Pathol. Oncol. Res. (2010) 16:569–577
DOI 10.1007/s12253-010-9248-8

# CD24 and Galectin-1 Expressions in Gastric Adenocarcinoma and Clinicopathologic Significance

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Received: 8 August 2009 / Accepted: 26 January 2010 / Published online: 23 February 2010


**Abstract** CD24 and galectin-1 expression in gastric adenocarcinoma and their clinicopathologic significance remained largely unknown. We aimed to evaluate expressions and staining intensities of CD24 and galectin-1 in gastric adenocarcinoma and to investigate the interrelation with clinicopathologic parameters including survival. 93 cases with gastric adenocarcinoma were reevaluated histopathologically and immunohistochemistry was performed with antibodies against CD24 and galectin-1. Staining intensities of both markers in tumor cells and staining intensity of galectin-1 in tumor-associated stromal cells were scored semiquantitatively. The relationship between expression and staining intensity of CD24 and galectin-1 and clinicopathologic variables were assessed. CD24 staining intensity was associated with lymphovascular invasion ($p$=0.007), serosal invasion ($p$=0.001), stage ($p$=0.001) and lymph node metastasis ($p$=0.005). Galectin-1 staining intensity in tumor-associated stromal cells was associated with tumor location ($p$=0.031), lymphovascular invasion ($p$=0.001), perineural invasion ($p$=0.001), serosal invasion ($p$=0.001), differentiation ($p$=0.003), stage ($p$=0.001) and lymph node metastasis ($p$=0.001). Staining intensity of CD24 ($p$=0.019) and gal-1 ($p$=0.018) were associated with patient survival. Staining intensity of CD24 in tumor cells and galectin-1 in tumor-associated stromal cells were related with certain clinicopathologic variables. Our findings suggest that these markers are independent prognostic indicators of poor survival and may serve as useful targets for novel therapies.

**Keywords** CD24 · Clinicopathologic variables · Galectin-1 · Gastric adenocarcinoma · Prognosis

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## Introduction

Gastric adenocarcinoma (GA) is the second-most common cause of cancer death in the world with an estimated 934.000 new cases per year in 2002 [1]. Although the clinical course of GA is variable, tumor progression and metastasis are clinically the most relevant prognostic determinants [2]. Identifying tumor-specific protein expression may be significant in understanding the molecular basis of GA and will contribute to the development of targeted therapy. Studies investigating CD24 in GA are limited in number [3, 4]. Furthermore, to the best of our knowledge, no previous studies have reported galectin-1 (gal-1) expression in GA. Thus, expression of CD24 and gal-1, and their clinicopathologic significance in GA have largely remained unknown. CD24, a glycosylphosphatidylinositol-anchored membrane protein, is thought to function as an adhesion molecule. It is expressed by pre-B lymphocytes, T cells, neutrophils, neuronal tissue, keratinocytes and renal tubular epithelium [5–7]. Because it is the ligand of adhesion receptor P-selectin (CD62P) expressed on activated endothelial cells and platelets, CD24 is considered to be associated with tumor metastasis [5, 8–15].

Galectins are a family of carbohydrate-binding proteins with an affinity for β-galactosides. A variety of biological functions including tissue development and differentiation,

regulation of immune responses, cell migration, apoptosis, tumor progression and immune-escape, and regeneration of the central nervous system after injury have been shown to be related to galectins. Gal-1 is a homodimer of 14 KDa subunits and the first protein discovered in this family [16–19]. Different expression profiles of gal-1 have been detected in tumor cells, tumor-associated stromal (TAS) cells and capillary endothelial cells in various neoplasms [20–23]. Increased expression of gal-1 in TAS cells correlates with progression of colon cancer [24]. The aim of this study was to investigate the immunohistochemical expression and staining intensity of CD24 and gal-1 in GA in correlation with clinicopathologic variables including survival.

## Material and Methods

### Pathologic Examination

This study included 93 cases which had been diagnosed as GA at the Department of Pathology in Zonguldak Karaelmas University School of Medicine from 2001 to 2009. Clinical features of the cases were extracted from hospital records. Cases with recurrent cancer, or unverifiable clinical, or pathologic data were excluded from the study. Formalin-fixed, paraffin-embedded samples were cut into 5 μm sections, and stained with hematoxylin and eosin (H&E). H&E stained slides were reviewed by the same pathologist (S.B). The following features were evaluated in all cases: primary location, size, Lauren's type, lymphovascular invasion, perineural invasion, serosal invasion, and the presence and number of lymph node metastasis. According to their degree of differentiation, tumors were subdivided into well, moderately and poorly differentiated categories. All tumors were reclassified according to the Tumor, Node, and Metastasis (TNM-UICC and AJCC) staging system. Survival was defined as the period from the first day of surgery to death or the last follow-up. Survival analysis included 44 patients with a minimum of 36 months post-operative follow-up, excluding patients with shorter follow-up periods. The patients who failed to return to the hospital as scheduled for follow-up were contacted by telephone. The survival period ranged from 7 to 2,640 days (median 510 days; mean ± SD, 721.56±696.09). By the end of the study, 15 patients (34%) were still alive and 29 (66%) had died.

