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Gastrointestinal Tract

Prognostic value of tumoral expression of galectin-9 in gastric cancer

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ABSTRACT

Background/Aims: Galectin-9 (Gal-9) is a member of the β-galactoside-binding lectin family. Our previous study revealed that Gal-9 suppresses migration, invasion, and epithelial-mesenchymal transition in gastric cancer cells. Gal-9 was reported to have anti-metastatic activity in patients with malignant melanoma, breast cancer, and hepatocellular carcinoma. Therefore, we aimed to evaluate the prognostic significance of Gal-9 in patients with gastric cancer.

Materials and Methods: The clinical significance of Gal-9 was explored using clinical and pathological data from 619 patients with gastric cancer who underwent gastrectomy at National Cancer Center, Korea. Tissue microarray and immunohistochemical analyses were used to evaluate Gal-9 expression. The median follow-up duration was 65.7 months (range 0-79 months). Kaplan-Meier method was used to evaluate survival. Log-rank test was used to assess the differences in survival.

Results: Based on the tumoral expression of Gal-9, 619 patients with gastric cancer were classified into two groups: Gal-9-positive patients (327, 52.8%) and Gal-9-negative patients (292, 47.2%). The Gal-9-positive group had a significantly lower overall ($p=0.001$, by log-rank test) and gastric cancer-specific mortalities ($p<0.001$) compared to the Gal-9-negative group. In multivariate analysis, which included the depth of invasion and lymph-node metastasis, Gal-9 positivity showed a trend toward improved prognosis but did not reach statistical significance (hazard ratio, 0.8; 95% confidence interval, 0.55-1.31).

Conclusion: Tumoral expression of Gal-9 may suppress tumor progression in patients with gastric cancer.

Keywords: Gastric cancer, Galectin-9, tumor progression suppressor, overall survival

Original Article

INTRODUCTION

Gastric cancer is the fourth most common type of cancer worldwide (1). Although the prognosis of patients with gastric cancer has improved due to early detection, radical resection, and development of adjuvant chemotherapy, gastric cancer still remains the second most common cause of cancer-related death (2). To date, the most important prognostic factors of gastric cancer are the depth of invasion and lymph-node metastasis (3,4). Our recent study showed that enhanced peroxisome proliferator-activated receptor γ (PPARγ) activity reduces migration, invasion, and epithelial-mesenchymal transition (EMT) in gastric cancer cells by the upregulation of galectin-9 (Gal-9). We also showed that the expression of PPARγ was associated with a good prognosis in patients with gastric cancer in an independent manner (5). However, the prognostic value of Gal-9 has not been investigated to date.

Galectins are proteins with a β-galactoside-binding affinity and harbor evolutionarily conserved sequences, and this sequences is known to be the carbohydrate-recognition domain (6,7). Until now, 15 mammalian galectins have been identified (8). Gal-9 was first identified as an eosinophil activation factor and chemoattractant (9-12). Previous studies reported that Gal-9, similar to other galectins, modulates various biological functions, such as tumor cell aggregation, adhesion, and apoptosis (13,14).

Recently, several studies have been published regarding the role of Gal-9 expression in malignant conditions. In several solid cancers, Gal-9 expression was linked to a decreased metastatic progression. In malignant melanoma patients, an increased tumoral expression of Gal-9 was closely associated with a decreased metastasis and a lower recurrence rate, and similar findings were

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observed in breast cancer (15,16). In another study, Gal-9 was found to be a potential new prognostic factor with anti-metastatic effects in patients with hepatocellular carcinoma (17). Assuming that Gal-9 would have a prognostic value, we tried to analyze clinical data of a large prospective gastric cancer patient cohort, stratified based on the Gal-9 expression status.

MATERIALS AND METHODS

Study Population and Treatment

Patients who had undergone gastrectomy with D2 or D1+ lymph-node dissection between January 2006 and December 2006 in the National Cancer Center, Korea were included in this study. Patients were enrolled consecutively and treated with total or subtotal gastrectomy according to the type and location of the tumor. Lymph-node dissection was performed following the recommendations by the Japanese Research Society for Gastric Cancer (18). Patients who could not be treated with curative resection (e.g., patients with distant metastasis) were excluded from the study. Those who had undergone a prior gastric surgery or chemotherapy were also excluded. Patients with postoperative pathologic stage II or more were treated with 5-fluorouracil-based adjuvant chemotherapy. Radiation therapy was not performed. Written informed consent was secured before surgical resection of gastric cancer from all patients for molecular analysis of surgical specimens. This study was approved by the institutional review board of National Cancer Center, Korea. (NCCNCS-12-603).

