	
I	11 (26.2%)
II	7 (16.7%)
III	4 (9.5%)
IV	20 (47.6%)
Tumor T status	
T1	14 (33.3%)
T2	12 (28.6%)
T3	3 (7.1%)
T4	13 (31.0%)
Lymph node status	
N0	27 (64.3%)
N1	4 (9.5%)
N2	10 (23.8%)
N3	1 (2.4%)
Distant metastasis	
M0	34 (81.0%)
M1	8 (19.0%)
Tumor diameter (cm)	
> 5	31 (73.8%)
≤ 5	11 (26.2%)

motif-containing molecules have been previously linked to the progression of various cancers [17, 18], and the expression of EGF-like domain 6 (EGFL6) in tumors suggests that it may also be linked to cancer [19-22].

EGFL6 has been shown to be expressed in fetal tissues and pancreatic, lung, ovarian and breast tumors [23, 24]. Since EGFL6 is expressed specifically in certain tumors but not in normal adult tissues, the EGFL6 gene product represents a potential marker of malignancy. However, the potential expression and role of EGFL6 in patients with colorectal cancer have yet to be elucidated. In this study, we investigated the association between the clinicopathological characteristics and level of EGFL6 in patients with colorectal cancer.

Materials and methods

Subjects and specimen collection

This study was approved by the Ethics Committee of Provincial Hospital Affiliated to Shandong University. Written informed consent was also provided by all patients enrolled in this study. In total, 42 pairs of CRC tissues and corresponding adjacent normal tissues were collected from CRC patients who were treated with surgical resection at the Provincial Hospital Affiliated to Shandong University between January 2011 and January 2017. Colorectal cancer was clinically staged at the time of diagnosis according to the TNM staging system of the American Joint Committee on Cancer (AJCC) Staging Manual, seventh edition. Medical information of the colorectal cancer patients including TNM clinical staging, primary tumor size, lymph node involvement, and histological grade was obtained from their medical records. Whole blood samples were collected from the patients and placed in tubes containing ethylene diamine tetraacetic acid. After immediate centrifugation at 3000 rpm, the supernatants were stored at -80°C.

Immunohistochemical assay

The samples of cancer tissues and adjacent tissues were incised into paraffin sections with a diameter of 4 μm. Histological sections were first soaked into dimethylbenzene for 10 min, followed by dehydrated alcohol for 5 min, and then in succession in the following alcohol solutions for 5 min each for dewaxing with 95%, 80%, and 75% alcohol. After washing with distilled water, tissue peroxidase was blocked with 3.0% hydrogen peroxide in methanol for 15 min at room temperature. For antigen retrieval using citric acid buffer, the slides were heated at 120°C for 20 min and then cooled for 45 min at room temperature. After washing, the slides were incubated with primary mouse monoclonal antibodies against EGFL6 overnight at 4°C. The slides were washed with PBS. EGFL6 stained slides were incubated with biotinylated anti-mouse IgG (Histofine Simplestain Max PO; Nichirei, Tokyo, Japan) as second antibody for 1 h at room temperature. The slides were washed with PBS again and then incubated with horse-

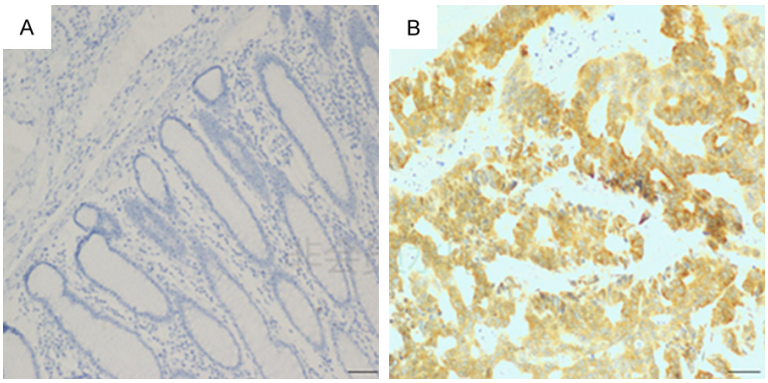

Figure 1. EGFL6 expression in colorectal cancer tissue and normal intestinal tissues by immunohistochemistry. A. EGFL6 negative expression in normal intestinal tissues, ×400; B. EGFL6 positive expression in colorectal cancer tissues, ×400.

