Circulating galectin-3 correlates with angiogenetic factors, indicators of nutritional condition and systemic inflammation in patients with thyroid cancer

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Abstract

Background and Aims: Several investigators have reported the significance of circulating galectin-3 in thyroid cancer patients. However, the precise meaning of circulating galectin-3 remains unclear. The aim of this study was to investigate the relationships between serum galectin-3 levels and angiogenetic factors, and nutritional and inflammatory indicators in patients with thyroid cancer.

Materials and Methods: Sixty-one patients with thyroid tumors were enrolled, comprising 47 pre-treatment thyroid cancer patients and 14 patients with benign thyroid diseases. Galectin-3, interleukin (IL)-6, vascular endothelial growth factor, granulocyte colony-stimulating factor (G-CSF), soluble form of intercellular adhesion molecule-1 (sICAM-1), retinol binding protein, prealbumin, albumin, and transferrin were measured. C-reactive protein (CRP), neutrophil count, lymphocyte count, and neutrophil/lymphocyte ratio (NLR) were also investigated.

Results: The amounts of circulating galectin-3 in benign disease and thyroid cancer were significantly higher than those of healthy volunteers (P < 0.001). Analysis of galectin-3 performance in distinguishing malignant disease from benign disease using a receiver operating characteristic curve revealed that the area under the curve was 0.555. There were statistically significant correlations between the circulating amount of galectin-3 and IL6, G-CSF, and sICAM-1. Serum galectin-3 showed statistically significant correlations with albumin, prealbumin, and transferrin. Circulating galectin-3 exhibited strong correlations with CRP, neutrophil count, lymphocyte count, and NLR.

Conclusions: Galectin-3 may be one of the key factors in the regulation of angiogenesis, inflammation, and nutrition.

Key Words: galectin-3, thyroid cancer, interleukin (IL)-6, soluble intercellular adhesion molecule-1 (ICAM-1), granulocyte colony-stimulating factor (G-CSF)

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Introduction

Thyroid cancer is the most common endocrine malignancy. Thyroid tumors represent 1% of all neoplasms and differentiated thyroid tumors (papillary and follicular type) account for 80–85% of all thyroid tumors.¹⁴ The main goal in the assessment of thyroid cancer is distinguishing thyroid cancer from benign nodules.⁵ Advances in defining diagnostic molecular markers, such as RET, RAS, BRAFV600E mutations, and galectin-3, have improved thyroid nodule diagnosis.⁶⁻¹⁰

Galectin-3, a beta-galactoside binding lectin, is a moonlighting protein, implicated in cell growth, differentiation, apoptosis, adhesion, malignant transformation, RNA processing, and angiogenesis.¹²⁻¹⁵. Accumulating evidences have revealed an elevated concentration of serum galectin-3 in various cancers including breast, colorectal,¹⁷,¹⁸ stomach,¹⁹ lung,⁶ bladder,²⁰ head and neck,²¹ liver,₂² melanoma,²³ pancreatic,²⁴ and thyroid.²⁵⁻²⁹ As for thyroid cancer, Yilmaz et al. reported that serum galectin-3 level was a significant biomarker to distinguish thyroid cancer from benign nodules.²⁵ However, the significance of circulating galectin-3 as a biomarker is still controversial.²⁶⁻²⁹

Recently, Chen et al. reported that galectin-3 induces secretion of metastasis-promoting cytokines, such as interleukin (IL)-6, granulocyte colony-stimulating factor
(G-CSF), granulocyte macrophage colony-stimulating factor and soluble intercellular adhesion molecule-1 (sICAM-1). Therefore, we sought to investigate the relationships between serum galectin-3 levels and angiogenic factors, and nutritional and inflammatory indicators in patients with thyroid cancer. Another aim of this study was to determine the usefulness of serum galectin-3 as a biomarker in distinguishing malignant tumors from benign nodules.

