Expression of CD44 according to Clinicopathologic Characteristics of Gastric Cancer

Min Sun Ryu[1]*, Hee Jung Park[2]*, Chang Mo Moon[1,3], Seong-Eun Kim[1], Hye-Kyung Jung[1], Ki-Nam Shim[1], Sung-Ae Jung[1], Min Sun Cho[4]

[1]Department of Internal Medicine, Ewha Womans University College of Medicine, [2]Department of Pathology, Yonsei University College of Medicine, [3]Tissue Injury Defense Research Center, Ewha Womans University, [4]Department of Pathology, Ewha Womans University College of Medicine, Seoul, Korea

Objectives: Cancer stem cells are defined as focal cluster of cells within a tumor that possess the capacity for self-renewal and differentiation into phenotypically heterogeneous cells. Cluster of differentiation 44 (CD44) is considered one of the gastric cancer stem cell markers. We aimed to investigate how the expression of CD44 varies according to the clinicopathologic characteristics in gastric cancer.

Methods: For this study, 157 patients who received an operation due to gastric cancer between May 1998 and December 2009 were selected. CD44 immunohistochemistry was reviewed using the semi-quantitative scoring of intensity and proportion. The sum of the intensity and proportion scores was calculated, and a score of 2 or less was deemed 'CD44-negative' and 3 or more as 'CD44-positive.'

Results: Among the final 143 subjects, 69 (48.3%) were CD44 positive. Older age, intestinal type gastric cancer, lymphatic invasion, and lymph node metastasis were significantly correlated with expression of CD44. In the multivariate analysis, older age was the only independent factor associated with CD44 expression (P=0.028). CD44 expression was correlated with overall survival, 5-year survival, and disease-free survival. In the multivariate analysis, older age, male gender, and lymphatic invasion were independent predictors of poor overall survival. Also, older age and lymphatic invasion were significant factors in 5-year survival, and lymphatic invasion was an independent factor of poor disease-free survival.

Conclusion: Older age (≥60 years) was independently associated with CD44 expression in gastric cancer patients. Also, CD44 expression was correlated with poor prognosis in gastric cancer patients. **(Ewha Med J 2018;41(3):63-74)**

Introduction

Gastric cancer is the third most common cause of cancer deaths, both worldwide and in Korea [1,2]. Human gastric carcinogenesis involves various gene alterations such as oncogenes, tumor-suppressor genes, cell-cycle regulators, cell adhesion molecules, and DNA repair genes [3]. Cancer stem cells (CSCs) are defined as focal clusters of cells within a tumor that possess the capacity for self-renewal and differentiation into phenotypically heterogeneous cells [4]. CSCs are more resistant to conventional chemotherapy or radiotherapy and are involved in relapse and metastasis [5]. Although conventional chemotherapy

for gastric cancer has improved, many patients still suffer from either a recurrence after cancer treatments or resistance to chemotherapy [6]. According to the CSC hypothesis, cancer relapse may be due to the presence of quiescent CSCs [5]. When conventional cancer treatments fail, CSCs are considered as a novel therapeutic target for tumors [7]. Therefore, finding ways to identify CSCs is a necessary starting point in making CSCs a therapeutic target in cancer therapy including gastric cancer.

Numerous genes, including the villin promoter, leucine-rich repeat-containing G-protein coupled receptor 5, cluster of differentiation 44 (CD44), and cluster of differentiation 133 (CD133), are known to be gastric stem cell markers, and of these, CD44 and CD133 are known to be strongly expressed in gastric cancer [7,8]. CD44 is a type I transmembrane protein and a primary adhesion molecule for hyaluronate, which is a component of the extracellular matrix [9]. CD44 is also involved in cell growth, differentiation, survival, and migration and allows cells to form colonies as a cell surface receptor for hyaluronate [9]. CD44 is considered to be one of the gastric CSC markers. Takaishi et al.[10] observed tumor formation after transplanting CD44-positive gastric cancer cells on the skin of severe combined immunodeficiency mice. Also, when confirming that the CD44-positive gastric cancer cells produced spheroid colonies, they found that gastric cancer initiating cells were present in the CD44 population and reported the first CSCs in gastric cancer using CD44 as a cell surface marker [10].