radish peroxidase-conjugated streptavidin (Histofine SAB-PO; Nichirei, Tokyo, Japan) for 30 min at room temperature. The immune reaction was demonstrated with DAB. The sections were then counterstained with Meyer's hematoxylin, dehydrated, and mounted.

Quantitative analysis of plasma EGFL6 level

The plasma EGFL6 concentration was determined quantitatively using an enzyme-linked immunosorbent assay (ELISA). One hundred microliters of plasma sample (100-fold dilution), standard control sample, and internal quality control were placed into microtiter plates coated with a monoclonal antibody against EGFL6 and incubated for 2 h at room temperature on a horizontal orbital shaker at 200 rpm. The absorbance was measured at 450 nm by using a microtest plate spectrophotometer (BioTek Instruments, Vemont, USA). EGFL6 levels were quantified with a calibration curve using human EGFL6 as the standard.

Statistical analysis

All analyses were performed using SPSS version 19.0 statistical software (SPSS Inc., Chicago, IL, USA). The demographic data are presented as number (%) and mean ± standard deviation (SD). Significances of differences between means were calculated using Student's t-test. In addition, gender, smoking status and drinking status were analyzed using the χ^2 test. A p value < 0.05 was considered significant.

Results

Patient characteristics

Forty-two patients with colorectal cancer were included in the analysis. **Table 1** presents the demographic data, and shows that 81% of the patients were smokers and 54.8% consumed alcohol. The TNM stage, Tumor T status, Lymph node status, Tumor diameter and distant metastasis of the patients are also shown in **Table 1**.

Expression of EGFL6

EGFL6 was primarily expressed in the cytoplasm and cell membrane with yellow-brown granules. EGFL6 positive expression rate was significantly higher in cancer tissues than that in adjacent normal tissues ($P < 0.01$, **Figure 1**).

Correlation between EGFL6 expression and patients' clinicopathological features

Correlation was found between the protein expression of EGFL6 and the TNM stage ($P < 0.05$), the Tumor T status ($P < 0.05$), distant metastasis ($P < 0.05$) and tumor diameter ($P < 0.05$). It was not correlated with patient's age ($P > 0.05$), gender ($P > 0.05$), smoking status ($P > 0.05$), drinking status ($P > 0.05$) and lymph node status ($P > 0.05$) in **Table 2**.

Correlation between plasma EGFL6 levels and clinicopathological characteristics of the patients

The mean plasma EGFL6 level was significantly higher in patients with colorectal cancer than in controls (298.67 ± 94.64 pg/mL vs 198.09 ± 54.16 pg/mL; $P < 0.001$) (**Figure 2A**). To verify our findings, TCGA colorectal cancer database was used in this study. The EGFL6 mRNA levels of colorectal cancer and normal tissues were evaluated. **Figure 2B** shows the EGFL6 mRNA levels were also significantly higher in patients with colorectal cancer tissues than in normal tissues. Moreover, the EGFL6 expression was also significantly increased in cancer tissue compared with that in the normal parts in colorectal cancer of the TCGA database (**Figure 2C**).

