Clinical significance of serum growth-regulated oncogene α (GROα) in patients with gynecological cancer

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Summary

Purpose of investigation: To assess the clinical relevance of serum growth-regulated oncogene α (GROα) levels in gynecological cancer, we investigated its concentration in distinguishing patients with cervical cancer, endometrial cancer, ovarian cancer, benign ovarian tumor and control. *Methods:* Preoperative serum GROα levels were measured in women with cervical cancer (n = 46), endometrial cancer (n = 39), ovarian cancer (n = 124), benign ovarian tumors (n = 52), and normal controls (n = 38) using an enzyme-linked immunosorbent assay. *Results:* Statistical analyses showed that the serum GROα concentration was significantly elevated in the cervical cancer, endometrial cancer and ovarian cancer patients compared with controls. Using GROα levels, the receiver operating characteristic (ROC) of cervical cancer (AUC ≈ 0.775), endometrial cancer (AUC ≈ 0.799), ovarian cancer (AUC ≈ 0.749) and benign ovarian tumors (AUC ≈ 0.568) vs controls were identified. *Conclusion:* Our findings suggest that serum GROα measurement as a molecular marker might contribute to detection and diagnosis of gynecological cancer.

Key words: Cancer; GRO; ELISA; Ovarian tumor; Serum; ROC.

Introduction

Growth-regulated oncogene α(GROα) is a neutrophil-activating chemokine and serves as a potent angiogenic factor [1, 2]. GROα was originally identified by its constitutive overexpression in transformed Chinese hamster fibroblasts [3]. Exogenously applied GROα exhibits growth-promoting activity toward melanoma cells [4]. The chemokine is also called a melanoma growth-stimulator, and increased GROα expression is frequently detected in melanoma [4, 5], squamous cell carcinoma [6, 7], colon cancer [8], gastric carcinoma [9], oral cancer [10], and ovarian cancer [11].

Serum chemokine levels have been investigated as diagnostic and prognostic markers in gynecological cancer [12]. Chemokine α or "CXC" family members are IL-8, GRO, platelet factor 4, and IP-10 [13]. Examples of chemokine family members include upregulated on activation, normal T-cell expression and secretion (RANTES), macrophage inflammatory protein-1 (MIP-1) and monocyte chemoattractant protein-1 (MCP-1), and they are the major determinants of macrophage and lymphocyte infiltration in carcinomas of the breast, ovary, and cervix [14, 15].

Yang *et al*. reported that GRO-1 was expressed at significantly higher amounts in ovarian cancer than in normal tissues and was higher in serum samples from women with ovarian cancer than in serum from women without ovarian cancer [16]. Also, a recent report suggests that increased GRO levels are detected in the plasma and ascites of ovarian cancer patients [11]. To our knowledge, there are no precise clinical reports of serum GROα levels in ovarian cancer and other gynecological cancer patients.

Here we measured serum concentrations of GROα in gynecological cancer patients and evaluated the utility of preoperative GROα levels in distinguishing malignant from benign or controls.

Materials and Methods

Subjects. Women with cervical cancer (n = 46), endometrial cancer (n = 39), ovarian cancer (n = 124) or benign ovarian tumors (n = 52), and healthy women as controls (n = 38) were enrolled between 1998 and 2010. The preoperative serum samples were reviewed in this study. The study protocol was approved by the Institutional Review Board of Nagoya City University and informed consent was obtained from all of the study subjects. Patients with inflammatory states, e.g., allergy and infections, were excluded from this study, as well as those who had bronchial asthma, rhinitis, and atrophic dermatitis [17, 18]. Also excluded were endometriosis patients and patients with positive blood cultures, and those who showed both CRP above 5 mg/dl and peripheral leukocyte counts above 10^4/mm^3 were also excluded because of the possibility of bacterial infection. The benign ovarian tumor group consisted of 52 women with ovarian cysts, with no evidence of malignancies, endometriosis or pelvic adhesions. In all of the groups, the diagnosis of benign ovarian tumors was confirmed histologically. The control group consisted of generally healthy hospital personnel.

