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ORIGINAL RESEARCH

Clinical Significance of Serum Vascular Endothelial Growth Factor, Pigment Epithelium–Derived Factor, Tumor Necrosis Factor Alpha, and Progranulin Levels in Patients with Gastric Cancer and Gastric Precancerous Lesions

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Abstract

Background The purpose of this study is to evaluate serum levels of vascular endothelial growth factor (VEGF), pigment epithelium–derived factor (PEDF), tumor necrosis factor alpha (TNF-α), and progranulin in patients with gastric cancer (GC) and precancerous lesions (PCL) and to determine the usefulness of these markers as diagnostic biomarkers in these diseases.

Method A total of 32 GC patients, 35 PCL patients, and 23 healthy controls participated in the study. The serum levels of VEGF, PEDF, TNF-α, and progranulin were measured by enzyme-linked immunosorbent assay (ELISA).

Results The mean serum VEGF levels were 30.6 ± 12.98 pg/mL in GC, 18.2 ± 5.72 pg/mL in PCL, and 17.5 ± 5.59 pg/mL in controls. GC VEGF levels were significantly higher than both PCL and control groups ($p < 0.001$). The mean serum PEDF levels were 1516.1 ± 993.8 pg/mL in GC, 1039.1 ± 1002.3 pg/mL in PCL, and 767.5 ± 661.5 pg/mL in controls. The serum PEDF level in the GC group was significantly higher than that in both PCL and control groups ($p = 0.004$ and $p = 0.038$, respectively). The mean serum TNF-α levels were 46.7 ± 14.82 pg/mL in GC, 38.4 ± 11.89 pg/mL in PCL, and 33.8 ± 12.77 pg/mL in controls. There was a significant difference between GC and controls ($p = 0.022$) in TNF-α levels. The mean serum progranulin levels in GC were 2496.6 ± 737.8 pg/mL, 2332.0 ± 482.1 pg/mL in PCL, and 1288.7 ± 830.9 pg/mL in controls. Progranulin levels in both GC and PCL groups were significantly higher than that in the control group ($p < 0.001$ for both).

Conclusion There were significant differences among patients with GC and PCL and healthy controls in terms of serum VEGF, PEDF, TNF-α, and progranulin levels.

Keywords Gastric cancer · Gastric atrophy · Intestinal metaplasia · VEGF · PEDF · TNF-α · Progranulin

Introduction

Gastric cancer (GC) is the fifth most frequently diagnosed cancer and the third most common cause of death due to cancer in the world [1]. The risk factors for GC are *Helicobacter pylori* infection, chronic atrophic gastritis, gastric polyps, obesity, deficiency of antioxidants, hypertrophic gastropathy, high salt intake, genetic alterations of oncogenes and tumor suppressor genes, and endogenous formation of N-nitroso compounds. The high mortality rate is partly due to the delayed diagnosis of asymptomatic patients at the early stages of the disease [2, 3].

Chronic inflammation of the gastric mucosa leads to atrophic changes and damage to glandular cells which are replaced by intestinal type epithelium. Gastric atrophy (GA)

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and intestinal metaplasia (IM) are classified as precancerous lesions (PCL) [4].

Angiogenesis plays an essential role in the growth, invasion, and metastatic spread of tumors. Vascular endothelial growth factor (VEGF) is the most important angiogenic factor in tumorigenesis [5]. In a research study, Ding et al. reported higher plasma VEGF-A levels in GC patients ($p < 0.01$) [6]. Another study by Vidal et al. showed that higher preoperative VEGF levels were associated with advanced disease, reduced recurrence-free status, and shorter disease specific survival in GC [7]. Pigment epithelium–derived factor (PEDF) is a multifunctional molecule which has neuroprotective, anti-oxidative, anti-inflammatory, and endogenous angiogenic inhibitor activities [8]. Previous studies suggest that PEDF induces apoptosis by several pathways and can be useful in tumor treatment. Zhang et al. demonstrated that growth and angiogenesis of gastric carcinoma were suppressed after PEDF injection in the xenograft model [9].