The stomach is anatomically divided into the cardia, fundus, corpus, and antrum. Cardial carcinoma has been reported to have different clinicopathologic features from noncardial carcinoma [11,12]. Intestinal-type gastric carcinogenesis progresses from intestinal metaplasia to carcinoma [13], and the precancerous intestinal metaplasia typically progresses from the antrum to the corpus [14]. Based on this, it can be assumed that the expression of CSC markers may be different between the antrum and corpus in noncardial carcinoma. Therefore, we aimed to evaluate how the expression of CD44 varies according to the clinicopathologic characteristics, in particular regarding the differentiation and location of gastric cancer. Also, we investigated the association between CD44 expression and the prognosis of gastric cancer patients.

Methods

1. Study subjects

The patients included in this study were selected from the data of a previous study that analyzed the clinicopathologic characteristics and prognosis of signet ring cell carcinoma [15]. The process of selecting patients is shown in Fig. 1. The records of 771 patients who underwent curative or palliative gastrectomy due to gastric cancer at Ewha Womans University Mokdong Hospital from May 1998 to December 2009 were retrospectively reviewed. Forty-eight patients with gastric cancer located in the cardia (n=34), fundus (n=6), or whole stomach (n=11) were excluded from this study to analyze the difference in CD44 expression between the antrum and corpus. Of the remaining 723 patients, the tumor was located in the antrum in 427 patients
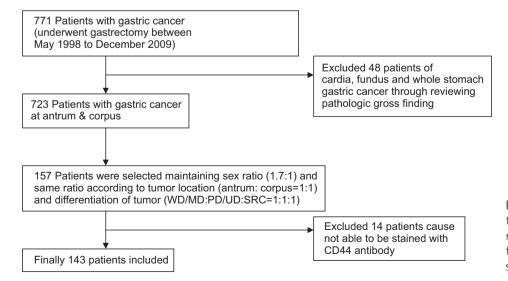

Fig. 1. Flow chart of patient selection for this study. WD, well differentiated; MD, moderately differentiated; PD, poorly differentiated; UD, undifferentiated; SRC, signet ring cell carcinoma.

and in the corpus in 296 patients. Of this group, 157 patients were randomly selected with the same sex ratio as the entire group and with an equal division based on tumor location (antrum vs. corpus) and degree of differentiation (well and moderately differentiated vs. poorly and un-differentiated vs. signet ring cell carcinoma). Fourteen patients were excluded because their specimens were not able to be stained with CD44 antibody due to technical difficulties. Finally, 143 patients were analyzed in this study. Patients were followed up until either death or the cutoff date of December 31, 2012. Additionally, the previously reported pathologic results were retrospectively reviewed.

The clinical data included age, sex, tumor location (antrum or corpus), CEA level (normal range, 0 to 5 ng/mL), CA19-9 level (normal range, 0 to 27 U/mL), date of operation, date of recurrence, date of death, cause of death, and purpose of operation. Recurrence was defined as evidence of either a local recurrence or distant metastasis based on radiologic findings and medical records after curative surgery. The pathologic data included a classification as either early gastric cancer or advanced gastric cancer, World Health Organization (WHO) histological classification, histologic grade of the tumor, Lauren classification, venous invasion, perineural invasion, lymphatic invasion, and TNM stage. Early gastric cancer was defined as carcinoma