### Immunohistochemical Staining

For each patient, a representative block containing adequate neoplastic and nonneoplastic tissues was selected. Briefly, archival paraffin-embedded tissue samples were cut in 4 μm sections and dewaxed in xylene and brought to water through graded alcohols. Antigen retrieval was achieved by microwaving in 0.01 mol/L citrate buffer for 30 min. Endogenous peroxidase was inhibited by 3% hydrogen peroxide in methanol. The sections were stained with labeled streptavidin biotin. The following primary antibodies were used: CD24 (Neomarkers, Clone SN3b, 1:50 dilution, Fremont, CA, USA) and galectin-1 (Novocastra, Clone 25C1, 1:100 dilution, Newcastle, United Kingdom). The primary antibodies were incubated at room temperature for 1 h and the slides were counterstained with hematoxylin. Tonsil and prostate tissues were used as a positive control for CD24 and gal-1, respectively. The results were interpreted by two independent pathologists (B.B., S.O.O.), who were unaware of the outcome of the patients.

CD24 staining intensity in tumor cells was semiquantitatively classified by using the following criteria: no positive cells = 0 (negative); 1–10% positive = 1 (weak); 11–50% positive = 2 (moderate) and >50% positive = 3 (strong). The staining patterns of CD24 (membranous and/or cytoplasmic) were also recorded. The preliminary analysis of membranous CD24 staining did not reveal any significant associations with clinicopathologic parameters including survival. Positive CD24 staining was defined as cytoplasmic pattern in statistical analysis. Negative CD24 staining was defined as negative cytoplasmic staining or only membranous staining.

Gal-1 staining intensity in tumor and TAS cells were scored semiquantitatively as follows: no positive cells = 0 (negative); 1–29% positive = 1 (weak); 30–60% positive = 2 (moderate) and >60% positive = 3 (strong). The staining patterns and intensity of CD24 and gal-1 in nonneoplastic, metaplastic and dysplastic mucosal epithelium adjacent to adenocarcinoma were also recorded.

*Statistical Analysis* Data were analyzed by SPSS for Windows, v.12 package. The $\chi^2$ and Fisher's exact test were used to evaluate the association between expression (negative versus positive) and staining intensity of CD24 and galectin-1 and clinicopathologic parameters. Pearson correlation was used to assess the correlation between staining of CD24 and gal-1. Univariate survival analysis was performed according to Kaplan-Meier; differences in survival curves were assessed with the log rank test. Multivariate analysis was performed using Cox's proportional hazard models. *P* values less than 0.05 were considered statistically significant.

## Results

### Patient Characteristics

Thirty-four patients (36.6%) were female, and 59 (63.4%) were male. Mean age ± standard deviation (SD) was

61.59 years ± 13.26 (range 30–84 years). The locations of tumors were as follows: 9 (9.7%) in the upper third, 48 (51.6%) in the middle third and 36 (38.7%) in the lower third. Maximum tumor diameter ranged from 0.4 to 19 cm (mean ± SD, 6.51±3.78). Tumors were categorized according to Lauren's type as: intestinal type in 55 (59.1%), diffuse type in 30 (32.3%) and mixed type in 8 (8.6%) out of 93 cases. Twelve (12.9%) tumors were well-differentiated, 35 (37.6%) moderately differentiated and 46 (49.5%) poorly differentiated. Lymphovascular invasion, perineural invasion and serosal invasion were determined in 75 (80.6%), 63 (67.7%) and 71 (76.3%) of the tumors, respectively. Lymph node metastasis was observed in 68 (73.1%) cases: 41 (44.6%) cases with 1–6, and 27 (29%) cases with ≥7 metastatic lymph nodes. Eighteen patients (19.4%) had stage I disease while 13 (14%) had stage II, 41 (44.1%) stage III and 21(22.6%) stage IV.

