Elevated serum galectin-3 is associated with poor prognosis in patients with colorectal carcinoma

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Abstract

Background: Recently, galectin-3 was reported to induce in vitro 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).

Materials and Methods: We investigated galectin-3 serum levels in 48 patients with colorectal carcinoma before treatment and 20 healthy volunteers in relation to angiogenetic factors, such as vascular endothelial growth factor (VEGF), IL-6, G-CSF and sICAM-1.

Results: The serum levels of galectin-3 (3.1 ± 1.4 [healthy volunteers] vs. 9.5 ± 4.5 ng/ml [patients]), IL-6 (5.3 ± 4.2 vs. 12.9 ± 12.0 pg/ml), VEGF (270.4 ± 108.7 vs. 429.2 ± 410.5 pg/ml), G-CSF (93.6 ± 40.4 vs. 140.6 ± 83.0 pg/ml), and sICAM-1 (189.7 ± 61.0 vs. 297.5 ± 145.6 ng/ml) were significantly higher in the patients with colorectal carcinoma than in the healthy volunteers (P < 0.01, P < 0.01, P = 0.02, P = 0.01, and P < 0.01, respectively). Serum galectin-3 level correlated with the level of IL-6 (γ = 0.360, P < 0.01), VEGF (γ = 0.408, P < 0.01), and sICAM-1 (γ = 0.474, P < 0.01). The patients with high serum galectin-3 (≥10 ng/ml) was associated with poorer prognosis than those with low galectin-3 (<10 ng/ml) in all the patients (P = 0.047), and in the patients with stage III disease (P = 0.04).

Conclusions: The concentration of serum galectin-3 correlated with IL-6, VEGF, and sICAM-1, and was associated with poorer prognosis in patients with colorectal carcinoma.

Key Words: galectin-3, interleukin (IL)-6, vascular endothelial growth factor (VEGF), soluble intercellular adhesion molecule-1 (sICAM-1), colorectal cancer

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The aim of the current study was to identify the relationship between galectin-3 and angiogenetic factors and investigate the relationship between circulating galectin-3 levels and prognosis in patients with colorectal carcinoma.

**Materials and Methods**

**Patients.** Blood samples were collected from 48 patients with colorectal cancer before starting treatment between February 2011 and August 2013. Sera were stored at −80°C until use. Each sample was used only once after thawing. The patient group included eight patients with stage I disease, 12 patients with stage II disease, 18 patients with stage III disease, and 10 patients with stage IV disease. The enrolled patients underwent surgery or chemotherapy for the treatment of histologically confirmed cancer at our department. In the prognosis analysis, 34 patients who underwent surgery at our department were enrolled including seven patients with stage I, 11 patients with stage II, 14 patients with stage III and two patients with stage IV disease. Simultaneous partial hepatectomy along with low anterior resection was performed on one of the stage IV patients who had a metastatic lesion. When patients had lymph-node involvement and/or had stage III disease, adjuvant chemotherapy was performed. In addition, samples from 20 healthy volunteers of similar age and gender distributions were used as controls. The study protocol was approved by the Ethics Committee of Fukushima Medical University and written informed consent was obtained from both the enrolled patients and healthy volunteers.

**Measurement of galectin-3, and angiogenetic factors.** Serum concentrations of galectin-3, IL-6, VEGF, G-CSF and sICAM-1 were measured using enzyme-linked immunosorbent assay (ELISA; R&D Systems, Minneapolis, MN, USA) according to the manufacturer’s instructions.

**Statistical analysis.** Categorical variables were evaluated by χ² test or Fisher’s exact test. Differences between groups were analyzed using the Student’s t-test. Associations between two variables were quantified using the Spearman’s rank correlation coefficient. The mean observation period was 45.0 months (range: 28.8–59.2). The final assessment of disease status was made on January 20, 2016. Overall survival was calculated using the Kaplan-Meier method and differences between the groups were assessed by the log-rank test. A P value of < 0.05 was considered to indicate statistically significant differences. All statistical calculations were performed using SPSS® version 22 (IBM Japan, Tokyo, Japan). Not all blood samples were of sufficient volume for all measurements.

**Results**

**Concentrations of galectin-3 and angiogenetic factors in patients with colorectal carcinoma**

Fig. 1 shows the results of serum levels of galectin-3 and angiogenetic factors in the healthy volunteers vs. those in the patients. Galectin-3 (3.1 ± 1.4 vs. 9.5 ± 4.5 ng/ml), IL-6 (5.3 ± 4.2 vs. 12.9 ± 12.0 pg/ml), VEGF (270.4 ± 108.7 vs. 429.2 ± 410.5 pg/ml), G-CSF (93.6 ± 40.4 vs. 140.6 ± 83.0 pg/ml), and sICAM-1 (189.7 ± 61.0 vs. 297.5 ± 145.6 ng/ml) were significantly higher in the patients with colorectal carcinoma than in the healthy volunteers (P < 0.01, P < 0.01, P = 0.02, P < 0.01, and P < 0.01, respectively).