Samples. Patient charts were reviewed to obtain data regarding age, diagnosis, histology, grade, International Federation of Gynecologists and Obstetricians (FIGO) stage, presence or absence of ascites, and operative findings. All of the patients were surgically staged according to the FIGO staging system. The pathology for all patients with cancer was reviewed by a gynecological pathologist. After clarification of the samples by centrifugation at 2000 g for 10 min, the supernatants were stored at –40°C until assayed.

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GROα-Immunoassay. Amounts of GROα in the serum were determined with a GROα-Immunoassay kit (R&D Systems, Minneapolis, MN, USA) following the manufacturer's protocol. Samples from all patients were measured in parallel and in duplicate to control for interassay variance. The optical density of each well was measured at dual wavelengths of 450/570 nm. Concentrations of GROα were calculated by interpolation from a standard curve. The sensitivity of the GROα ELISA was 16 pg/ml.

Statistical Analyses. Calculated values were expressed as medians and interquartile ranges. All analyses were performed in the R statistical computing environment for Windows (version 2.6) [19]. All data were analyzed with the Mann-Whitney and Kruskal-Wallis non-parametric tests, a p value < 0.05 being regarded as statistically significant. Logistic regression models were used to analyze the influence of serum GROα levels on the probability of malignancy. Using the logistic regression models, sensitivity and specificity were calculated for each possible threshold value of the estimated probability of malignancy. Based on the values, receiver operator characteristic (ROC) curves were constructed to visualize the relationship among gynecological cancers [20].

Results

The cervical cancer group (n = 46, 51 ± 2.0: mean age ± SEM), endometrial cancer group (n = 39, 60 ± 2.4), ovarian cancer group (n = 124, 56 ± 1.3), benign ovarian tumor group (n = 52, 51 ± 2.5) and normal control group (n = 38, 45 ± 1.5) were matched for age. Serum samples from the women with ovarian cancers (n = 124) contained significantly higher concentrations of GRO (median 142 pg/ml, interquartile range 43 - 756 pg/ml) than those of the group with benign ovarian tumors (99 pg/ml, 44 - 345 pg/ml) by the Mann-Whitney U-test (p = 0.0004, Table 1 and Figure 1).

Table 1 shows descriptive statistics for preoperative GROα values by patient characteristics among those with gynecological cancers. The GROα levels were significantly higher in all gynecological cancer patients as compared to the controls. The ovarian cancer patients were staged from I to IV depending on the severity of disease based on the FIGO classification. The stage distribution at surgery was as follows: Stage I, n = 51; Stage II, n = 16; Stage III, n = 48; Stage IV, n = 9 (Table 2). There was no significant difference (p = 0.36) in GROα levels among the benign ovarian tumor group, the ovarian cancer Stage I, stage II, Stage III, and Stage IV groups by the Kruskal-Wallis test.

To determine if preoperative GROα levels can be useful for differentiating benign adnexal masses from Stage I ovarian cancer, the distribution of GROα levels among patients with Stage I ovarian cancer was compared to with those with benign tumors. There were 51 patients with Stage I ovarian cancer with a mean GROα level of 114 pg/ml (median = 118 pg/ml, range 52-756 pg/ml) and the 52 patients with benign tumors had a mean GROα level of 119 pg/ml. A significant difference between these GROα distributions was found based on the Mann-Whitney U test (p = 0.03).