limited to the mucosa and/or submucosa regardless of lymph node metastasis [16]. Gastric cancer was classified as either tubular adenocarcinoma, papillary adenocarcinoma, mucinous adenocarcinoma, or signet ring cell carcinoma according to the WHO histological classification, and tubular adenocarcinoma was further classified as either well-differentiated, moderately differentiated, or poorly differentiated [17]. The Lauren classification identified the gastric cancer as either intestinal type, diffuse type, or mixed type [18]. The TNM stage was classified according to the 7th edition of the American Joint Committee on Cancer/International Union Against Cancer TNM staging system [19]. The depth of the tumor (T stage) was classified as either Tis, T1, T2, T3, or T4; lymph node metastasis (N stage) as either N0, N1, or N2; and distant metastasis (M stage) as either M0 or M1. This study was approved by the institutional review board of Ewha Womans University Mokdong Hospital (2014-09-010-003).

2. Immunohistochemical staining and scoring of CD44

Tissues were cut in serial sections (3.5-μm thickness), fixed in 10% formalin, embedded in paraffin, and stained using standard histological methods. For each tissue sample, one slide was stained with hematoxylin-eosin, and immunohistochemi-
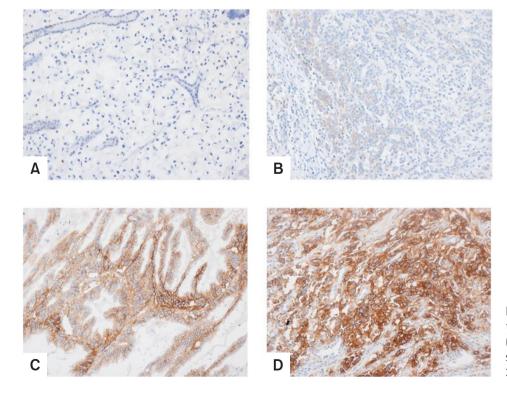
Fig. 2. Intensity of CD44 immunohistochemical staining for gastric cancer (×200). (A) 0, no staining; (B) 1, weak staining; (C) 2, moderate staining; and (D) 3, strong staining.

Table 1. Baseline clinicopathologic characteristics of patients with gastric cancer

Characteristics		All cases (n=143)
Clinical characteristics	Mean age (yr)	60.7±12.9 (27–84)
	Sex	
	Male	91 (63.6)
	Female	52 (36.4)
	Tumor location	
	Antrum	74 (51.7)
	Corpus	69 (48.3)
	Median overall survival (mo)	45 (0–155)
	Died patient	66 (46.2)
	Cause of death	
	Gastric cancer	47 (32.9)
	Hepatocellular carcinoma	5 (3.5)
	Liver cirrhosis	2 (1.4)
	Cardiologic disease	2 (1.4)
	End stage renal disease	1 (0.7)
	Pneumonia	1 (0.7)
	Another cancer	1 (0.7)
	Could not identify cause	7 (4.9)
	Purpose of operation	
	Curative	117 (81.8)
	Palliative	26 (18.2)
	Recurrent patient	21 (14.7)
	CEA (n=130)	
	≤5	115 (88.5)
	>5	15 (11.5)
	CA19-9 (n=124)	
	≤27	104 (83.9)
	>27	20 (16.1)
Pathologic characteristics	Early gastric cancer	58 (40.6)
	Advanced gastric cancer	85 (59.4)
	WHO classification	
	Tubular adenocarcinoma	90 (62.9)
	Well differentiated	18 (12.6)
	Moderate differentiated	34 (23.8)
	Poorly differentiated	38 (26.6)
	Papillary adenocarcinoma	1 (0.7)
	Mucinous adenocarcinoma	9 (6.3)
	Signet ring cell carcinoma	43 (30.1)

Table 1. Continued

Characteristics	All cases (n=143)
Lauren classification	
Intestinal	68 (47.6)
Diffuse & mixed	75 (52.4)
Vascular invasion (n=140)	
No	108 (77.1)
Yes	32 (22.9)
Lymphatic invasion	
No	80 (55.9)
Yes	63 (44.1)
Perineural invasion (n=140)	
No	99 (70.7)
Yes	41 (29.3)
T stage	
Tis, T1	55 (38.5)
T2, T3, T4	88 (61.5)
N stage	
N0	66 (46.2)
N1, N2, N3	77 (53.8)
M stage	
M0	131 (91.6)
M1	12 (8.4)

Values are presented as mean±SD (range) or number (%).
WHO, World Health Organization.

cal staining of CD44 was performed on the remaining sections using standard procedures. An antigen retrieval process was performed with hydrocitrate buffer at pH 6.0, and the sections were incubated with an anti-CD44 monoclonal antibody (1:100 dilution; SC-7297, Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 37°C for 15 minutes.