## CD24 Staining and Association with Clinicopathologic Variables

CD24 was not expressed in normal mucosal epithelium, whereas weak cytoplasmic or luminal staining was found in metaplastic and dysplastic mucosal epithelium adjacent to tumor. CD24 staining showed strong cytoplasmic and membranous expression in lymphovascular tumor thrombi (Fig. 1a). In GA, CD24 expression was detected in 81 (87%) of 93 tumors. Eleven (11.8%) of these tumors showed only membranous CD24 staining, whereas 70 (75.2%) displayed cytoplasmic staining (Fig. 1b–d). CD24 staining intensity in cases with cytoplasmic expression was classified as weak in 20 (21.5%), moderate in 16 (17.2%), strong in 34 (36.6%) cases. In cross-tables, CD24 staining intensity was significantly associated with lymphovascular invasion ($p$=0.007), serosal invasion ($p$=0.001), stage ($p$=0.001) and the presence of lymph node metastasis ($p$=0.005), but not with gender, age, tumor location, tumor size, differentiation, Lauren's type, perineural invasion, and the number of lymph node metastasis. However, CD24 expression was significantly associated only with Lauren's type ($p$=0.03). Table 1 summarizes CD24 expression and staining intensity as well as clinicopathologic features.

## Galectin-1 Staining and Association with Clinicopathologic Variables

Gal-1 was not expressed in normal, metaplastic and dysplastic mucosal epithelium, whereas weak or moderate staining was observed in vascular endothelial cells associated with the adenocarcinoma cells (Fig. 2a). Gal-1 expression in tumor cells was detected in 17 (18.3%) of 93 tumors (Fig. 2b and c). The gal-1 staining intensity in tumor cells was weak in 14 (15.1%) and moderate in 3 (3.2%) cases; no strong expression was observed. In cross-tables, gal-1 expression and staining intensity of in tumor cells was not significantly associated with any of the clinicopathologic parameters.

Gal-1 expression in TAS cells was detected in 92 (98.9%) of 93 tumors (Fig. 2d). The gal-1 staining intensity

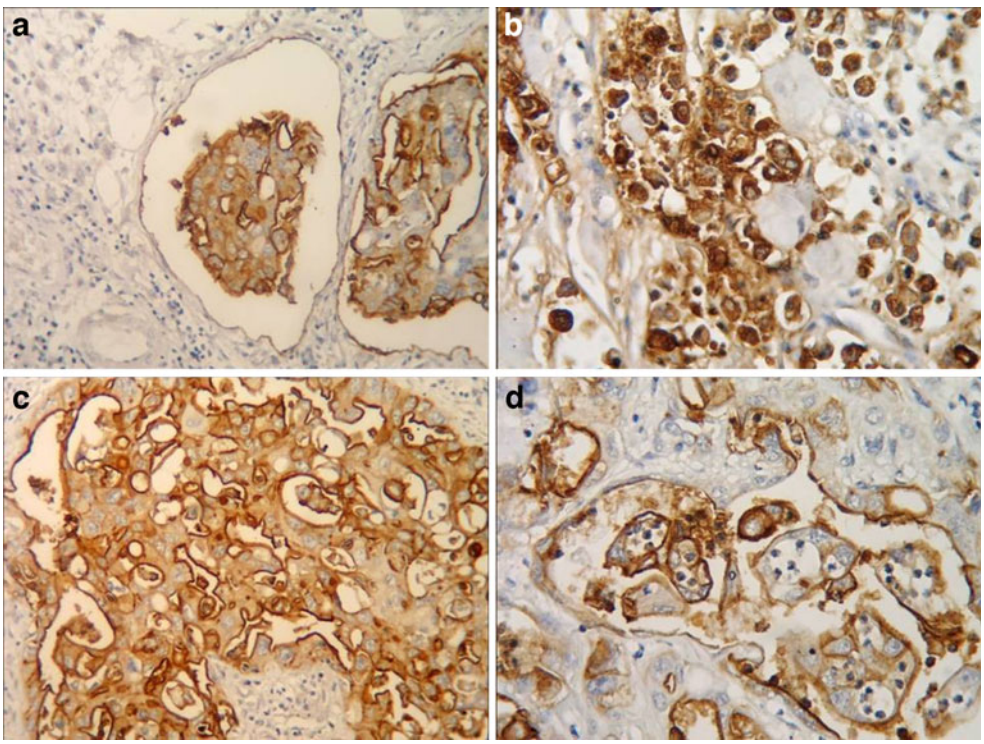


**Fig. 1** CD24 immunohistochemistry. **a** Strong membranous and cytoplasmic CD24 expression in lymphovascular tumor thrombi. **b** Diffuse type adenocarcinoma cells with strong cytoplasmic staining for CD24. **c** Neoplastic glandular cells with strong cytoplasmic and membranous CD24 expression. **d** Adenocarcinoma cells with only membranous staining for CD24