**Materials and Methods**

**Patients.** Blood samples were collected from 61 patients with thyroid tumors, comprising 47 patients with thyroid cancer before starting any treatment and 14 patients with benign thyroid diseases (nine patients with follicular adenoma, three patients with adenomatous goiter, and two patients with Hashimoto’s disease), between March 2011 and March 2013. Sera from the patients were stored at −80°C until use. Each sample was used only once after thawing. The patients’ demographics are summarized in Table 1. The TNM stage of thyroid cancer was defined according to the General Rules for the Description of Thyroid Cancer (6th edition) by the Japanese Society of Thyroid Surgery. There were ten patients with stage I, two patients with stage II, 5 patients with stage III, 16 patients with stage IVA, seven patients with stage IVB, and seven patients with stage IVC. All patients underwent surgery or chemotherapy for the treatment of histologically confirmed cancer at the Department of Thyroid and Endocrinology in Fukushima Medical University Hospital. In addition, samples from 20 healthy volunteers of similar age and gender distributions to those of the patients were used as controls. The study protocol was approved by the ethics committee of Fukushima Medical University and written informed consent was obtained from the enrolled patients and healthy volunteers.

**Measurement of Galectin-3, IL-6, vascular endothelial growth factor (VEGF), G-CSF, and sICAM-1.** The serum concentrations of galectin-3, IL-6, VEGF, G-CSF, and sICAM-1 were measured using an enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN, USA) according to the manufacturer’s instructions.

**Parameters for nutritional status and inflammation.** The patients’ nutritional status was determined by measuring the serum concentrations of albumin (by nephelometry), RBP (by latex agglutination immunoassay), prealbumin (by turbidimetric immunoassay) and transferrin (TF) (by turbidimetric immunoassay). Neutrophil and lymphocyte counts, as well as NLR, were used as indicators of inflammation.

**Statistical analysis.** Differences between the groups were analyzed using the Student’s t-test. Associations between two variables were quantified using the Spearman’s rank correlation coefficient. To assess the performance characteristics of the serum galectin-3 concentrations, we generated receiver operating characteristic curves and calculated the area under the curve. A *P* value of < 0.05 was considered statistically significant. All statistical calculations were performed using SPSS® version 22 (IBM Japan, Tokyo, Japan). Not all blood samples were of suf-

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<th>Table 1 Demographics of the patients</th>
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*NA: not applicable*
Results

Circulating galectin-3 in patients with thyroid cancer and benign thyroid disease

The concentrations of galectin-3 in the 20 healthy volunteers (3.1 ± 1.4 ng/ml), benign thyroid disease patients (7.4 ± 2.6 ng/ml), and thyroid cancer patients (9.2 ± 5.5 ng/ml) are shown in Fig. 1A. The amounts of circulating galectin-3 in the benign disease and thyroid cancer patients were significantly higher than those of the healthy volunteers \((P < 0.001)\). However, no statistically significant differences were observed between those of the benign disease patients and the thyroid cancer patients \((P = 0.097)\). The performance analysis of galectin-3 in distinguishing malignant disease from benign disease using a receiver operating characteristic curve revealed that the area under the curve was 0.555.

Fig. 1B shows the galectin-3 concentrations according to the stage of thyroid cancer. The amounts of circulating galectin-3 in stage I \((n = 10)\), II \((n = 2)\), III \((n = 5)\), IVA \((n = 16)\), IVB \((n = 7)\), and IVC \((n = 7)\) were 8.2 ± 3.9, 4.3 ± 0.6, 5.5 ± 2.2, 7.8 ± 4.3, 13.6 ± 7.6, 13.6 ± 5.6 ng/ml, respectively (Fig. 1B). There were statistically significant differences in the amount of galectin-3 between those in stage I vs. II \((P = 0.015)\), stage II vs. IVC \((P = 0.004)\), stage III vs. IVB \((P = 0.032)\), and stage III vs. IVC \((P = 0.008)\).

The amount of circulating galectin-3 in patients with papillary carcinoma \((n = 33)\), follicular carcinoma \((n = 3)\), medullary carcinoma \((n = 3)\), and anaplastic carcinoma \((n = 7)\) were 8.6 ± 5.3, 5.0 ± 1.3, 7.4 ± 2.4, and 14.5 ± 5.8 ng/ml, respectively (Fig. 1C). There were statistically significant differences in the amount of serum galectin-3 between those with papillary vs. follicular \((P = 0.012)\), papillary vs. anaplastic \((P = 0.036)\), follicular vs. anaplastic \((P = 0.004)\), and medullary and anaplastic carcinoma \((P = 0.026)\).

Relationship between circulating galectin-3 and angiogenic factors

Fig. 2 shows the relationships between galectin-3 serum level and angiogenic factors. There were statistically significant correlations between the circulating amount of galectin-3 and IL6 \((r = 0.389, P = 0.008)\), G-CSF \((r = 0.405, P = 0.006)\), and sICAM-1 \((r = 0.469, P = 0.001)\). However, the concentration of serum galectin-3 exhibited no significant correlation with VEGF \((r = 0.185, P = 0.223)\). The same analysis was performed on the benign thyroid disease patients; however, no statistically significant differences were observed.