The stained slides were evaluated by one pathologist who was blinded as to the patients' information. At ×200 magnification, three hot spots, defined as the most highly stained areas, were identified. Expression of CD44 was measured using intensity and proportion in the semi-quantitative method [20,21]. First, intensity was represented as either 0 (no staining), 1 (weak staining), 2 (moderate staining), or 3 (strong staining). A sample of the scoring intensity is shown in Fig. 2. The overall intensity score was calculated as the mean value of the three measured points. Second, the proportion was determined as the percentage of stained cells in the observed areas and scored

Table 2. Association of CD44 expression with clinicopathologic characteristics in patients with gastric cancer

Clinical parameters	CD44 positive (n=69)	CD44 negative (n=74)	P-value
Age (yr)			0.003
<60	21 (30.4)	41 (55.4)	
≥60	48 (69.6)	33 (44.6)	
Sex			0.077
Male	49 (71.0)	42 (56.8)	
Female	20 (29.0)	32 (43.2)	
Tumor location			0.442
Antrum	38 (55.1)	36 (48.6)	
Corpus	31 (44.9)	38 (51.4)	
Early gastric cancer	24 (34.8)	34 (45.9)	0.174
Advanced gastric cancer	45 (65.2)	40 (54.1)	
WHO classification			0.061
Tubular adenocarcinoma	51 (73.9)	39 (52.7)	
Papillary adenocarcinoma	0 (0.0)	1 (1.4)	
Mucinous adenocarcinoma	3 (4.3)	6 (8.1)	
Signet ring cell carcinoma	15 (21.7)	28 (37.8)	
Lauren classification			0.038
Intestinal	39 (56.5)	29 (39.2)	
Diffuse & mixed	30 (43.5)	45 (60.8)	
Vascular invasion (n=140)			0.497
No	50 (74.6)	58 (79.5)	
Yes	17 (25.4)	15 (20.5)	
Lymphatic invasion			0.010
No	31 (44.9)	49 (66.2)	
Yes	38 (55.1)	25 (33.8)	
Perineural invasion (n=140)			0.104
No	43 (64.2)	56 (76.7)	
Yes	24 (35.8)	17 (23.3)	
T stage			0.224
Tis, T1	23 (33.3)	32 (43.2)	
T2, T3, T4	46 (66.7)	42 (56.8)	

Table 2. Continued

Clinical parameters	CD44 positive (n=69)	CD44 negative (n=74)	P-value
N stage			0.022
N0	25 (36.2)	41 (55.4)	
N1, N2, N3	44 (63.8)	33 (44.6)	
M stage			0.233
M0	61 (88.4)	70 (94.6)	
M1	8 (11.6)	4 (5.4)	
CEA (n=130)			0.787
≤5	60 (89.6)	55 (87.3)	
>5	7 (10.4)	8 (12.7)	
CA19-9 (n=124)			0.810
≤27	56 (84.8)	48 (82.8)	
>27	10 (15.2)	10 (17.2)	

Values are presented as number (%).
CD44, cluster of differentiation 44.

from 0% to 100% in 5% increments. The average proportion measured in the three hot spots was calculated and categorized as either 0 (0% positive cells), 1 (1% to 25% positive cells), 2 (26% to 75% positive cells), or 3 (>75% positive cells). Based on a previous study on evaluating immunohistochemistry, each intensity and proportion score was added to obtain the final score [22]. A final score of 2 or less was deemed 'CD44-negative' and 3 or more as 'CD44-positive.'