**Table 1** CD24 expression and intensity of staining and clinicopathologic characteristics of the 93 patients with gastric adenocarcinoma

| Variable | CD24 staining | | | CD24 intensity | | | |
|---|---|---|---|---|---|---|---|
| | Positive *n* (%) | Negative *n* (%) | *P* value | Weak *n* (%) | Moderate *n* (%) | Strong *n* (%) | *P* value |
| Gender | | | >0.05 | | | | >0.05 |
| Male | 43 (46.2) | 16 (17.2) | | 14 (20) | 10 (14) | 19 (27) | |
| Female | 27 (29.1) | 7 (7.5) | | 6 (8.5) | 6 (8.5) | 15 (22) | |
| Age | | | >0.05 | | | | >0.05 |
| ≤60 | 33 (35.5) | 9 (9.7) | | 8 (11.4) | 9 (12.9) | 16 (22.8) | |
| >60 | 37 (39.8) | 14 (15) | | 12 (17) | 7 (10) | 18 (25.7) | |
| Tumor location | | | >0.05 | | | | >0.05 |
| Upper third | 7 (7.5) | 2 (2.2) | | 1 (1.4) | 3 (4.3) | 3 (4.3) | |
| Middle third | 35 (37.7) | 13 (14) | | 14 (20) | 10 (14) | 11 (15.7) | |
| Lower third | 28 (30) | 8 (8.6) | | 5 (7.1) | 3 (4.3) | 20 (28.6) | |
| Tumor size | | | >0.05 | | | | >0.05 |
| ≤5 cm | 26 (28) | 7 (7.5) | | 11 (15.7) | 5 (7.1) | 10 (14) | |
| 6–10 cm | 37 (39.8) | 14 (15) | | 7 (10) | 9 (12.9) | 21 (30) | |
| >10 cm | 7 (7.5) | 2 (2.2) | | 2 (2.9) | 2 (2.9) | 3 (4.3) | |
| Lauren's type | | | **0.03** | | | | >0.05 |
| Intestinal | 36 (38.7) | 19 (20.4) | | 11 (15.7) | 9 (12.9) | 16 (22.8) | |
| Diffuse | 27 (29) | 3 (3.2) | | 8 (11.4) | 4 (5.7) | 15 (22) | |
| Mixed | 7 (7.5) | 1 (1.1) | | 1 (1.4) | 3 (4.3) | 3 (4.3) | |
| Differentiation | | | > 0.05 | | | | >0.05 |
| Well | 8 (8.6) | 4 (4.3) | | 5 (7.1) | – | 3 (4.3) | |
| Moderately | 23 (24.7) | 12 (12.9) | | 6 (8.5) | 6 (8.5) | 11 (15.7) | |
| Poorly | 39 (42) | 7 (7.5) | | 9 (12.9) | 10 (14) | 20 (28.6) | |
| LVI | | | > 0.05 | | | | **0.007** |
| Negative | 12 (12.9) | 6 (6.5) | | 8 (11.4) | 3 (4.3) | 1 (1.4) | |
| Positive | 58 (62.3) | 17 (18.3) | | 12 (17) | 13 (18.6) | 33 (47) | |
| PNI | | | > 0.05 | | | | >0.05 |
| Negative | 20 (21.5) | 10 (10.8) | | 8 (11.4) | 5 (7.1) | 7 (10) | |
| Positive | 50 (53.7) | 13 (14) | | 12 (17) | 11 (15.7) | 27 (38.6) | |
| Serosal invasion | | | > 0.05 | | | | **0.001** |
| Negative | 17 (18.2) | 5 (5.4) | | 14 (20) | 3 (4.3) | – | |
| Positive | 53 (57) | 18 (19.4) | | 6 (8.5) | 13 (18.6) | 34 (48.6) | |
| LN metastasis | | | > 0.05 | | | | **0.001** |
| Negative | 18 (19.4) | 7 (7.5) | | 11 (15.7) | 3 (4.3) | 4 (5.7) | |
| Positive | 52 (55.9) | 16 (17.2) | | 9 (12.9) | 13 (18.6) | 30 (42.9) | |
| Stage | | | > 0.05 | | | | **0.001** |
| I | 13 (14) | 5 (5.4) | | 11 (15.7) | 2 (2.9) | | |
| II | 11 (11.8) | 2 (2.2) | | 4 (5.7) | 3 (4.3) | 4 (5.7) | |
| III | 30 (32.2) | 11 (11.8) | | 3 (4.3) | 7 (10) | 20 (28.6) | |
| IV | 16 (17.2) | 5 (5.4) | | 2 (2.9) | 4 (5.7) | 10 (14) | |