Tumor necrosis factor-alpha (TNF-α) is a pro-inflammatory cytokine that plays role in tumorigenesis by mediating gene expressions of cytokines, adhesion molecules, and pro-angiogenic molecules [10]. Forones et al. reported that serum TNF-α levels were associated with advanced GC and poor prognosis [10].

Progranulin, also called granulin-epithelin precursor, proepithelin, or acrogranin, belongs to a new class of growth factors that is involved in cell development, cell cycle progression, cell motility, and tumorigenesis. High expressions of progranulin and its association with tumor progression and poor prognosis reported in breast cancer, liver cancer, and ovarian cancer [11]. Line at al. reported elevated progranulin levels in GC patients for the first time [12]. Yang et al. demonstrated that higher progranulin expressions are correlated with lymph node metastasis, lymphatic invasion, and advance clinical stage [13].

It is crucial to differentiate PCL from normal tissue and overt cancerous tissue for an early diagnosis. Endoscopic evaluation and histological examination is the most effective procedure for this distinction; however, it is an expensive and invasive procedure with potential complications.

In this study, we aimed to evaluate serum levels of VEGF, PEDF, TNF-α, and progranulin in patients with GC and PCL and to determine the usefulness of these markers as diagnostic biomarkers in these diseases.

Materials and Methods

Study Population

The study included 32 GC and 35 PCL patients (IM and/or GA) and 23 healthy controls. Patients were diagnosed based on endoscopic and histopathological findings at Keçiören Training and Research Hospital Gastroenterology Clinic between January 2018 and December 2018.

Patients younger than 18 years old, patients with a history of abdominal surgery, systemic disease, patients receiving proton-pump inhibitors or antibiotics for the last 6 weeks were excluded from the study.

This study was approved by the Institutional Review Board of Keçiören Training and Research Hospital and signed informed consent was obtained from all patients.

Endoscopic and Histopathological Evaluation

Endoscopic and histopathological evaluation was carried out by a trained gastroenterologist and pathologist. GA, IM, and *Helicobacter pylori* infection were described according to the updated Sydney System Classification. The operative link on gastric atrophy (OLGA) and operative link on gastric intestinal metaplasia (OLGIM) stages were recorded for the patients with precancerous lesions.

Laboratory Tests

Peripheral fasting blood samples were collected from all patients and centrifuged at 5000 ppm, and serum samples were stored at – 80 °C until analysis. The serum concentrations of VEGF, PEDF, TNF-α, and progranulin were measured by enzyme-linked immunosorbent assay using commercially available kits according to the manufacturer's instructions (ELISA, Boster Immunoleader, USA). Serum VEGF, PEDF, TNF-α, and progranulin levels were presented as pg/mL. The intra-assay and inter-assay coefficient of variations were less than 10%.

Statistical Analysis

Statistical analyses were performed using the computer program Statistical Package for the Social Sciences (SPSS) 22.0 (IBM, USA). Kolmogorov-Smirnov and Shapiro-Wilk tests of normality were used to test the distribution of variables. One-way ANOVA was used for comparison of group means. Relationship between inflammatory markers and gastric cancer diagnosis was assessed using with regression analysis, odds ratio (OR), and 95% confidence interval (CI). Cut-off values for each marker were assessed using a receiver operating characteristic (ROC) curve and sensitivity and specificity were presented. Data were presented as means ± standard deviation or number and percentage. Differences were considered significant at $p < 0.05$.

Ethical Procedure

All procedures performed involving human participants were in accordance with the ethical standards of the institutional

research committee (Keçiören Training and Research Hospital with reference number 01.2018/1601) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

Results

Patient Characteristics

Thirty-two patients with GC, 35 patients with PCL, and 23 healthy control subjects were enrolled in this study. There was no difference between groups in terms of age, gender, and *Helicobacter pylori* presence. *Helicobacter pylori* infection was 50% in the GC group and 51.4% in the PCL group. All of the GC patients had adenocarcinoma. In the PCL group, OLGIM stages were stage III for 60% and stage IV for 40% of patients, and OLGA stages were stage III for 45.7% and stage IV for 54.3% of patients (Table 1).