Tissue microarray (TMA) and Immunohistochemical (IHC) Analysis of Gal-9

A TMA was prepared with paraffin-embedded blocks of 619 tumor samples using a tissue array device (Beecher Instruments Inc., Sun Prairie, WI, USA). For each case, core biopsies (2 mm in diameter) of the tumor area were acquired from previously prepared paraffin blocks. TMA blocks were cut into 3 µm thickness and then dried at 56°C for 1 hour, dewaxed in xylene, rehydrated with EZ Prep (Ventana Medical Systems, Tucson, AZ, USA), and washed with Tris-buffered saline. The antigens were retrieved through heat treatment for 30 minutes in a pH 8.0 Tris-EDTA buffer (CC1, Ventana Medical Systems, Tucson, AZ, USA) at 95°C. Subsequently, 3% H_2O_2 was applied for 10 minutes at room temperature to block endogenous peroxidase activity. A ready-to-use protein blocker solution (Ventana Medical Systems, Tucson, AZ, USA) was applied for 20 minutes at room temperature to block nonspecific binding. A primary antibody against Gal-9 (2 µg/mL) (rabbit polyclonal, ab69630, Abcam, Cambridge, UK) was applied to the slide section for 32 minutes at 42°C. Next, the sections were incubated with an HRP multimer-labeled secondary antibody (ultraView Universal DAB Detection Kit, Ventana Medical Systems, Tucson, AZ, USA) for 20 minutes at room temperature, stained using an ultraView Universal DAB Detection Kit (Ventana Medical Systems; Tucson, AZ, USA) for 8 minutes, and counterstained using hematoxylin. The signal strength of Gal-9 staining in normal epithelial cells was strong. We defined the sample as positive if more than 10% of the tumor cells showed strength similar to foveolar epithelial cells. If there was no signal or less than 10% of the tumor cells showed a positive signal, the sample was regarded as negative (Figure 1). Normal gastric epithelial cells and stromal cells (fibroblasts and lymphocytes) were used as positive and negative controls, respectively, in each immunohistochemistry assay. All slides that were immunohistochemically stained for Gal-9 were read by a single experienced pathologist blinded to other clinical information.

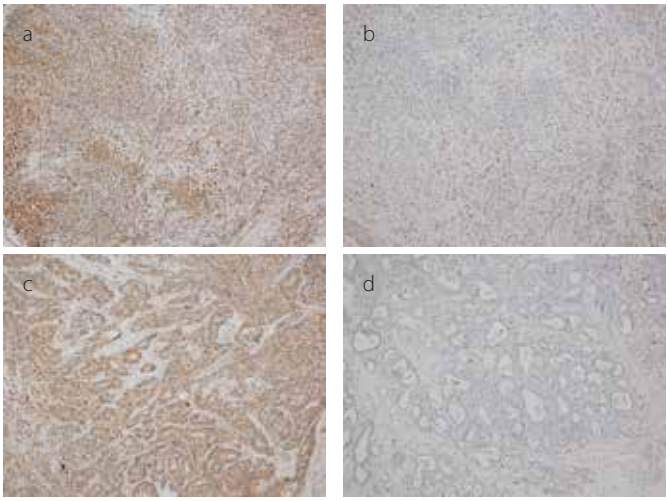

Figure 1. a-d. Evaluation of galectin-9 expression with immunohistochemical analysis (original magnification ×100). Positive and negative expression in diffuse-type (a, b) and intestinal-type (c, d) gastric cancers

Clinical Data Collection

Clinical data from a prospective cohort of 619 patients who underwent surgery for gastric cancer at the National Cancer Center, Korea from January 2006 to December 2006 were reviewed retrospectively. Clinicopathological characteristics, such as age, sex, location and size of the tumor, depth of invasion, lymph-node metastasis, and survival were analyzed. Regardless of the lymph-node status, tumor within the mucosa or submucosa was defined as early gastric cancer (EGC), whereas tumor that invaded proper muscles or beyond was considered to be advanced gastric cancer (AGC) (19). Pathological tumor staging followed the 7th edition of the International Union Against Cancer and American Joint Committee on Cancer TNM classification system (20). The location of the tumor was described according to the Japanese Classification of Gastric Cancer, which described location of the tumor based on three categories: upper, middle, and lower (18).