*LVI* lymphovascular invasion, *LN* lymph node, *PNI* perineural invasion

in TAS cells was weak in 21 (22.6%), moderate in 26 (28.0%), strong in 45 (48.4%) cases. In cross-tables, gal-1 staining intensity in TAS cells was significantly associated with tumor location ($p$=0.031), lymphovascular invasion ($p$=0.001), perineural invasion ($p$=0.001), serosal invasion ($p$=0.001), differentiation ($p$=0.003), stage ($p$=0.001) and presence of lymph node metastasis ($p$=0.001), but not with other parameters. Gal-1 expression in TAS cells was not significantly associated with any of the clinicopathologic parameters. Table 2 summarizes gal-1 expression and

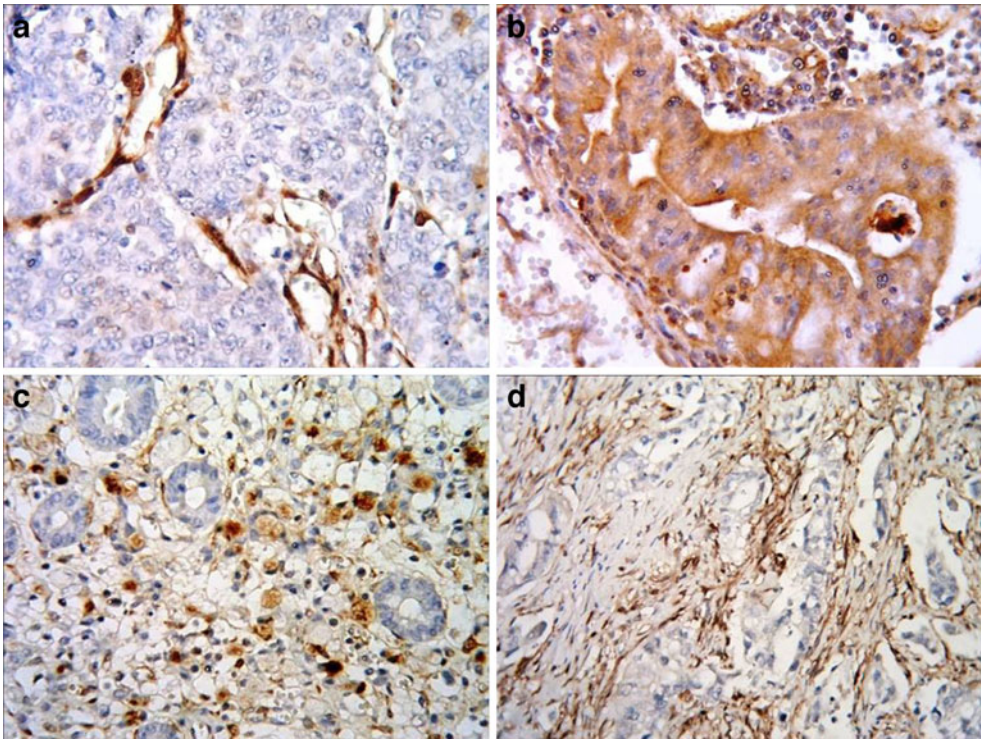


**Fig. 2** Galectin-1 immunohistochemistry. **a** Galectin-1 expression in vascular endothelial cells associated with the adenocarcinoma cells. **b** Intestinal type adenocarcinoma cells with strong cytoplasmic staining for galectin-1. **c** Galectin-1 expression in diffuse type adenocarcinoma cells but negative reaction in normal glandular cells. **d** Galectin-1 expression in tumor-associated stromal cells but negative reaction in tumoral glands

staining intensity as well as clinicopathologic features. A significant positive correlation was found between staining intensities of gal-1 in TAS cells and CD24 in tumor cells ($r$: 0.35, $p$=0.01).