Serum VEGF, PEDF, TNF-α, and Progranulin Levels of Patients

The mean serum VEGF levels were 30.6 ± 12.98 pg/mL in GC, 18.2 ± 5.72 pg/mL in PCL, and 17.5 ± 5.59 pg/mL in controls. GC VEGF levels were significantly higher than both the PCL and control groups ($p < 0.001$ for both); however, there was no significant difference between the PCL and control groups ($p > 0.05$). The mean serum PEDF levels were 1516.1 ± 993.8 pg/mL in GC, 1039.1 ± 1002.3 pg/mL in PCL, and 767.5 ± 661.5 pg/mL in controls. GC PEDF levels were significantly higher than both the PCL and control groups ($p = 0.038$ and $p = 0.004$, respectively), meanwhile there was no significant difference between the PCL and control groups ($p > 0.05$). The mean serum TNF-α levels were 46.7 ± 14.82 pg/mL in GC, 38.4 ± 11.89 pg/mL in PCL, and 33.8 ± 12.77 pg/mL in controls. GC TNF-α levels were significantly higher than the control group ($p = 0.022$). The mean serum progranulin levels were 2496.6 ± 737.8 pg/mL in GC, 2332.0 ± 482.1 pg/mL in PCL, and 1288.7 ± 830.9 pg/mL in controls. Both GC and PCL group progranulin levels were significantly higher than the control group ($p < 0.001$ for both); however, there was no difference between the GC and PCL groups ($p > 0.05$) (Table 2).

The OR levels for all biomarkers confirmed that elevated serum VEGF, PEDF, TNF-α and progranulin levels were risk factors for GC (Table 3). Furthermore, we determined cut-off values for each cytokine using ROC analysis, which showed moderate values for the areas under the curve. There was no significant difference between PCL and controls or GC. The only significant difference was between the GC and control groups. Optimal cut-off point for VEGF is 18.9 pg/ml with 94% sensitivity and 64% specificity (ROC area under the curve, 0.858; $p < 0.001$). Optimal cut-off point for PEDF is 707.9 pg/ml, with 88% sensitivity and 59% specificity (ROC area under the curve, 0.729; $p < 0.001$). Optimal cut-off point for TNF-α is 33.1 pg/ml with 81% sensitivity and 52% specificity (ROC area under the curve, 0.714; $p = 0.001$). Optimal cut-off point for progranulin is 1682 pg/ml with 90% sensitivity and 42% specificity (ROC area under the curve, 0.685; $p = 0.004$) (Fig. 1).

Discussion

We analyzed serum levels of VEGF, PEDF, TNF-α, and progranulin levels for GC and PCL patients and healthy controls. We found significantly increased levels of VEGF and PEDF in the GC group compared with the PCL group. Although VEGF, PEDF, and TNF-α levels were similarly lower in the PCL and control groups compared with GC, progranulin levels were significantly higher both in the GC and PCL groups compared with healthy controls.

In scientific literature, there are copious amounts of data on the relationship between overall survival and tumor angiogenesis, local invasion, and distant metastasis. Not all tumors are angiogenic at the beginning of their developmental stages. It is crucial to detect the disease in the stage where angiogenic factors are secreted from the tumor microenvironment.

Wang et al. observed a significantly increased VEGF expression in tissues with gastritis, atrophy, dysplasia, and gastric stromal tumor compared with normal gastric mucosa of animal models [14]. The expression of VEGF in tissue with dysplasia was higher than that in tissue with inflammation and atrophy (10.8 ± 1.96 pg/mL vs. 7.62 ± 0.25 pg/mL, $p = 0.029$; 10.8 ± 1.96 pg/mL vs. 6.26 ± 0.76 pg/mL, $p = 0.033$, respectively); the expression of VEGF in tissue with gastritis and atrophy was not significantly different ($p > 0.05$) [14]. Feng et al. demonstrated that the positive immunostaining rate for VEGF was very low in chronic atrophic gastritis, slightly increased in IM and dysplasia, and significantly increased in GC. There were significant differences between GC and both chronic atrophic gastritis and IM. There was no significant difference between dysplasia and GC [15]. Yakut et al. found no significant difference in VEGF levels between *Helicobacter pylori* positive chronic non-atrophic gastritis, chronic atrophic gastritis, intestinal metaplasia, and dysplasia groups [16]. We had no patients with dysplasia. But our findings were similar with the existent reports.

