Clinical significance of serum expression of GROβ in esophageal squamous cell carcinoma

Qiao-Mei Dong, Jin-Qiang Zhang, Qian Li, Jacqueline C Bracher, Denver T Hendricks, Xiao-Hang Zhao

AIM: To determine the association between serum levels of growth-related gene product β (GROβ) and clinical parameters in esophageal squamous cell carcinoma (ESCC).

METHODS: Using enzyme-linked immunosorbent assay, serum GROβ levels were measured in ESCC patients (n = 72) and healthy volunteers (n = 83). The association between serum levels of GROβ and clinical parameters of ESCC was analyzed statistically.

RESULTS: The serum GROβ levels were much higher in ESCC patients than in healthy controls (median: 645 ng/L vs 269 ng/L, P < 0.05). Serum GROβ levels were correlated positively with tumor size, lymph node metastasis, and tumor-node-metastasis (TNM) staging, but not with gender or the histological grade of tumors in ESCC patients. The sensitivity and specificity of the assay for serum GROβ were 73.61% and 56.63%, respectively.

CONCLUSION: GROβ may function as an oncogene product and contribute to tumorigenesis and metastasis of ESCC.

© 2011 Baishideng. All rights reserved.

Key words: GROβ; Esophageal squamous cell carcinoma; Metastasis; Cytokine; Tumor markers

Peer reviewer: Yu-Yuan Li, Professor, Department of Gastroenterology, First Municipal People’s Hospital of Guangzhou, 1 Panfu Road, Guangzhou 510180, Guangdong Province, China

INTRODUCTION

Esophageal squamous cell carcinoma (ESCC) is one of the most aggressive malignancies in China, South Africa, and some other developing countries[1,2]. In China, patients with ESCC have a poor prognosis, with an average 5-year survival rate of less than 30%[3-5]. It is thus imperative to find new markers, especially serum protein markers, to facilitate the early detection and diagnosis of ESCC.
of the CXC chemokine family, a group of small cytokines involved in the response to injury and inflammatory reactions as well as tumor progression, metastasis, and angiogenesis. CXCL16 expression is increased in multiple tumor tissues and cell lines, and overexpression is associated with tumor progression and metastasis. The plasma levels of CXCL4 and CXCL6 are much higher in patients with osteosarcoma than in healthy controls, and elevated plasma CXCL4 and CXCL6 levels are associated with poor prognosis. Growth-related gene product (GRO) is a member of the CXC chemokine family, which is composed of GROα, GROβ and GROγ. GROα is highly expressed in a variety of tumors and plays an important role in tumor proliferation, angiogenesis, and metastasis. Compared with GROα, the roles of GROβ in tumors are poorly understood.

Recently, authors from South Africa reported that GROβ is highly expressed in ESCC tissues and cell lines. Although this suggests that GROβ may be secreted from tumor tissues into the extracellular milieu, no report has yet investigated serum GROβ expression levels in human ESCC. In this study, we measured the serum levels of GROβ in ESCC patients and healthy controls by enzyme-linked immunosorbent assay (ELISA), and analyzed the association between clinical parameters of ESCC and serum GROβ levels.

**MATERIALS AND METHODS**

**Clinical specimen collection and preparation**

Serum samples were collected from 72 ESCC patients (52 males and 20 females; mean age, 61 ± 7.7 years (SD); range 41-76 years) and 83 healthy controls (45 males and 38 females; mean age, 57 ± 8.4 years (SD); range 41-70 years) after the informed consent was obtained. Pathological diagnosis was independently conducted by two senior pathologists. The healthy individuals were negative for hepatitis B virus, hepatitis C virus, and human immunodeficiency virus. Abdominal ultrasound examination, routine blood tests, and biochemistry tests were performed for the healthy controls, and the results were within normal ranges. Sample preparation was referred to the reference. The clinical characteristics of the serum samples are shown in Table 1.

**ELISA for GROβ**

A human GROβ ELISA development kit (PeproTech, Rocky Hill, NJ, USA) was used to measure GROβ in human serum. Briefly, 96-well ELISA microplates were coated overnight with 100 μL GROβ antibody (PeproTech, Rocky Hill, NJ, USA) at a final concentration of 0.25 mg/L in PBS. After washing with PBS/0.05% (w/v) Tween-20 (PBST, pH 7.4), the wells were blocked with blocking buffer at room temperature for 1 h. Then, 100 μL diluted serum samples (at 1:5 dilution) were added and incubated at room temperature for 2 h. Similarly, 100 μL PBST lacking antibody was used as a negative control. Following three washes with PBST, 100 μL antibody diluted to a concentration of 0.25 mg/L was added. After incubation at room temperature for 2 h, 100 μL avidin-horseradish peroxidase-conjugated secondary antibody (at 1:2000 dilution) was added, and plates were incubated at room temperature for 30 min. The excess conjugate was removed by washing the plates three times with PBST. The amount of bound conjugate was determined by adding ABTS liquid substrate solution to each well, and plates were incubated at room temperature for color development. The absorbance was measured at 405 nm using a Model 680 microplate reader (Bio-Rad Lab. Inc., Hercules, CA, USA). All analyses were performed in triplicate. The coefficient of variation was lower than 15% between analyses.