### Survival Analysis

Kaplan-Meier analysis demonstrated a significant impact of tumor size ($p$=0.0009), serosal invasion ($p$=0.016), stage ($p$=0.005), the number of metastatic lymph nodes ($p$=0.001), CD24 staining intensity ($p$=0.019) in tumor cells and gal-1 staining intensity in TAS cells ($p$=0.018) on patient survival. Cases with a strong cytoplasmic CD24 intensity ($p$=0.005) and strong Gal-1 intensity ($p$=0.016) in TAS cells showed a significantly shortened mean survival time compared to the cases with weaker staining (Figs. 3 and 4). CD24 staining intensity in both intestinal-type ($p$=0.02) and diffuse-type ($p$=0.01) GA was significantly associated with survival. Gal-1 staining intensity in TAS cells showed significant association with survival in only intestinal-type ($p$=0.01).

A multivariate analysis based on the Cox's proportional hazard model was performed for all variables studied and revealed that staining intensities of CD24 in tumor cells ($p$=0.03) and gal-1 in TAS cells ($p$=0.02), serosal invasion ($p$=0.018) and stage ($p$=0.001) remained significant independent prognostic factors. Additionally, CD24 staining intensity was a significant independent prognostic factor in both intestinal-type ($p$=0.03) and diffuse-type ($p$=0.02) GA. Gal-1 staining intensity in TAS cells was a significant independent prognostic factors in only intestinal-type ($p$=0.06) GA.

## Discussion

CD24 and gal-1 molecules have recently raised considerable attention in tumor biology [25–28]. Although these molecules have been investigated in various solid tumors, specific functions and clinicopathologic significance of CD24 and gal-1 expressions in GA are unclear. CD24 expression may be cytoplasmic and/or membranous. Many studies have reported that cytoplasmic expression is significantly associated with clinicopathologic parameters, as observed in our study [11, 12, 15, 23, 29–34]. CD24 staining intensity is significantly enhanced in malignant tumors of colon, breast and endometrium [29–31, 35]. Accordingly, we determined moderate and strong staining intensity for CD24 in more than of the cases.

CD24 has been identified as a ligand to P-selectin and it is considered to play an important role in tumor progression and metastasis. P-selectin is a surface molecule expressed by activated endothelial cells and platelets. The ability of tumor cells to bind to platelets in the blood is considered important, because stabilized platelets-tumor thrombi can protect tumor cells from destruction and promote tumor extravasation and tissue penetration [8, 25, 26]. It has been reported that CD24 expression increases tumor cell proliferation, supports rapid cell spreading and strongly induces cell motility and invasion [5]. Strong CD24 expression in

**Table 2** Galectin-1 expression and intensity of staining in tumor-associated stromal cells and clinicopathologic characteristics of the 93 patients with gastric adenocarcinoma