Zhang et al. studied the effect of PEDF on gastric carcinoma. They showed that growth and angiogenesis of gastric carcinoma were suppressed after PEDF injection by downregulating hypoxia-inducible factor-1α and VEGF [9]. In the present study, serum VEGF and PEDF levels showed similar

Table 1 Demographic characteristics of patients

	GC ($n = 32$)	PCL ($n = 35$)	Control ($n = 23$)	p
Age (years) (mean ± SD)	59.9 ± 9.94	57.3 ± 9.49	58.6 ± 8.72	$p > 0.05$
Sex % (M/F)	59.4/40.6	37.1/62.9	56.5/43.5	$p > 0.05$
Hp (+) (n, %)	16 (50.0)	18 (51.4)	–	$p > 0.05$
Location (n, %)				
Corpus	10 (31.3)	–	–	
Cardia	7 (21.9)	–	–	
Antrum	15 (46.9)	–	–	
OLGIM (n, %)				
III	–	21 (60.0)	–	
IV	–	14 (40.0)	–	
OLGA (n, %)				
III	–	16 (45.7)	–	
IV	–	19 (54.3)	–	
Severity of intestinal metaplasia (n, %)				
++	–	13 (37.1)	–	
+++	–	22 (62.9)	–	
Gastric atrophy (n, %)				
+	–	8 (22.9)	–	
++	–	26 (74.3)	–	

Hp, Helicobacter pylori; *GC*, gastric cancer; *PCL*, precancerous lesion

trends between groups. We believe that PEDF levels increased in response to the increase in VEGF levels.

Senthilkumar et al. reported overexpression of TNF-α in chronic gastritis, intestinal metaplasia, dysplasia, and gastric adenocarcinoma patients. [17]. Sánchez-Zauco et al. found no significant difference in TNF-α levels between GC patients and controls [18]. Ertürk et al. found that the baseline serum TNF-α concentrations of the GC patients were significantly higher ($p = 0.001$) than controls [19]. Our findings were similar with these data. For TNF-α levels, only significant difference was between the GC and control groups.

Yang et al. defined expression levels of progranulin in gastric cancer tissues with immunohistochemistry, and high expression of progranulin was correlated with lymph node metastasis, lymphatic invasion, advanced clinical stage, and poor prognosis [13]. Loei et al. detected that granulin is expressed in GC tissues but not in normal gastric epithelial cells. They observed a progressively increased expression with inflammation, metaplasia, dysplasia, and GC. They also showed increased serum granulin levels of GC patients compared with healthy controls. Increased expression of granulin in the precancerous stage implies that it may have an important role in GC initiation [20]. Our results were in concordance with these studies where GC and PCL patients had a higher progranulin level compared with healthy controls; however, there were no clear difference between GC and PCL groups.

The major limitation of our study is the absence of patients with dysplasia and the lack of evaluation of these biomarker expression levels in the gastric mucosa. There were significant differences between GC patients and healthy controls in terms of serum VEGF, PEDF, TNF-α, and progranulin levels. Serum VEGF, PEDF, and TNF-α levels did not significantly differ

Table 2 Serum VEGF, PEDF, TNF-α, and progranulin levels of patients (pg/mL)