**Statistical analysis**

Statistical significance was determined with the nonparametric Mann-Whitney U-test (differences between two groups) and the Kruskal-Wallis nonparametric test (differences among more than two groups) with SPSS 16.0 software (Chicago, IL, USA). Values of P < 0.05 were considered statistically significant.

**RESULTS**

**Serum GROβ levels in ESCC patients and healthy controls**

A total of 155 participants were enrolled in this study. The serum levels of GROβ in ESCC patients (n = 72) and healthy controls (n = 83) were assessed by ELISA.
Serum concentrations of GROβ ranged from 146 ng/L to 3721 ng/L (median = 645 ng/L) in ESCC patients, and from 0 ng/L to 2781 ng/L (median = 269 ng/L) in healthy controls. GROβ levels were significantly higher in ESCC patients than in healthy controls (Mann-Whitney U-test, P < 0.001, Figure 1A). The sensitivity and specificity of serum GROβ were 73.61% and 56.63%, respectively.

**Association between serum concentrations of GROβ and clinical parameters in ESCC**

We assessed the potential correlations between serum levels of GROβ and clinical parameters, including tumor-node-metastasis (TNM) stage, tumor size, lymph node metastasis, histological grade, and gender. The median levels of GROβ in the ESCC patients at different stages were: stage I, 284 ng/L (range, 146-1141 ng/L); stage II, 360 ng/L (range, 211-2453 ng/L); and stage III, 966 ng/L (range, 165-3721 ng/L). The serum GROβ levels were significantly different (Kruskal-Wallis nonparametric test, P = 0.006) among the three TNM stages, and significantly higher in patients at stage III than in those at stage II (Mann-Whitney U-test, P = 0.041, Figure 1B) and stage I (Mann-Whitney U-test, P = 0.004, Figure 1B). However, there was no significant difference between stage I and stage II (Mann-Whitney U-test, P = 0.135). Furthermore, patients with tumor diameters ≥ 5 cm had higher serum levels of GROβ (median = 786 ng/L, range, 191-3721 ng/L) than those with smaller tumors (median = 456 ng/L, range, 146-2008 ng/L; Mann-Whitney U-test, P = 0.016; Figure 1C). Serum levels of GROβ in patients with lymph node metastasis (median = 819 ng/L, range, 165-3721 ng/L) were significantly higher than those without lymph node metastasis (median = 330 ng/L, range, 146-2453 ng/L; Mann-Whitney U-test, P = 0.020; Figure 1D). However, there was no significant difference between serum levels of GROβ in patients with poorly differentiated tumors (median = 607 ng/L, range, 191-2705 ng/L) and well-differentiated tumors (median = 1178 ng/L, range, 239-3721 ng/L; Mann-Whitney U-test, P = 0.110, Figure 1E). Serum levels of GROβ were not gender dependent (Mann-Whitney U-test, P = 0.650, Figure 1F).

**DISCUSSION**

Identification of targets for early detection of ESCC is important to improve the prognosis of the patients with this pernicious disease. Currently, carcinoembryonic antigen, cytokeratin 19 fragments, and squamous cell carcinoma-associated antigen are routinely used as serum markers for detection of ESCC. Due to the low sensitivity and specificity of detection of these markers, additional serum markers must be established for early detection and diagnosis of ESCC.

GROβ, also known as the chemokine CXCL2, is a member of the CXC chemokine family and shares the same receptor with interleukin (IL)-8, interleukin-8Rβ/CXCR2. GROβ is widely expressed in the central nervous system[17] and possesses important functions, such as attracting neutrophils to sites of inflammation, mobilizing stem cells[18,19], and modulating neurotransmitter release[20]. The roles of GROβ in tumor formation and development have been investigated[21,22], and a recent report described significant elevation of GROβ mRNA in colon cancer tissues[23]. Upregulation of GROβ promotes colony formation by melanocytes in soft agar and tumor formation in nude mice[24,25]. Conversely, decreased expression of GROβ inhibits the proliferation and colonization capacity of esophageal cancer cells[26]. Through binding to its receptor CXCR2, GROβ forms an autocrine loop that activates the Ras-Erk1/2 signaling pathway, which is important for cell proliferation[17,25,26,31]. This pathway in turn activates the transcription and expression of the early growth response 1 gene (egr1), a transcription factor that regulates the expression of downstream factors related to cell growth and cell cycle regulation (p65, p27), thereby promoting tumor growth[22,34].