Patients were followed until death or the predefined cut-off date of June 30th, 2012. Overall mortality was defined as death from any cause. For analyses for gastric cancer-specific mortality, deaths from other causes except gastric cancer were excluded.

Statistical Analysis

According to the Gal-9 expression status, patients were categorized into two groups: Gal-9-positive group and Gal-9-negative group. All continuous variables were analyzed using the Student's t-test. Categorical variables were compared using the χ^2 test or Fisher's exact test.

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Table 1. Comparison of clinicopathological characteristics of galectin-9-positive and galectin-9-negative patients with gastric cancer (n=619)

	Galectin-9 negative (n=292)	Galectin-9 positive (n=327)	p
Age (years), mean ± SD	58.6±11.9	58.7±11.5	0.888
Sex, n (%)			**0.037**
Male	207 (70.9)	206 (63.0)	
Female	85 (29.1)	121 (37.0)	
Diagnosis, n (%)			**<0.001**
Early gastric cancer	94 (32.2)	258 (78.9)	
Advanced gastric cancer	198 (67.8)	69 (21.1)	
Location, n (%)			**0.385**
Upper third	47 (16.1)	40 (12.2)	
Middle third	60 (20.5)	71 (21.7)	
Lower third	185 (63.4)	216 (66.1)	
Tumor size (cm)[a], mean±SD	5.2±3.1	3.7±2.7	<0.001
Depth of invasion, n (%)			**<0.001[b]**
T1	94 (32.2)	258 (78.9)	
T2	131 (44.9)	42 (12.8)	
T3	58 (19.9)	25 (7.6)	
T4	9 (3.1)	2 (0.6)	
Lymph-node metastasis, n (%)			**<0.001**
N0	139 (47.6)	269 (82.3)	
N1	95 (32.5)	38 (11.6)	
N2	36 (12.3)	11 (3.4)	
N3	22 (7.5)	9 (2.8)	
pStage[c], n (%)			**<0.001[b]**
I	154 (52.7)	283 (86.5)	
II	74 (25.3)	17 (5.2)	
III	53 (18.2)	25 (7.6)	
IV	11 (3.8)	2 (0.6)	

SD: standard deviation
[a]Long diameter of tumor; [b]Fisher's exact test; [c]Postoperative stage evaluated pathologically

Kaplan-Meier method was used for survival analyses, and the difference of survival between groups was evaluated using log-rank test. To evaluate the association between Gal-9 expression and mortality while controlling for other confounding factors, the Cox proportional hazards regression model was used. Multivariate Cox model was used to evaluate the interaction between variables, and a likelihood ratio test was performed. A p value of less than 0.05 was considered statistically significant. Statistical analyses were performed using Statistical Package for Social Sciences version 20.0 software (IBM Corp.; Armonk, NY, USA).