Relationship between circulating galectin-3 and parameters of nutritional condition

Fig. 3 shows the relationships between galectin-3 serum level and nutritional parameters. The serum concentration of galectin-3 showed statistically significant correla-
tions with albumin \(r = -0.348, P = 0.020\), prealbumin \(r = -0.300, P = 0.040\), and TF \(r = -0.338, P = 0.033\). However, no statistically significant correlation was observed between serum galectin-3 and RBP \(r = 0.084, P = 0.575\). The same analysis was performed on the benign thyroid disease patients; however, no statistically significant differences were observed.

**Relationship between circulating galectin-3 and parameters of systemic inflammation**

Fig. 4 shows the relationships between the galectin-3 concentration and inflammation indicators. The amount of circulating galectin-3 exhibited strong correlations with CRP \(r = 0.452, P = 0.002\), neutrophil count \(r = 0.485, P = 0.001\), lymphocyte count \(r = -0.419, P = 0.004\), and NLR \(r = 0.444, P = 0.002\). The same analysis was performed on the benign thyroid disease patients; however, no statistically significant differences were observed.

**Discussion**

Angiogenesis is essential for tumor cells to survive and proliferate in primary foci as well as in forming metastatic foci. Progression into invasive phenotypes requires degradation of the matrix surrounding tumor cells and cellular motility. Tumor cell adhesion to the blood vascular endothelium is also critical for dissemination to distant organs. After adhesion to the endothelium, fenestration of endothelial lining, penetration of basement
membranes of capillary vessels, and re-proliferation are prerequisite to survive in newly formed foci in the distant organ. Thus, forming metastatic foci involves complicated phenomenon, regulated by multiple interactions between tumor cells and host cells\(^{31}\). Galectin-3 has been reported to play a role in each of the following steps: anti-apoptosis, tumor proliferation, tumor progression, cellular adhesion, and angiogenesis\(^{18-25}\). Consistent with Chen et al.’s findings in an in vitro examination\(^{30}\), we observed correlations in this study between galectin-3 and angiogenetic factors, such as IL-6, G-CSF, and sICAM-1.

IL-6 is a pleiotropic cytokine that plays diverse roles as a regulator of immunological responses. Galectin-3 plays a role in regulating the production of IL-6\(^{18, 32}\). Circulating sICAM-1 inhibits T cell interaction with tumor cells\(^{30}\), and also inhibits natural killer (NK) cell-mediated toxicity\(^{30}\). Galectin-3 also inhibits NK cell-mediated tumor immunity by binding the natural cytotoxicity receptor, NKp30, or NKG2D binding site of major histocompatibility complex I-related chain A\(^{35, 36}\). G-CSF stimulates the bone marrow to produce granulocytes. Administration of G-CSF via the tail vein of nude mice facilitates lung metastasis of human breast cancer cells\(^{37}\). Taken together, galectin-3 cooperates with these angiogenetic factors in forming metastatic foci.

Circulating galectin-3 has been reported to be a biomarker to distinguish malignant tumors from benign nodules\(^{25}\). In the present study, however, analysis using a receiver operating characteristic curve revealed that serum galectin-3 was not a useful biomarker. The amount of serum galectin-3 in the thyroid cancer patients showed no statistically significant differences from that in the benign thyroid disease patients. The number of enrolled patients with benign diseases in the present study was small, and said benign diseases contained non-tumor diseases; adenomatous goiter and Hashimoto’s disease. So, further investigation is required.

Assessment of nutritional status is essential for a diagnosis of nutritional compromise, and measurements of serum concentrations of rapid turnover proteins, such as RBP, prealbumin, and TF, have been reported to provide a more accurate assessment than the measurement of albumin\(^{38-40}\). In the current study, galectin-3 exhibited correlations with indicators of systemic inflammation and an inverse correlation with nutritional condition. Persistent inflammation can lead to malnutrition as well. The key mechanisms leading to cancer cachexia in which nutritional impairment is a major clinical issue, are mostly immune reactions caused by chronic inflammation. Galectin-3 may be one of the key factors in the regulation of angiogenetic, inflammation, and nutrition.

**References**