Table 2. Correlation between expression levels of EGFL6 and clinicopathologic findings in 42 colorectal cancer patients

Variable	No. of case (%)	EGFL6 expression		*P* value
	n = 42	Positive	Negative	
Age (years)				0.468
< 55	19 (45.2%)	13	6	
≥ 55	23 (54.8%)	16	7	
Gender				0.673
Male	28 (66.7%)	19	17	
Female	14 (33.3%)	8	6	
Smoking status				0.463
No	12 (28.6%)	7	5	
Yes	30 (71.4%)	22	8	
Drinking status				0.525
No	19 (45.2%)	11	8	
Yes	23 (54.8%)	16	7	
TNM stage				0.019*
I + II	18 (42.9%)	8	10	
III + IV	24 (57.1%)	20	4	
Tumor T status				0.01*
T1 + T2	26 (61.9%)	12	14	
T3 + T4	16 (38.1%)	14	2	
Lymph node status				0.102
N0	27 (64.3%)	14	13	
N1 + N2 + N3	15 (35.7%)	12	3	
Distant metastasis				0.016*
M0	34 (81.0%)	18	16	
M1	8 (19.0%)	8	0	
Tumor diameter (cm)				0.03*
> 5	31 (73.8%)	16	15	
≤ 5	11 (26.2%)	10	1	

Note: *$P < 0.05$.

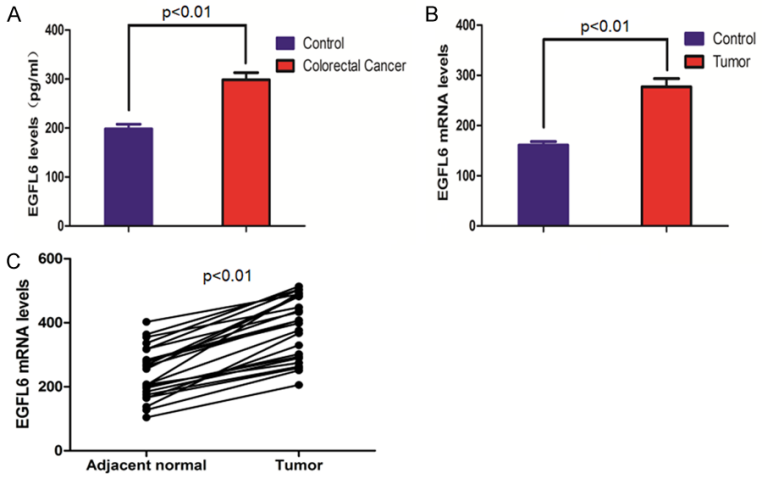

Figure 2. ELISA-determined plasma EGFL6 level of colorectal cancer patients. A. EGFL6 levels were compared according to normal control and colorectal cancer patients. B. EGFL6 mRNA expressions were compared according to normal tissue and colorectal cancer patient tissues from the TCGA database. C. Relative expression of EGFL6 in 24 pairs of colorectal tumor tissues and their corresponding adjacent non-cancerous tissues.

The relationships between plasma EGFL6 levels and various clinicopathologic parameters of the patients are summarized in **Table 3**. Plasma levels of EGFL6 protein were not correlated with age, gender, smoking status, drinking status, and lymph node metastasis. However, they were significantly higher in patients with higher TNM stage (stage III + stage IV; P = 0.024), advanced Tumor T status (T3 + T4; P = 0.021), distant metastasis (M1; P = 0.000) and larger tumor diameter (> 5; P < 0.001). Detailed comparisons of plasma EGFL6 levels between the patients with different disease severity are illustrated in **Figure 3**. With regards to TNM stage, the levels of EGFL6 were significantly higher in the patients with stage IV (332.83 pg/mL) compared to those with an early stage (stage I: 274.22 pg/mL) (**Figure 3**). The levels of EGFL6 were significantly higher in the patients with advanced tumor T status (T4: 347.73 pg/mL) compared to those with early T status (T1: 273.67 pg/mL and T2: 286.64 pg/mL; P = 0.004 and P = 0.020) (**Figure 4**).