	GC ($n = 32$)	PCL ($n = 35$)	Control ($n = 23$)	p
VEGF	30.6 ± 12.98	18.2 ± 5.72	17.5 ± 5.59	$p^a < 0.001$, $p^b > 0.05$, $p^c < 0.001$
PEDF	1516.1 ± 993.8	1039.1 ± 1002.3	767.5 ± 661.5	$p^a = 0.038$, $p^b > 0.05$, $p^c = 0.004$
TNF-α	46.7 ± 14.82	38.4 ± 11.89	33.8 ± 12.77	$p^a > 0.05$, $p^b > 0.05$, $p^c = 0.022$
Progranulin	2496.6 ± 737.8	2332.0 ± 482.1	1288.7 ± 830.9	$p^a > 0.05$, $p^b < 0.001$, $p^c < 0.001$

GC, gastric cancer; *PCL*, precancerous lesion; p^a, difference between GC and PCL groups; p^b, difference between PCL and control groups; p^c, difference between GC and control groups

Table 3 Logistic regression analysis for inflammatory marker associated with the risk of gastric cancer

	OR	95% CI	*p*
VEGF	0.807	(0.733–0.888)	< 0.001
PEDF	0.999	(0.999–1.000)	0.011
TNF-α	0.975	(0.951–0.999)	0.039
Progranulin	0.999	(0.998–1.000)	0.003

between the PCL and control groups; however, they showed an increasing trend in PCL groups. Furthermore, progranulin levels were significantly higher in the PCL compared with the control group, which might imply that progranulin levels can be used in PCL diagnosis. The lack of difference in progranulin levels between the PCL and GC groups might be due to the role of progranulin in the early stage of carcinogenesis. VEGF and PEDF seem to contribute to carcinogenesis at the late stages or after the cancer formation.