Table 1. — *Characteristics of gynecological cancer patients and associated serum GROα levels (pg/ml).*

	n	Median	Range	p value
Controls	38	90	45-237	–
Cervical cancer	46	142	76-851	0.00006
Endometrial cancer	39	145	70-279	0.00002
Ovarian cancer	124	142	43-756	0.000007
Benign ovarian tumors	52	99	44-345	0.27

Table 2. — *Characteristics of ovarian cancer patients and associated serum GROα levels (pg/ml).*

	n	Median	Range
Patient age (yrs)	124	142	43-756
< 50	44	118	52-756
≧ 50	80	147	43-687
FIGO stage			
I	51	118	52-756
II	16	149	43-429
III	48	147	52-687
IV	9	147	68-260
Histology			
Serous	62	144	52-687
Non-serous			
Mucinous	22	115	65-756
Endometrioid	7	200	132-347
Clear cell	17	118	43-303
Others	16	186	59-571

In endometrial cancer, the serum levels of GROα were not associated with age, histological types and stage. Also there were no significant differences associated with age, histological types and stage in serum GROα levels of cervical cancer patients.

In ovarian cancer, studying the differences of GROα levels based on histology (serous n = 62; non-serous n = 62: mucinous n = 22, endometrioid n = 7, clear cell n = 17), higher concentrations of GROα were observed in the patients with endometrioid carcinoma (n = 7) than the other non-serous carcinoma (n = 55, p = 0.048) or the others (n = 117, p = 0.049). At a cutoff GROα level of 170 pg/ml, the GROα levels in endometrioid carcinoma patients were detected with a sensitivity of 86% as compared to the other carcinoma and benign ovarian tumor patients.

To evaluate the utility of preoperative GROα levels in predicting malignancy, sensitivity and specificity calculations were performed for various cutoff values of GROα in predicting malignancy using all of the patients. In the ROC curves of cervical cancer, endometrial cancer, ovarian cancer, or benign ovarian tumor versus controls, the area under the curve (AUC) was examined [20]. Using GROα levels, the ROC curves of ovarian cancer (AUC ≈ 0.749), cervical cancer (AUC ≈ 0.775), endometrial cancer (AUC ≈ 0.799), and benign ovarian tumors (AUC ≈ 0.568) are shown in Figure 2. In ROC curves of ovarian cancer of endometrioid type versus non-endometrioid ovarian cancer and benign ovarian tumor, the AUC showed high values using GROα levels (AUC ≈ 0.833). In ovarian tumors, at a cutoff GROα level of 110

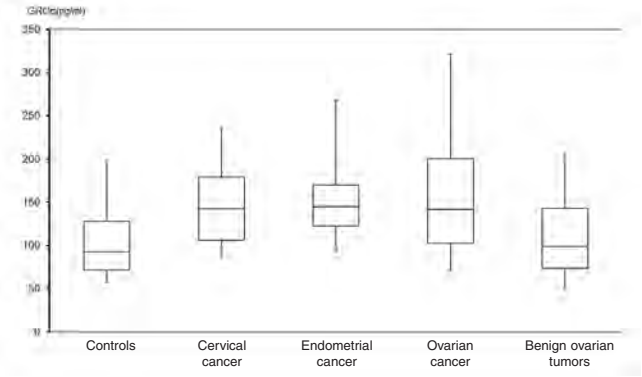

Figure 1. — Concentrations of serum GRO determined by ELISA in gynecological cancer patients. The boxes delimit values falling between the 25th and 75th percentiles and the horizontal lines refer to the median scores.

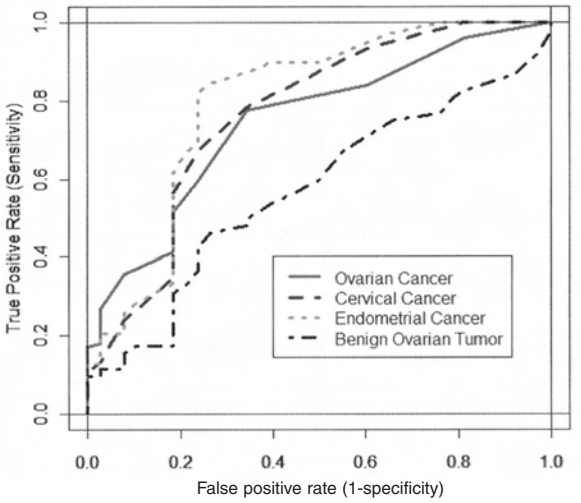

Figure 2. — Receiver operator characteristics (ROC) curves of gynecological cancers or benign ovarian tumors vs controls are shown.

pg/ml, the sensitivity was 67%, the specificity was 73%, the positive predictive value was 81%, and the negative predictive value was 58% for predictive malignancy.