Discussion

We investigated the levels of EGFL6 in colorectal cancer patients, and found that levels of EGFL6 were correlated with TNM stage, tumor T status,

Table 3. Correlation between plasma levels of EGFL6 and clinicopathologic findings in 42 colorectal cancer patients

Variable	No. of case (%)	EGFL6 level	*P* value
	n = 42	Mean + SD (pg/ml)	
Age (years)			
< 55	19 (45.2%)	297.75 ± 101.59	0.955
≥ 55	23 (54.8%)	299.42 ± 90.82	
Gender			
Male	28 (66.7%)	299.53 ± 90.33	0.967
Female	14 (33.3%)	298.23 ± 98.35	
Smoking status			
No	12 (28.6%)	301.97 ± 100.94	0.889
Yes	30 (71.4%)	297.34 ± 93.77	
Drinking status			
No	19 (45.2%)	314.93 ± 79.60	0.402
Yes	23 (54.8%)	290.92 ± 101.31	
TNM stage			
I + II	18 (42.9%)	261.65 ± 90.50	0.024*
III + IV	24 (57.1%)	326.80 ± 89.36	
Tumor T status			
T1 + T2	26 (61.9%)	273.31 ± 88.61	0.021*
T3 + T4	16 (38.1%)	339.86 ± 92.05	
Lymph node status			
N0	27 (64.3%)	316.44 ± 92.29	0.107
N1 + N2 + N3	15 (35.7%)	266.67 ± 93.32	
Distant metastasis			
M0	34 (81.0%)	270.68 ± 82.90	0.000*
M1	8 (19.0%)	417.60 ± 9.78	
Tumor diameter (cm)			
> 5	31 (73.8%)	242.67 ± 67.67	0.049*
≤ 5	11 (26.2%)	318.54 ± 95.70	

Note: *$P < 0.05$.

distant metastasis, and tumor diameter. EGFL6 protein levels were significantly higher in patients with higher TNM stage (stage III + stage IV; P = 0.024), advanced tumor T status (T3 + T4; P = 0.021), distant metastasis (M1; P = 0.000) and tumor diameter (> 5; P = 0.000). Previous reports have shown that tumor invasion and metastasis are related to cell adhesion, migration, invasion, and angiogenesis [25-30]. In addition, the EGFL6 protein has been reported to induce cell migration of endothelial cells [31-35]. These findings suggested that EGFL6 may promote colorectal cancer tumor invasion and metastasis by promoting cell migration and angiogenesis. Our results also suggest that EGFL6 may play an important role in the carcinogenesis of colorectal cancer.

Several EGF-like superfamily members have been identified, including EGFL2, EGFL3, EGFL5, EGFL6, EGFL7, EGFL8, and EGFL9. EGFL2, EGFL5 and EGFL9 contain transmembrane domains, however EGFL3, EGFL6, EGFL7 and EGFL8 lack transmembrane domains and are secreted as proteins. The EGFL6 gene maps to the human Xp22 chromosome and encodes a secreted protein containing multiple EGF repeat motifs, which is highly expressed in certain tumors and fetal tissues, suggesting a role as a growth factor [34, 36].

Previous studies have reported that the EGFL6 protein induces migration and angiogenesis of endothelial cells, but that endothelial cells themselves do not express EGFL6. Several signaling pathways during angiogenesis have been reported to be potentially activated, such as the integrin/FAK-mediated pathway, MAPK pathway, and the PIK3/Akt pathway [37, 38]. Chim et al. reported that extracellular signal-regulated kinase (ERK) is activated by the EGFL6 protein, and that inhibition of the ERK signaling pathway blocks EGFL6-induced ERK activation and endothelial cell migration. They further validated that EGFL6 promotes endothelial cell migration and angiogenesis via activation of the ERK pathway.

In addition to colorectal cancer, the overexpression of plasma EGFL6 has been observed in several tumors including brain, lung, ovarian, and breast tumors, but generally not in normal adult tissues [39-41]. EGFL6 has also been proposed to be a new target for diagnostic and therapeutic interventions in patients with breast cancer, which shows promise for new areas of basic research in tumor biology [41]. Combined with our results, the plasma level of