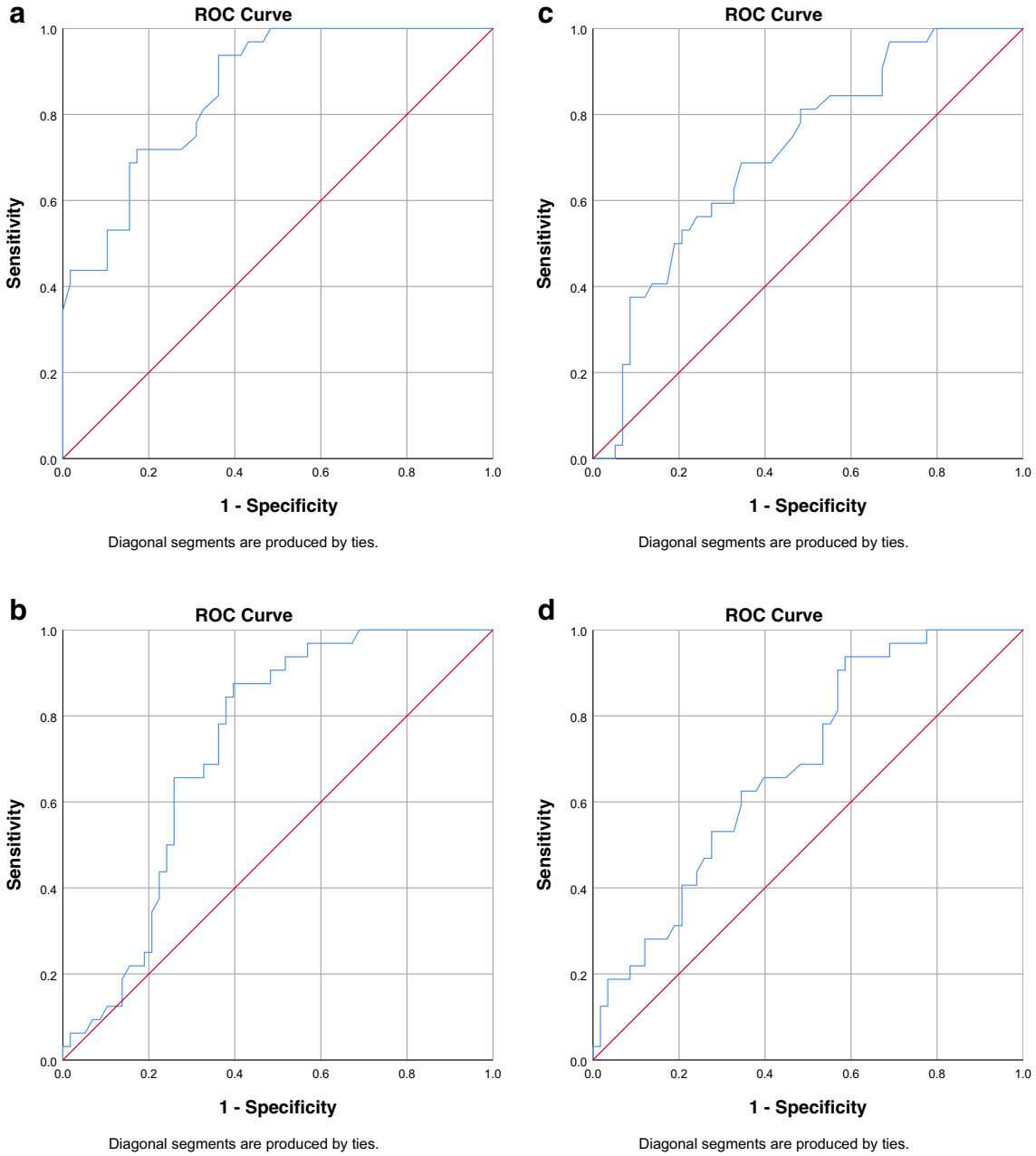

Fig. 1 ROC analyses for GC patients. **a** For VEGF, ROC area under the curve 0.858, $p < 0.001$, optimal cut-off point 18.9 pg/mL, 94% sensitivity and 64% specificity. **b** For PEDF, ROC area under the curve 0.729, $p < 0.001$, optimal cut-off point 707.9 pg/mL, 88% sensitivity and 59% specificity. **c** For TNF-α, ROC area under the curve 0.714, $p = 0.001$, optimal cut-off point 33.1 pg/mL, 81% sensitivity, and 52% specificity. **d** For progranulin, ROC area under the curve 0.685, $p = 0.004$, optimal cut-off point 1682 pg/mL, 90% sensitivity, and 42% specificity

Compliance with Ethical Standards

All procedures performed involving human participants were in accordance with the ethical standards of the institutional research committee (Keçiören Training and Research Hospital with reference number 01.2018/1601) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

Conflict of Interest The authors declare that they have no conflict of interest.