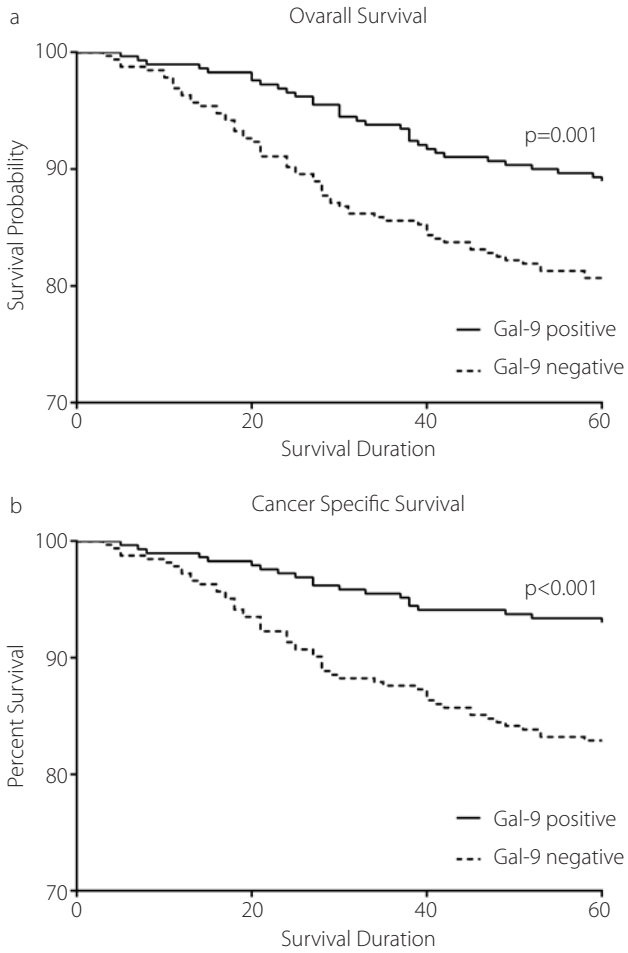

Figure 2. a, b. Kaplan-Meier survival curves of study population. Overall survival (a) and gastric cancer-specific survival (b) of 619 patients with gastric cancer based on the Gal-9 expression status

RESULTS

Clinical and Pathological Features

Gal-9 was expressed in 327 (52.8%) of the 619 patients with gastric cancer, as shown by IHC staining. Table 1 shows the baseline characteristics of patients according to the Gal-9 status: 327 patients in the Gal-9-positive group and 292 in the Gal-9-negative group. Significant differences were found in sex, diagnosis, tumor size, the depth of invasion, lymph-node metastasis, and postoperative stage. Gal-9 negativity was associated with a higher T and N stage, resulting in advanced stage disease.

Survival Analysis

The median follow-up duration was 65.7 months (range 0-79 months). Survival curves were plotted using Kaplan-Meier method (Figure 2). Significantly lower overall mortality was observed in patients with Gal-9-positive tumors compared with that in patients with Gal-9-negative tumors (Figure 2a, p=0.001, by log-rank test). Gastric cancer-specific survival curves are also shown in Figure 2b, demonstrating that the Gal-9-positive group had a significantly lower gastric cancer-specific mortality compared with the Gal-9-negative group (p<0.001, by log-rank test).

Table 2. Cox multivariate analysis of factors independently associated with overall mortality in patients with gastric cancer

	Univariate HR	95% CI	p	Multivariate HR[a]	95% CI	p
Location						
Upper third	1	Referent		1	Referent	
Middle third	0.61	0.33-1.11	0.106	0.90	0.49-1.66	0.728
Lower third	0.61	0.37-1.00	0.050	1.01	0.60-1.71	0.968
Tumor size	1.18	1.14-1.22	<0.001	1.08	1.02-1.14	0.007
Depth of invasion						
T1	1	Referent		1	Referent	
T2	2.08	1.23-3.54	0.006	1.11	0.55-2.23	0.773
T3	9.18	5.69-14.80	<0.001	2.44	1.12-5.34	0.025
T4	17.17	7.81-37.72	<0.001	4.36	1.62-11.74	0.004
Lymph-node metastasis						
N0	1	Referent		1	Referent	
N1	2.05	1.20-3.49	0.008	1.21	0.62-2.36	0.582
N2	8.28	4.92-13.94	<0.001	3.35	1.63-6.88	0.001
N3	18.58	11.06-31.21	<0.001	6.53	3.10-13.73	<0.001
Gal-9 expression						
Negative	1	Referent		1	Referent	
Positive	0.51	0.35-0.76	0.001	0.85	0.55-1.31	0.467

CI: confidence interval; HR: hazard ratio; Gal-9: galectin-9
[a]Cox multivariate regression model included tumor location, tumor size, depth of invasion, lymph-node metastasis, and Gal-9 expression

In univariate analysis, tumor size, T stage, N stage, and Gal-9 expression were significantly associated with overall survival, with a hazard ratio (HR) of 0.51 [95% confidence interval (CI), 0.35-0.76] for Gal-9 positivity (Table 2). In multivariate analysis, which took into account age, sex, tumor location and size, the depth of invasion, and lymph-node metastasis, the Gal-9 expression status was not significantly associated with the overall survival (Table 2). However, there was a slight association toward better prognosis (HR, 0.8; 95% CI, 0.55-1.31).