Discussion

We found concentrations of GRO in serum to be significantly elevated in women with gynecological cancer as compared to controls. Higher concentrations of GRO were also observed in patients with ovarian cancer, especially in endometrioid type rather than benign ovarian tumors.

Lee *et al*. suggest that GROα is generated in ascites, likely by ovarian cancer cells, and migrates to the peripheral circulation [11]. Although ovarian cancer cells in culture elaborate GROα, under serum-free conditions, these cells stopped producing GROα, suggesting that the chemokine is not constitutively expressed by ovarian cancer cells but is rather responsive to growth factors in serum. Our findings suggest that in patients with ovarian cancer, especially with endometrioid type, higher GROα may be produced than in benign ovarian tumors.

A new technique, the cytokine bead array has been developed for simultaneous analysis of multiple cytokines in serum samples. Serum levels of epidermal growth factor (EGF), MCP-1, CA125, vascular endothelial growth factor (VEGF), IL-6 and IL-8 showed significant differences between early-stage ovarian cancer and control groups based on the cytokine bead array [21]. A panel of these cytokines resulted in a higher sensitivity and specificity than CA125 alone in distinguishing early-stage ovarian cancer from control groups [21]. Using the cytokine bead array, Lambeck *et al*. reported that IL-7 levels were found to be strongly associated with ovarian cancer and could be used in combination with CA125 to distinguish between malignant and benign ovarian tumors [12]. Our data suggest that preoperative GROα levels without a combination of CA125 could also be useful in distinguishing ovarian tumor cases from malignancy.

CA125 has been shown to contribute to the early diagnosis of epithelial cancer [18, 19, 22]. However, only about 50% of early-stage ovarian cancers will be associated with elevated serum CA125 [23]. Elevation in serum GROα is likely to be an early event during the development of ovarian cancer of endometrioid type, although the number of serum from the patients was too small. The findings indicate that GROα might be a potentially useful biomarker for endometrioid type in distinguishing women with ovarian cancer from benign ovarian tumor patients.

Recent findings suggest that a panel of four serum biomarkers (apolipoprotein A-1, transthyretin, transferring and CA125) effectively detected early-stage ovarian cancers with the highest reported overall sensitivity of 96% [24]. They also reported that endometrioid tumors were detected at early-stages with a sensitivity of 98%. Moore *et al*. reported utility of a serum tumor biomarker HE4 in patients with endometrioid adenocarcinoma of the uterus [25]. Our present data suggest that preoperative serum GRO levels might have a role in the diagnostic clinical setting for discerning benign from gynecological cancers, especially ovarian cancer of endometrioid type.

GROα promotes chemoattraction, wound healing and angiogenesis through the seven-transmembrane G-protein-coupled receptor CXCR2 [26]. Recent data suggest that CXCR2 regulates the cell cycle, apoptosis and angiogenesis through multiple signaling pathways, including mitogen-activated protein kinase and NF- B, in ovarian cancer [27]. CXCR2 thus has potential as a therapeutic target and for use in ovarian cancer.

In conclusion, GROα might play a role in oncogenesis development and metastasis of gynecological cancer, and

can be readily detected in the serum of these patients. This study has shown that GROα detection by ELISA as a molecular marker can contribute to detection and diagnosis of gynecological cancer, especially for ovarian cancer of endometrioid type. Further studies will be performed to assess how GROα production is basically regulated and to evaluate GROα and its receptor CXCR2 as a diagnostic and prognostic marker for gynecological cancer. Proof of the effectiveness will require a large prospective study in the future.

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