References

1. World Health Organization. GLOBOCAN 2012: Estimated cancer incidence, mortality and prevalence worldwide. 2012, http://globocan.iarc. fr/Pages/fact_sheets_cancer. aspx (accessed June 20, 2016).
2. Yusefi AR, Bagheri Lankarani K, Bastani P, Radinmanesh M, Kavosi Z. Risk factors for gastric cancer: a systematic review. Asian Pac J Cancer Prev. 2018;19(3):591–603. https://doi.org/10.22034/APJCP.2018.19.3.591.
3. Arrington AK. Timing of chemotherapy and survival in patients with resectable gastric adenocarcinoma. World J Gastrointest Surg. 2013;5(12):321–8.
4. Correa P. Human gastric carcinogenesis: a multistep and multifactorial process–first American Cancer Society award lecture on cancer epidemiology and prevention. Cancer Res. 1992;52:6735–40.
5. Folkman J. Tumor angiogenesis: therapeutic implications. N Engl J Med. 1971;285:1182–6. https://doi.org/10.1056/NEJM197111182852108.
6. Ding S, Lin S, Dong X, Yang X, Qu H, Huang S, et al. Potential prognostic value of circulating levels of vascular endothelial growth factor-A in patients with gastric cancer. In Vivo. 2005;19:793–5.
7. Vidal O, Metges JP, Elizalde I, Valentíni M, Volant A, Molina R, et al. High preoperative serum vascular endothelial growth factor levels predict poor clinical outcome after curative resection of gastric cancer. Br J Surg. 2009;96:1443–51.
8. Fernandez-Garcia NI, Volpert OV, Jimenez B. Pigment epithelium-derived factor as a multifunctional antitumor factor. J Mol Med (Berl). 2007;85(1):15–22. https://doi.org/10.1007/s00109-006-0111-z.
9. Zhang Y, Han J, Yang X, Shao C, Xu Z, Cheng R, et al. Pigment epithelium-derived factor inhibits angiogenesis and growth of gastric carcinoma by down-regulation of VEGF. Oncol Rep. 2011;26(3):681–6. https://doi.org/10.3892/or.2011.1318.
10. Forones NM, Mandowsky SV, Lourenço LG. Serum levels of interleukin-2 and tumor necrosis factor-alpha correlate to tumor progression in gastric cancer. Hepatogastroenterology. 2001;48(40):1199–201.
11. Demorrow S. Progranulin: a novel regulator of gastrointestinal cancer progression. Transl Gastrointest Cancer. 2013 Jul;2(3):145–51. https://doi.org/10.3978/j.issn.2224-4778.2013.02.02.
12. Linē A, Stengrēvics A, Slucka Z, Li G, Jankevics E, Rees RC. Serological identification and expression analysis of gastric cancer-associated genes. Br J Cancer. 2002;86(11):1824–30.
13. Yang D, Li R, Wang H, Wang J, Han L, Pan L, et al. Clinical implications of progranulin in gastric cancer and its regulation via a positive feedback loop involving AKT and ERK signaling pathways. Mol Med Rep. 2017;16(6):9685–91. https://doi.org/10.3892/mmr.2017.7796.
14. Wang XY, Wang LL, Zheng X, Meng LN, Lyu B, Jin HF. Expression of p-STAT3 and vascular endothelial growth factor in MNNG induced precancerous lesions and gastric tumors in rats. World J Gastrointest Oncol. 2016;8(3):305–13. https://doi.org/10.4251/wjgo.v8.i3.305.
15. Feng CW, Wang LD, Jiao LH, Liu B, Zheng S, Xie XJ. Expression of p53, inducible nitric oxide synthase and vascular endothelial growth factor in gastric precancerous and cancerous lesions: correlation with clinical features. BMC Cancer. 2002;29(2):8.
16. Yakut M, Örmeci N, Erdal H, Keskin O, Karayel Z, Tutkak H, et al. The association between precancerous gastric lesions and serum pepsinogens, serum gastrin, vascular endothelial growth factor, serum interleukin-1 beta, serum toll-like receptor-4 levels and Helicobacter pylori cag A status. Clin Res Hepatol Gastroenterol. 2013;37(3):302–11. https://doi.org/10.1016/j.clinre.2012.09.013.
17. Senthilkumar C, Niranjali S, Jayanthi V, Ramesh T, Devaraj H. Molecular and histological evaluation of tumor necrosis factor-alpha expression in Helicobacter pylori-mediated gastric carcinogenesis. J Cancer Res Clin Oncol. 2011;137(4):577–83. https://doi.org/10.1007/s00432-010-0921-9.
18. Sánchez-Zauco N, Torres J, Gómez A, Camorlinga-Ponce M, Muñoz-Pérez L, Herrera-Goepfert R, et al. Circulating blood levels of IL-6, IFN-γ, and IL-10 as potential diagnostic biomarkers in gastric cancer: a controlled study. BMC Cancer. 2017;17(1):384. https://doi.org/10.1186/s12885-017-3310-9.
19. Erturk K, Tastekin D, Serilmez M, Bilgin E, Bozbey HU, Vatansever S. Clinical significance of serum interleukin-29, interleukin-32, and tumor necrosis factor alpha levels in patients with gastric cancer. Tumour Biol. 2016;37(1):405–12. https://doi.org/10.1007/s13277-015-3829-9.
20. Loei H, Tan HT, Lim TK, Lim KH, So JB, Yeoh KG, et al. Mining the gastric cancer secretome: identification of GRN as a potential diagnostic marker for early gastric cancer. J Proteome Res. 2012;11(3):1759–72. https://doi.org/10.1021/pr201014h.

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