Using cDNA microarray analysis, authors from South Africa showed that GROβ was highly expressed in ESCC tissues and cultured cells[18]. As a chemokine, GROβ may be secreted into the extracellular matrix. However, no report has investigated the clinical significance of serum GROβ levels in ESCC. To compare the levels of serum GROβ in patients with ESCC and healthy controls, we measured
serum GROβ concentrations with ELISA. The level of serum GROβ was much higher in patients with ESCC than in healthy controls, which supported the hypothesis that GROβ may function as an oncogene in ESCC.

We assessed the potential correlations between serum levels of GROβ and several clinical parameters of ESCC, including tumor size, TNM stage, lymph node metastasis, histological grade and gender. Serum levels of GROβ were correlated significantly with tumor size and the extent of metastasis. Patients with larger tumors (> 5 cm in diameter) had higher levels of serum GROβ than those with smaller tumors (P < 0.05). Likewise, serum GROβ levels in patients with lymph node metastasis were significantly higher than those without lymph node metastasis (P < 0.05). The elevation of serum GROβ in ESCC patients may therefore reflect the enhanced potential of tumor cells to proliferate and metastasize, which is consistent with TNM staging[3]. Indeed, GROβ serum levels were significantly increased in stage III patients compared with stage II and stage I patients (P < 0.05). The histological grade of tumors is also an important indicator associated with the development and prognosis of cancer[36]. However, no significant differences were found in the serum levels of GROβ in ESCC patients with different histological tumor grades (P > 0.05) in our study. Because our study involved a relatively small number of ESCC patients and the sensitivity and specificity of our assay to measure serum GROβ were not sufficiently high, further confirmation in a larger sample size is warranted.

In conclusion, we found that serum GROβ levels were increased in ESCC patients and correlated positively with tumor size, TNM stage and lymph node metastasis, but not with gender or the histological grade of ESCC. GROβ may function as an oncogene product and play a role in tumor formation, progression and metastasis. Examining and monitoring serum GROβ levels may be useful in estimating the prognosis of ESCC.

ACKNOWLEDGMENTS

We thank Drs. Yu-Lin Sun, You-Sheng Mao, Fang Liu and Lan-Ping Zhou for critical reading of the manuscript and sample preparations. Additional correspondence authorship: Zhao X, Center of Basic Medical Sciences, Navy General Hospital, Beijing 100048, China.

REFERENCES

1. Yang CS. Research on esophageal cancer in China: a review. Cancer Res 1980; 40: 2633-2644
2. Hendricks D, Parker MI. Oesophageal cancer in Africa. IUBMB Life 2002; 53: 263-268
3. Lu CL, Lang HC, Luo JC, Liu CC, Lin HC, Chang FY, Lee SD. Increasing trend of the incidence of esophageal squamous cell carcinoma, but not adenocarcinoma, in Taiwan. Cancer Causes Control 2010; 21: 269-274
4. Wang GQ, Abnet CC, Shen Q, Wen J, Sun XD, Roth MJ, Qiao YL, Mark SD, Dong ZW, Taylor PR, Dawsey SM. Histological precursors of esophageal squamous cell carcinoma: results from a 13 year prospective follow up study in a high risk population. Gut 2005; 54: 187-192
5. Li L, Lu F, Zhang S. [Analyses of variation trend and short-term detection of Chinese malignant tumor mortality during twenty years]. Zhonghua Zhongliu Zazhi 1997; 19: 3-9
6. Rotondi M, Chiovato L, Romagnani S, Serio M, Romagnani P. Role of chemokines in endocrine autoimmune diseases. Endocr Rev 2007; 28: 492-520
7. Clarke CN, Kuboki S, Tevar A, Lentsch AB, Edwards M. CXC chemokines play a critical role in liver injury, recovery, and regeneration. Am J Surg 2009; 198: 415-419
8. Fang Y, Zhao L, Yan F. Chemokines as novel therapeutic targets in autoimmune thyroiditis. Recent Pat DNA Gene Seq 2010; 4: 52-57
9. Keeley EC, Mehrad B, Strieter RM. CXC chemokines in cancer angiogenesis and metastases. Adv Cancer Res 2010; 106: 91-111
10. Li Y, Flores R, Yu A, Okeu MF, Murray J, Chintagumpala M, Hicks J, Lau CC, Man TK. Elevated expression of CXC chemokines in pediatric osteosarcoma patients. Cancer 2011; 117: 207-217
11. Deng L, Chen N, Li Y, Zheng H, Lei Q. CXCR6/CXCL16 functions as a regulator in metastasis and progression of cancer. Biochim Biophys Acta 2010; 1806: 42-49
12. Modi WS, Yoshimura T. Isolation of novel GRO genes and a phylogenetic analysis of the CXC chemokine subfamily in mammals. Mol Biol Evol 1999; 16: 180-193
13. Wang D, Yang W, Du J, Devalaraja MN, Liang P, Matsumoto K, Tsubakimoto K, Endo T, Richmond A. MGSA/GRO-mediated melanocyte transformation involves induction of Ras expression. Oncogene 2000; 19: 4647-4659
14. Wang JM, Deng X, Gong W, Su S. Chemokines and their role in tumor growth and metastasis. J Immune Methods 1998; 220: 1-17