DISCUSSION

This study demonstrated that Gal-9-positive patients with gastric cancer had less advanced tumors with lower T and N stages. Our previous study showed that Gal-9 acts as a suppressor of EMT and is a downstream target of PPARγ. It suggests that a decreased Gal-9 expression directly correlates with an increased cancer cell invasion and migration, resulting in higher T and N stages, and this is consistent with the findings of this study. Furthermore, comparison between the Gal-9-positive and Gal-9-negative groups showed that Gal-9-positive patients had improved survival compared with Gal-9-negative patients largely due to less advanced tumors in patients with a high Gal-9 expression.

The role of Gal-9 expression in malignant tumors has received much attention recently. Gal-9 suppresses metastasis by inhibiting multiple steps that are required for tumor metastasis: invasion of the extracellular matrix (ECM), detachment from tumors, and adherence to the vascular endothelium. Several studies reported decreased invasion and migration of malignant tumors with Gal-9 expression (15-17). In other words, Gal-9 expression may inhibit detachment of tumor cells from primary lesions and reduce adhesion of cancer cells to ECM. Adhesion of cancer cells to ECM is an essential step in tumor cell invasion because the adhesive interaction between the metastatic tumor cells and ECM components appears to be an essential step for successful target organ colonization (16,21). In a recent study, decreased Gal-9 expression was associated with a marked increase in the endothelial adhesion and transendothelial invasion of HepG2 cancer cells (17)**.** Because the invasion of cancer cells into the vascular endothelium is also an essential step in metastasis, this potential anti-invasive effect of Gal-9 may prevent tumor cells from disseminating to the blood vessel endothelium (22,23). Unlike other types of cancer, the association between gastric cancer and Gal-9 has not been thoroughly investigated. A recent study conducted by Jiang et al. (24) evaluated Gal-9 expression using IHC staining and found that patients with a higher age, a higher TNM stage, lymphovascular invasion, lymph-node metastasis, and distant metastasis had a significantly lower Gal-9 expression. They also reported significant association between increased expression of Gal-9 and lower survival compared with other groups. However, the study participants had been enrolled for more than 10 years, and there was no information about surgical resection, which suggests a bias in patient selection. Our study recruited consecutive patients for a year, and more patients (619 vs. 305) were examined compared to the previous study (24).

There are several limitations to our study. First, this study used data from a patient cohort at a single center. Although we recruited a large number of patients with gastric cancer, external validation of the current finding should be undertaken with other prospective cohorts. Second, although univariate analysis showed that Gal-9 expression was significantly associated with improved overall survival, this association turned out to be statistically insignificant in multivariate analysis. This may be due to the inclusion of T and N stages in multivariate analysis. Because T and N stages are strongly associated with the clinical outcome, the effect of other variables may have been mitigated when analyzed together, concealing the correlation of Gal-9 expression and survival. Third, because this was a retrospective review of the prospectively collected data, we were not able to evaluate several factors that may have affected recurrence, such as smoking and diet. Further investigation and inclusion of other clinical data that may influence the patient outcome will give us a better understanding of this issue.

In conclusion, we evaluated Gal-9 expression in patients with gastric cancer and found that Gal-9 positivity was associated with early-stage disease and a trend toward better overall survival. This finding suggests that Gal-9 expression may be involved in the suppression of tumor progression, implying that Gal-9 may be a potential therapeutic target for managing gastric cancer in future.

Ethics Committee Approval: This study was approved by the institutional review board of National Cancer Center, Korea on June 25th, 2012 (Approval number: NCCNCS-12-603).

Informed Consent: Informed consent was obtained from each patient.

Peer-review: Externally peer-reviewed.

Author contributions: Concept - S.J.C.; Design - S.J.C.; Supervision - C.G.K., Y.W.K.; Resource - S.J.C.; Materials - M.C.K., C.G.K., Y.W.K., S.J.C.; Data Collection and/or Processing - K.W.S., M.C.K.; Analysis and/or Interpretation - S.I.C., K.W.S., S.J.C.; Literature Search - S.I.C., K.W.S., S.J.C.; Writing - S.I.C., K.W.S.; Critical Reviews - M.C.K., C.G.K., Y.W.K.

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