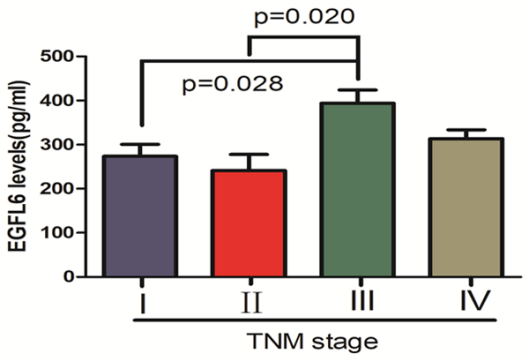

Figure 3. EGFL6 levels were compared according to stage. The levels of EGFL6 were significantly higher in patients with stage III (393.84 pg/ml) compared to those with early stage status (I: 273.64 pg/ml and II: 241.53 pg/ml; P = 0.028 and P = 0.020).

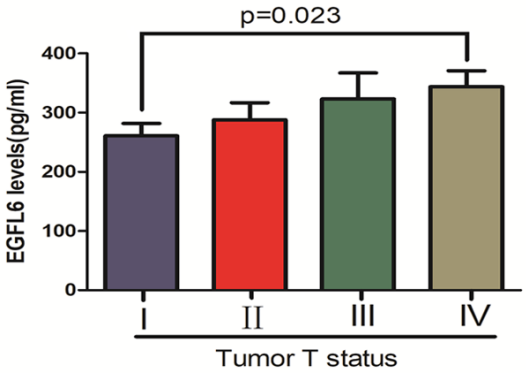

Figure 4. EGFL6 levels were compared according to T status. The levels of EGFL6 were significantly higher in patients with advanced tumor T status (T4: 343.68 pg/ml) compared to those with early T status (T1: 260.76 pg/ml; P = 0.023).

the EGFL6 protein appears to be a likely candidate biomarker for various human cancers.

To the best of our knowledge, this is the first report to examine the association between EGFL6 level and clinicopathologic characteristics for patients with colorectal cancer with regards to the possible application of this molecule as a tumor marker. We suggest that EGFL6 may play an important role in the carcinogenesis of colorectal cancer.

In summary, we found that a substantial increase in the plasma level of EGFL6 by ELISA is useful to assess disease progression, especially in patients with colorectal cancer with an advanced T status, higher TNM stage, longer distant metastasis and larger tumor diameter. As a secreted protein, EGFL6 may not only play an important role in the carcinogenesis of colorectal cancer, but also find clinical applications as a biomarker for disease diagnosis and in planning therapy for colorectal cancer.

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Disclosure of conflict of interest

None.

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References

[1] Jemal A, Siegel R, Xu J and Ward E. Cancer statistics, 2010. CA Cancer J Clin 2010; 60: 277-300.

[2] Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D and Bray F. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 2015; 136: E359-386.

[3] Andrews L. Dietary flavonoids for the prevention of colorectal cancer. Clin J Oncol Nurs 2013; 17: 671-672.

[4] Altobelli E, Lattanzi A, Paduano R, Varassi G and di Orio F. Colorectal cancer prevention in europe: burden of disease and status of screening programs. Prev Med 2014; 62: 132-141.

[5] Sugarbaker PH. Colorectal cancer: prevention and management of metastatic disease. Biomed Res Int 2014; 2014: 782890.

[6] Scheer A and Auer RA. Surveillance after curative resection of colorectal cancer. Clin Colon Rectal Surg 2009; 22: 242-250.

[7] Haggar FA and Boushey RP. Colorectal cancer epidemiology: incidence, mortality, survival, and risk factors. Clin Colon Rectal Surg 2009; 22: 191-197.

[8] Davies RJ, Miller R and Coleman N. Colorectal cancer screening: prospects for molecular stool analysis. Nat Rev Cancer 2005; 5: 199-209.