COMMENTS

Background

Esophageal squamous cell carcinoma (ESCC) is one of the most aggressive malignancies in China, South Africa and some other developing countries. In China, prognosis of the patients with ESCC is poor, with a 5-year survival rate of less than 30%. It is important to find new markers, especially serum protein markers to facilitate the early detection and diagnosis of ESCC.

Research frontiers

Growth-related gene product β (GROβ) was reported to be highly expressed in ESCC tissues and cell lines. As a chemokine, GROβ could be secreted from cells to extracellular matrix. But no report has yet investigated serum GROβ expression levels in human ESCC. In the present study, serum level of GROβ between patients with ESCC and healthy controls were firstly measured, and the association between clinical parameters of ESCC and serum GROβ levels were also analyzed.

Innovations and breakthroughs

The serum level of GROβ was increased in ESCC patients and was positively correlated with tumor size, tumor-node-metastasis staging and lymph node metastasis of the patients. GROβ might serve as an oncogene and contribute to tumorigenesis and metastasis of ESCC.

Applications

Examining and monitoring serum GROβ levels are useful in estimating the prognosis of ESCC patients.

Terminology

GROβ, also known as CXCL2 chemokine, is a member of the CXC chemokine family. It is widespread in the central nervous system and involved in many important functions, such as attracting neutrophils to inflammation sites, mobilization of stem cells and neurotransmitter release modulation.

Peer review

GRO, a member of the CXC chemokine subfamily, plays a major role in inflammation and wound healing. CXC chemokines have been found to be associated with tumorigenesis, angiogenesis and metastasis. The roles of GROβ in tumor formation and development were unclear. The papers from tumor cell lines, animal and human studies on this field showed controversial results.
June 7, 2011 | Volume 17 | Issue 21 | WJG | www.wjgnet.com

Dong QM et al. Serum GROβi expression and ESCC

15 Belperio JA, Keane MP, Arenberg DA, Addison CL, Ehler JE, Burdick MD, Strieter RM. CXC chemokines in angiogenesis. *J Leukoc Biol* 2000; 68: 1-8

16 Cyster JG. Chemokines and cell migration in secondary lymphoid organs. *Science* 1999; 286: 2098-2102

17 Wang B, Hendricks DT, Wamunyokoli F, Parker MI. A growth-related oncogene/CXC chemokine receptor 2 autocrine loop contributes to cellular proliferation in esophageal cancer. *Cancer Res* 2006; 66: 3071-3077

18 Sun Y, Mi W, Cai J, Ying W, Liu F, Lu H, Qiao Y, Jia W, Bi X, Lu N, Liu S, Qian X, Zhao X. Quantitative proteomic signature of liver cancer cells: tissue transglutaminase 2 could be a novel protein candidate of human hepatocellular carcinoma. *J Proteome Res* 2008; 7: 3847-3859

19 Kawaguchi H, Ohno S, Miyazaki M, Hashimoto K, Egashira A, Saeki H, Watanabe M, Sugimachi K. CYFRA 21-1 determination in patients with esophageal squamous cell carcinoma: clinical utility for detection of recurrences. *Cancer* 2000; 89: 1413-1417

20 Hesselgesser J, Horuk R. Chemokine and chemokine receptor expression in the central nervous system. *J Neurosci* 1999; 5: 13-26

21 Charo IF, Ransohoff RM. The many roles of chemokines and chemokine receptors in inflammation. *N Engl J Med* 2006; 354: 610-621