| Variable | Galectin-1 staining | | | Galectin-1 intensity | | | |
|---|---|---|---|---|---|---|---|
| | Positive *n* (%) | Negative *n* (%) | *P* value | Weak *n* (%) | Moderate *n* (%) | Strong *n* (%) | *P* value |
| Gender | | | >0.05 | | | | >0.05 |
| Male | 59 (63.4) | – | | 14 (15.2) | 15 (16.3) | 30 (32.6) | |
| Female | 33 (35.5) | 1 (1.1) | | 7 (7.6) | 11 (12) | 15 (16.3) | |
| Age | | | > 0.05 | | | | >0.05 |
| ≤60 | 42 (45.2) | – | | 11 (12) | 13 (14.1) | 18 (19.6) | |
| >60 | 50 (53.8) | 1 (1.1) | | 10 (10.9) | 13 (14.1) | 27 (29.3) | |
| Tumor location | | | >0.05 | | | | **0.03** |
| Upper third | 9 (9.7) | – | | 1(1.1) | – | 8 (8.7) | |
| Middle third | 48 (51.6) | – | | 16 (17.4) | 13 (14.1) | 19 (20.7) | |
| Lower third | 35 (37.6) | 1 (1.1) | | 4 (4.3) | 13 (14.1) | 18 (19.6) | |
| Tumor size | | | >0.05 | | | | >0.05 |
| ≤5 cm | 33 (35.5) | – | | 14 (15.2) | 5 (5.4) | 14 (15.2) | |
| 6–10 cm | 50 (53.8) | 1 (1.1) | | 7 (7.6) | 17 (18.5) | 26 (28.3) | |
| >10 cm | 9 (9.7) | – | | – | 4 (4.3) | 5 (5.4) | |
| Lauren's type | | | >0.05 | | | | >0.05 |
| Intestinal | 54 (58) | 1 (1.1) | | 14 (15.2) | 13 (14.1) | 27 (29.3) | |
| Diffuse | 30 (32.3) | – | | 5 (5.4) | 11 (12) | 14 (15.2) | |
| Mixed | 8 (8.6) | – | | 2 (2.2) | 2 (2.2) | 4 (4.3) | |
| Differentiation | | | >0.05 | | | | **0.003** |
| Well | 11 (11.8) | 1 (1.1) | | 6 (6.5) | 1 (1.1) | 4 (4.3) | |
| Moderately | 35 (37.6) | – | | 9 (9.8) | 6 (6.5) | 20 (21.7) | |
| Poorly | 46 (49.5) | – | | 6 (6.5) | 19 (20.7) | 21 (22.8) | |
| LVI | | | >0.05 | | | | **0.001** |
| Negative | 17 (18.3) | 1 (1.1) | | 14 (15.2) | – | 3 (3.3) | |
| Positive | 75 (80.6) | – | | 7 (7.6) | 26 (28.3) | 42 (45.7) | |
| PNI | | | >0.05 | | | | **0.001** |
| Negative | 29 (31.2) | 1 (1.1) | | 14 (15.2) | 6 (6.5) | 9 (9.8) | |
| Positive | 63 (67.7) | – | | 7 (7.6) | 20 (21.7) | 36 (39.1) | |
| Serosal invasion | | | >0.05 | | | | **0.001** |
| Negative | 21 (22.6) | 1 (1.1) | | 19 (20.7) | 2 (2.2) | – | |
| Positive | 71 (76.3) | – | | 2 (2.2) | 24 (26) | 45 (48.9) | |
| LN metastasis | | | >0.05 | | | | **0.001** |
| Negative | 24 (25.8) | 1 (1.1) | | 17 (18.5) | 7 (7.6) | – | |
| Positive | 68 (73.1) | | | 4 (4.3) | 19 (20.7) | 45 (48.9) | |
| Stage | | | >0.05 | | | | **0.001** |
| I | 17 (18.3) | 1 (1.1) | | 16 (17.4) | 1 (1.1) | – | |
| II | 13 (14) | – | | 5 (5.4) | 8 (8.7) | – | |
| III | 41 (44) | – | | – | 15 (16.3) | 26 (28.3) | |
| IV | 21 (22.6) | – | | – | 2 (2.2) | 19 (20.7) | |

*LVI* lymphovascular invasion, *LN* lymph node, *PNI* perineural invasion

lymphovascular tumor thrombi in our study may support the idea that tumor cells with increased CD24 expression spread more easily because of their capacity to form tumor thrombi with activated platelets and then adhere with endothelial cells at the distal metastatic sites [3]. Conversely, some studies suggested that CD24 cross-linking may inhibit migration, hence tumor progression in breast cancer [36]. Recent studies have reported CD24 expression is closely related with tumor metastasis and poor prognosis in several epithelial neoplasms including non small cell lung

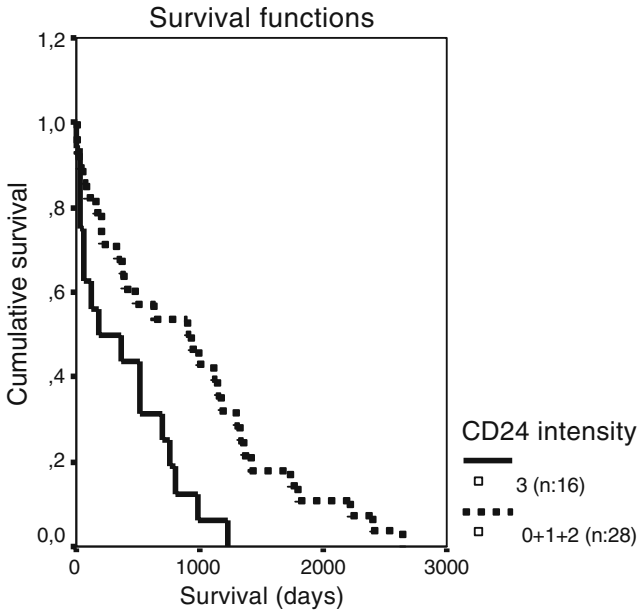


**Fig. 3** Kaplan-Meier survival curve for 44 gastric adenocarcinoma with CD24 staining intensity 3 and (0+1+2) ($p$=0.005) in tumor cells

carcinoma, breast cancer, colorectal adenocarcinoma, esophageal squamous cell carcinoma, prostatic cancer, ovarian cancer, renal cell carcinoma and cholangiocarcinoma [9–15, 31, 37].