[9] Su SC, Lin CW, Yang WE, Fan WL and Yang SF. The urokinase-type plasminogen activator (uPA) system as a biomarker and therapeutic

target in human malignancies. Expert Opin Ther Targets 2016; 20: 551-566.
[10] Yang JS, Lin CW, Su SC and Yang SF. Pharmacodynamic considerations in the use of matrix metallo proteinase inhibitors in cancer treatment. Expert Opin Drug Metab Toxicol 2016; 12: 191-200.
[11] Ho HY, Lin CW, Chien MH, Reiter RJ, Su SC, Hsieh YH and Yang SF. Melatonin suppresses TPA-induced metastasis by down-regulating matrix metalloproteinase-9 expression through JNK/SP-1 signaling in nasopharyngeal carcinoma. J Pineal Res 2016; 61: 479-492.
[12] Su SC, Hsieh MJ, Yang WE, Chung WH, Reiter RJ and Yang SF. Cancer metastasis: Mechanisms of inhibition by melatonin. J Pineal Res 2017; 62.
[13] Davis CG. The many faces of epidermal growth factor repeats. New Biol 1990; 2: 410-419.
[14] Singh AB and Harris RC. Autocrine, paracrine and juxtacrine signaling by EGFR ligands. Cell Signal 2005; 17: 1183-1193.
[15] Carter TH and Kung HJ. Tissue-specific transformation by oncogenic mutants of epidermal growth factor receptor. Crit Rev Oncog 1994; 5: 389-428.
[16] Rusch V, Mendelsohn J and Dmitrovsky E. The epidermal growth factor receptor and its ligands as therapeutic targets in human tumors. Cytokine Growth Factor Rev 1996; 7: 133-141.
[17] Birk D, Gansauge F, Gansauge S, Formentini A, Lucht A and Beger HG. Serum and correspondent tissue measurements of epidermal growth factor (EGF) and epidermal growth factor receptor (EGF-R). Clinical relevance in pancreatic cancer and chronic pancreatitis. Int J Pancreatol 1999; 25: 89-96.
[18] Panin VM, Papayannopoulos V, Wilson R and Irvine KD. Fringe modulates notch-ligand interactions. Nature 1997; 387: 908-912.
[19] Bai S, Ingram P, Chen YC, Deng N, Pearson A, Niknafs Y, O'Hayer P, Wang Y, Zhang ZY, Boscolo E, Bischoff J, Yoon E and Buckanovich RJ. EGFL6 regulates the asymmetric division, maintenance, and metastasis of ALDH+ ovarian cancer cells. Cancer Res 2016; 76: 6396-6409.
[20] Larimer BM and Deutscher SL. Identification of a peptide from *in vivo* bacteriophage display with homology to EGFL6: a candidate tumor vasculature ligand in breast cancer. J Mol Biomark Diagn 2014; 5.
[21] Wang X, Gong Y, Wang D, Xie Q, Zheng M, Zhou Y, Li Q, Yang Z, Tang H, Li Y, Hu R, Chen X and Mao Y. Analysis of gene expression profiling in meningioma: deregulated signaling pathways associated with meningioma and EGFL6 overexpression in benign meningioma tissue and serum. PLoS One 2012; 7: e52707.
[22] Buckanovich RJ, Sasaroli D, O'Brien-Jenkins A, Botbyl J, Hammond R, Katsaros D, Sandaltzopoulos R, Liotta LA, Gimotty PA and Coukos G. Tumor vascular proteins as biomarkers in ovarian cancer. J Clin Oncol 2007; 25: 852-861.
[23] Yeung G, Mulero JJ, Berntsen RP, Loeb DB, Drmanac R and Ford JE. Cloning of a novel epidermal growth factor repeat containing gene EGFL6: expressed in tumor and fetal tissues. Genomics 1999; 62: 304-307.
[24] Sjoblom T, Jones S, Wood LD, Parsons DW, Lin J, Barber TD, Mandelker D, Leary RJ, Ptak J, Silliman N, Szabo S, Buckhaults P, Farrell C, Meeh P, Markowitz SD, Willis J, Dawson D, Willson JK, Gazdar AF, Hartigan J, Wu L, Liu C, Parmigiani G, Park BH, Bachman KE, Papadopoulos N, Vogelstein B, Kinzler KW and Velculescu VE. The consensus coding sequences of human breast and colorectal cancers. Science 2006; 314: 268-274.