22 Rossi EM, Pyllkänen L, Koivisto AJ, Vippola M, Jensen KA, Miettinen M, Sirola K, Nykäsenoja H, Karisola P, Stjernwall T, Vanhala E, Kiiunen M, Pasanen P, Mäkinen M, Häméri K, Joutsensaari J, Tuomi T, Jokiniemi J, Wolff H, Savolainen K, Mattikainen S, Aelenius H. Airway exposure to silica-coated TiO2 nanoparticles induces pulmonary neutrophilia in mice. *Toxicol Sci* 2010; 113: 422-433

23 Pelus LM. Peripheral blood stem cell mobilization: new regimens, new cells, where do we stand. *Curr Opin Hematol* 2008; 15: 285-292

24 Thorburn E, Kolesar L, Brabcova E, Petrickova K, Petrickev M, Jaresova M, Slavcev A, Striz I. CXC and CC chemokines induced in human renal epithelial cells by inflammatory cytokines. *APMIS* 2009; 117: 477-487

25 Ragozzino D, Giovannelli A, Mileo AM, Limatola C, Santoni A, Eusebi F. Modulation of the neurotransmitter release in rat cerebellar neurons by GRO beta. *Neuroreport* 1998; 9: 3601-3606

26 Limatola C, Mileo AM, Giovannelli A, Vacca F, Ciotti MT, Mercanti D, Santoni A, Eusebi F. The growth-related gene product beta induces sphingomyelin hydrolysis and activation of c-Jun N-terminal kinase in rat cerebellar granule neurons. *J Biol Chem* 1999; 274: 36537-36543

27 Daller B, Müssch W, Röhr J, Tumanov AV, Nedospasov SA, Männel DN, Schneider-Bracht W, Hohlgas T. Lymphotixin-β receptor activation by lymphotixin-α(1)(β(2)) and LIGHT promotes tumor growth in an NFκB-dependent manner. *Int J Cancer* 2011; 128: 1363-1370

28 Doll D, Keller L, Maak M, Boulesteix AL, Siewert JR, Holzmann B, Janssen KP. Differential expression of the chemokines GRO-2, GRO-3, and interleukin-8 in colon cancer and their impact on metastatic disease and survival. *Int J Colorectal Dis* 2010; 25: 573-581

29 Owen JD, Strieter R, Burdick M, Haghnegahdar H, Nanney L, Shattuck-Brandt R, Richmond A. Enhanced tumor-forming capacity for immortalized melanocytes expressing melanoma growth stimulatory activity/growth-regulated cytokine beta and gamma proteins. *Int J Cancer* 1997; 73: 94-103

30 Bruyère C, Lonez C, Duray A, Cludts S, Ruyschaert JM, Saussez S, Yeaton P, Kiss R, Mijatovic T. Considering temozolomide as a novel potential treatment for esophageal cancer. *Cancer* 2010; Epub ahead of print

31 Mukhopadhyay P, Rajesh M, Pan H, Patel V, Mukhopadhyay B, Bátikai S, Gao B, Haskó G, Pacher P. Cannabinoid-2 receptor limits inflammation, oxidative/nitrosative stress, and cell death in nephropathy. *Free Radic Biol Med* 2010; 48: 457-467

32 Wang B, Khachigian LM, Esau L, Birrer MJ, Zhao X, Parker MI, Hendricks DT. A key role for early growth response-1 and nuclear factor-kappaB in mediating and maintaining GRO/CXCR2 proliferative signaling in esophageal cancer. *Mol Cancer Res* 2009; 7: 755-764

33 Redmond KL, Crawford NT, Farmer H, D’Costa ZC, O’Brien GJ, Buckley NE, Kennedy RD, Johnston PG, Harkin DP, Mullan PB. T-box 2 represses NDRG1 through an EGR1-dependent mechanism to drive the proliferation of breast cancer cells. *Oncogene* 2010; 29: 3252-3262

34 Dong Q, Zhang J, Hendricks DT, Zhao X. GROβ and its downstream effector EGR1 regulate cisplatin-induced apoptosis in WHCO1 cells. *Oncol Rep* 2011; 25: 1031-1037

35 Ito Y, Ichihara K, Matsuoka H, Fukushima M, Inoue H, Kihara M, Tomoda C, Higashiyama T, Takamura Y, Kobayashi K, Miya A, Miyauchi A. Establishment of an intraoperative decision system for papillary thyroid carcinoma. *Surgery* 2010; 148: 103-110

36 Derwinger K, Kodeda K, Bexe-Lindskog E, Taflin H. Tumour differentiation grade is associated with TNM staging and the risk of node metastasis in colorectal cancer. *Acta Oncol* 2010; 49: 57-62