Chou et al. were the first to study CD24 expression in GA [3]. They reported immunohistochemical CD24 expression in 50% of GA, compared to higher expression (75.2%) in the present study. They reported that cytoplasmic CD24 expression was associated with lymphovascular invasion, serosal invasion, and stage in GA and that CD24 expression correlated with poor prognosis only in diffuse-type GA. Considering the current study, CD24 staining intensity was also associated with lymphovascular invasion, serosal invasion, stage and lymph node metastasis, indicating tumor progression and metastasis. On the other hand, survival analysis demonstrated that staining intensity significantly correlated with patient survival not only in intestinal-type but in diffuse-type GA as well. This may be explained by the fact that cytoplasmic CD24 expression is associated with various tumor types independent of their molecular pathways. Possibly, CD24 positive tumor cells spread more easily resulting in decreased survival time.

Different expression profiles for gal-1 have been detected in cancer cells and surrounding host tissues such as in clolangiocarcinoma, transitional-cell carcinoma, pancreatic, prostate, throid, ovary and breast carcinomas [22, 23, 38–42]. However, this is first study investigating the clinicopathologic significance of gal-1 expression in tumor cells and TAS cells in GA. In this study, gal-1 staining was observed in vascular endothelial cells but not in normal, metaplastic or dysplastic mucosal epithelium. Gal-1 expression in tumor cells was detected in only 17 cases most of which displayed weak staining. Conversely, Gal-1 expression in TAS cells was higher by far than that of tumor cells. The presence of gal-1 in TAS cells may be explained by two possible mechanisms. First, gal-1 expressing tumor cells may synthesize and secrete gal-1 into stromal cells [43]. Second, gal-1 may be synthesized by stromal cells, especially fibroblasts, as they get stimulated by tumor cells [39, 44]. Gal-1 expression in TAS cells was also proved to correlate with progression from precancerous to cancerous tissue in colon cancer [18, 45]. The relationship between gal-1 staining intensity in TAS cells and clinicopathologic parameters in this study suggests that higher gal-1 intensity in TAS cells is associated with progression of GA. Furthermore, hypoxia-induced gal-1 expression may confer tumor immune privilege [46]. Therefore, gal-1 expression in TAS cells in GA may also reflect such a host response in the host–tumor interaction.

To our knowledge, there are only two studies in the literature evaluating the correlation between gal-1 expression and survival. In these studies, gal-1 expression in tumor cells significantly correlated with short survival in astrocytic neoplasms and in colon cancer [24, 47]. In the current study, we found that staining intensity of gal-1 in TAS cells were significant associated with short patient survival in only intestinal type GA. Additionally, the correlation between staining intensities of gal-1 in TAS cells and CD24 in tumor cells may reflect an increase in migration properties and progression of GA.

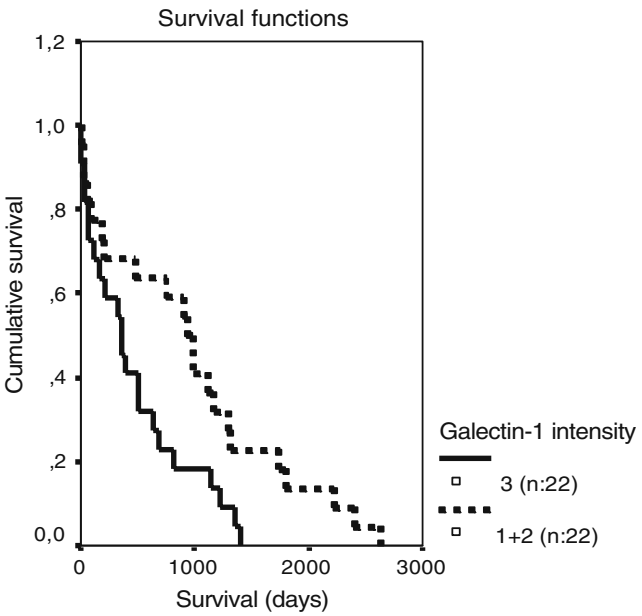


**Fig. 4** Kaplan-Meier survival curve for 44 gastric adenocarcinoma with galectin-1 staining intensity 3 and (1+2) ($p$=0.016) in tumor-associated stromal cells

In conclusion, our findings supported that overexpressions of CD24 in tumor cells and gal-1 in TAS cells were related with tumor invasiveness and progression as well as with short patient survival in GA. Gal-1 can act a key role in stromal cell–GA interaction. Therefore, these molecules may enhance metastatic potential of tumor cells and result in poor prognosis. Thus, these markers may represent independent prognostic indicators for survival and novel therapeutic targets in GA.

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