[25] Hanahan D and Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011; 144: 646-674.
[26] Buck E, Eyzaguirre A, Barr S, Thompson S, Sennello R, Young D, Iwata KK, Gibson NW, Cagnoni P and Haley JD. Loss of homotypic cell adhesion by epithelial-mesenchymal transition or mutation limits sensitivity to epidermal growth factor receptor inhibition. Mol Cancer Ther 2007; 6: 532-541.
[27] Friedl P and Wolf K. Plasticity of cell migration: a multiscale tuning model. J Cell Biol 2010; 188: 11-19.
[28] Gocheva V, Wang HW, Gadea BB, Shree T, Hunter KE, Garfall AL, Berman T and Joyce JA. IL-4 induces cathepsin protease activity in tumor- associated macrophages to promote cancer growth and invasion. Genes Dev 2010; 24: 241-255.
[29] Bergers G and Benjamin LE. Tumorigenesis and the angiogenic switch. Nat Rev Cancer 2003; 3: 401-410.
[30] Hanahan D and Folkman J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. Cell 1996; 86: 353-364.
[31] Chim SM, Qin A, Tickner J, Pavlos N, Davey T, Wang H, Guo Y, Zheng MH and Xu J. EGFL6 promotes endothelial cell migration and angiogenesis through the activation of extracellular signal-regulated kinase. J Biol Chem 2011; 286: 22035-22046.
[32] Mehta VB and Besner GE. HB-EGF promotes angiogenesis in endothelial cells via PI3-kinase and MAPK signaling pathways. Growth Factors 2007; 25: 253-263.
[33] 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.
[34] Schmidt M, Paes K, De Maziere A, Smyczek T, Yang S, Gray A, French D, Kasman I, Klumperman J, Rice DS and Ye W. EGFL7 regulates the collective migration of endothelial cells by restricting their spatial distribution. Development 2007; 134: 2913-2923.
[35] Campagnolo L, Leahy A, Chitnis S, Koschnick S, Fitch MJ, Fallon JT, Loskutoff D, Taubman MB and Stuhlmann H. EGFL7 is a chemoattractant for endothelial cells and is up-regulated in angiogenesis and arterial injury. Am J Pathol 2005; 167: 275-284.
[36] Yeung G, Mulero JJ, Berntsen RP, Loeb DB, Drmanac R and Ford JE. Cloning of a novel epidermal growth factor repeat containing gene EGFL6: expressed in tumor and fetal tissues. Genomics 1999; 62: 304-307.
[37] Buchner G, Orfanelli U, Quaderi N, Bassi MT, Andolfi G, Ballabio A and Franco B. Identification of a new EGF-repeat-containing gene from human Xp22: a candidate for developmental disorders. Genomics 2000; 65: 16-23.
[38] Kim HS, Shin HS, Kwak HJ, Cho CH, Lee CO and Koh GY. Betacellulin induces angiogenesis through activation of mitogen-activated protein kinase and phosphatidylinositol 3'-kinase in endothelial cell. FASEB J 2003; 17: 318-320.
[39] Lamalice L, Le Boeuf F and Huot J. Endothelial cell migration during angiogenesis. Circ Res 2007; 100: 782-794.
[40] Januchowski R, Zawierucha P, Rucinski M and Zabel M. Microarray-based detection and expression analysis of extracellular matrix proteins in drugresistant ovarian cancer cell lines. Oncol Rep 2014; 32: 1981-1990.
[41] Sjoblom T, Jones S, Wood LD, Parsons DW, Lin J, Barber TD, Mandelker D, Leary RJ, Ptak J, Silliman N, Szabo S, Buckhaults P, Farrell C, Meeh P, Markowitz SD, Willis J, Dawson D, Willson JK, Gazdar AF, Hartigan J, Wu L,Liu C, Parmigiani G, Park BH, Bachman KE, Papadopoulos N, Vogelstein B, Kinzler KW and Velculescu VE. The consensus coding sequences of human breast and colorectal cancers. Science 2006; 314: 268-274.