![Fig.1. Serum levels of galectin-3 and angiogenetic factors in healthy volunteers and patients with colorectal cancer. Galectin-3 (3.1 ± 1.4 vs. 9.5 ± 4.5 ng/ml), IL-6 (5.3 ± 4.2 vs. 12.9 ± 12.0 pg/ml), VEGF (270.4 ± 108.7 vs. 429.2 ± 410.5 pg/ml), G-CSF (93.6 ± 40.4 vs. 140.6 ± 83.0 pg/ml), and sICAM-1 (189.7 ± 61.0 vs. 297.5 ± 145.6 ng/ml) were significantly higher in the patients with colorectal carcinoma than in the healthy volunteers (P < 0.01, P < 0.01, P = 0.02, P < 0.01, and P < 0.01, respectively).](image-url)
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Relationship between galectin-3 and angiogenetic factors

As shown in Fig. 2, serum galectin-3 level correlated with the level of IL-6 (r = 0.360, P = 0.03), VEGF (r = 0.408, P < 0.01), and sICAM-1 (r = 0.474, P < 0.01). However, the level of serum galectin-3 had no correlation with the level of G-CSF (r = 0.127, P = 0.45).

Prognosis of the patients according to the level of serum galectin-3

The mean level of galectin-3 in 34 patients who underwent surgery was 9.5 ± 4.9 ng/ml. We decided to classify the patients into a low serum galectin-3 group (< 10.0 ng/ml) and a high serum galectin-3 group (≥ 10.0 ng/ml) in order to analyze prognosis. The patients’ backgrounds are shown in Table 1. There were no statistically significant differences between the two groups. Fig. 3 shows the overall survival of the patients. High serum galectin-3 was associated with poorer prognosis than low galectin-3 (P = 0.047). In the analysis involving patients with stage III disease, those with high serum galectin-3 also showed poorer prognosis than those with low serum galectin-3 (P = 0.04), although there were no significant differences in the analysis involving patients with other stages.

Discussion

A previous study reported that galectin-3 has a correlation with angiogenetic factors, such as IL-6, G-CSF and sICAM-1 only in patients with metastasis\(^9\). However, our results showed a correlation in all enrolled patients.

Table 1. Demographics of the patients

| Level of serum galectin-3 | Low (n=19) | High (n=15) | P       |
|--------------------------|-----------|------------|---------|
| Age                      | 62.7 ± 12.8 | 67.5 ± 11.9 | 0.623   |
| Gender                   |            |            | 0.201   |
| Male                     | 13         | 7          |         |
| Female                   | 6          | 8          |         |
| T category               |            |            | 0.718   |
| T1 or T2                 | 5          | 5          |         |
| T3 or T4                 | 14         | 10         |         |
| N category               |            |            | 0.300   |
| 0                        | 8          | 9          |         |
| ≥ 1                      | 11         | 6          |         |
| M category               |            |            | 1.000   |
| 0                        | 18         | 14         |         |
| 1                        | 1          | 1          |         |
| Stage                    |            |            | 0.464   |
| I or II                  | 9          | 9          |         |
| III or IV                | 10         | 6          |         |

Fig. 2. Correlation of serum galectin-3 level with IL-6, VEGF, and sICAM-1. Serum galectin-3 level correlated with the level of IL-6 (A: r = 0.360, P = 0.03), VEGF (B: r = 0.408, P < 0.01), and sICAM-1 (C: r = 0.474, P < 0.01).
To the best of our knowledge, the present study reports for the first time that serum galectin-3 level also correlated with VEGF serum concentration. VEGF, previously known as vascular permeability factor\(^\text{18}\), has a molecular weight of 45 kDa and belongs to a family of platelet-derived growth factors. Several isoforms have been identified, including isoforms A, B, C, D, and E\(^\text{18, 19}\). In addition, VEGF has been reported not only as an angiogenic factor but also as an immunomodulator. Elevated VEGF levels are reportedly associated with advanced-stage melanoma, along with negative immune reactions, including type 2 helper T cell (Th2) dominance and impaired dendritic cell function\(^\text{20}\). Galectin-3 has been shown to reduce the affinity of the T-cell receptor\(^\text{21}\), influence the strength of antigen activation in dendritic cells\(^\text{22, 23}\), internalize the T-cell receptor\(^\text{24}\), and induce apoptosis of T cells\(^\text{25}\). Galectin-3 has also been reported to have inhibitory effects on IL-12 production by dendritic cells\(^\text{26}\), resulting in Th2 dominance.

IL-6 is a pleiotropic cytokine that plays diverse roles as a regulator of immunological responses. Elevated serum levels have been reported in colorectal carcinoma\(^\text{27}\). IL-6 has also been reported to stimulate the release of angiogenic factors such as VEGF and basic fibroblast growth factor\(^\text{28}\). Galectin-3 plays a role in regulating the production of IL-6\(^\text{5, 20}\). Taken together, there might be positive feedback among galectin-3, VEGF and IL-6.

The soluble form of ICAM-1, sICAM-1, binds to macrophage antigen-1 and integrin lymphocyte function-associated antigen-1. Elevated serum concentrations of sICAM-1 have been reported to be associated with TNM stage in colorectal carcinoma\(^\text{29}\). Circulating sICAM-1 inhibits T cell interaction with tumor cells\(^\text{30}\), and natural killer (NK) cell-mediated toxicity\(^\text{31}\). Galectin-3 also inhibits NK cell-mediated tumor immunity by binding to the natural cytotoxicity receptor, NKp30 or NKG2D binding site of major histocompatibility complex class I-related chain A\(^\text{32, 33}\).

Thus, increased circulation of galectin-3 correlated with angiogenic factors, such as IL-6, VEGF, and sICAM-1, which are also immunological regulators. This study has limitations that the number of cases is small to draw a definite conclusion regarding the relationship between survival and serum galectin-3 levels. Further investigations with larger series of colorectal cancer patients are mandatory.

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