3. Statistical analysis

To analyze the relationships between CD44 expression and clinicopathologic features, Pearson's chi-square test and Fisher's exact test were used. The odds ratios (ORs) and corresponding 95% confidence intervals (CIs) of factors affecting CD44 expression were calculated from the coefficients in the logistic regression models. The overall survival was defined from the operation date to the date of death or the last follow-up date. The 5-year survival was evaluated from patients who were alive at 5 years after surgery. Disease-free survival was calculated for patients who either died or experienced a recurrence through the last follow-up date after curative surgery. Patients who died due to other causes were censored. The Kaplan-Meier method was used to analyze the overall, 5-year, and disease-free survival rates. Also, the differences between the curves were measured using the log-rank test. The hazard ratios (HRs) and 95% CIs

of the factors affecting overall survival and disease-free survival were computed using the Cox regression model. All statistical procedures were performed using IBM SPSS Statistics ver. 20.0 (IBM Corp., Armonk, NY, USA). P-values <0.05 were considered statistically significant.

Results

1. Baseline clinicopathologic characteristics of patients

The baseline clinicopathologic data of the study subjects are summarized in Table 1. The mean age of the study subjects was 60.7±12.9 years (range, 27 to 84 years), and 91 (63.6%) were male. The primary tumor was located in the antrum in 74 patients (51.7%) and in the corpus in 69 patients (48.3%). The median overall survival was 45 months (range, 0 to 155 months). Among the 66 patients who died during the follow-up period, 47 (71.2%) died of gastric cancer. One hundred seventeen patients (81.8%) received curative surgery, and 26 patients (18.2%) received palliative surgery. Recurrence of gastric cancer occurred in 21 patients (14.7%). The prevalence of advanced gastric cancer was 59.4%. Following WHO classification for histopathology of gastric cancer, tubular adenocarcinoma was observed in 90 (62.9%) patients and signet ring cell carcinoma in 43 (30.1%) patients. According to the Lauren classification, intestinal, diffuse, and mixed type carcinoma was observed in 68 (47.6%), 54 (37.8%), and 21 (14.7%) patients, respectively.

Table 3. Independent risk factors associated with CD44 expression

	Odds ratio (95% CI)	P-value
Age		
<60	1 (reference)	
≥60	2.27 (1.09–4.70)	0.028
Lauren classification		
Diffuse & mixed type	1 (reference)	
Intestinal type	1.99 (0.96–4.09)	0.063
Lymphatic invasion		
No	1 (reference)	
Yes	2.09 (0.86–5.07)	0.104
N stage		
N0	1 (reference)	
N1, N2, N3	1.32 (0.54–3.24)	0.543

CI, confidence interval.

Thirty-two patients (22.9%) had vascular invasion, 63 patients (44.1%) had lymphatic invasion, and 41 patients (29.3%) had perineural invasion. Among the patients with intestinal type, 67.6% (46/68) were older than 60 years, and 57.4% of those with diffuse type (31/54) were younger than 60 years (P=0.021).

2. The association of CD44 expression with clinicopathologic factors

CD44 expression was analyzed to determine its association with various clinicopathologic factors (Table 2). In the univariate analysis, CD44 expression was significantly higher in patients with age ≥60 years (vs. age <60 years, P=0.003), those with intestinal type (vs. diffuse and mixed type, P=0.038), those with the presence of lymphatic invasion (vs. no lymphatic invasion, P=0.010), and those with lymph node metastasis (P=0.022). CD44 expression was not statistically different between the antrum and corpus (P=0.442). When only patients with intestinal type gastric cancer were analyzed, there was no significant difference in CD44 expression between the antrum and corpus (P=0.468). Additionally, there was no difference in CD44 expression according to sex, pathologic classification, vascular invasion, perineural invasion, T or M stage, CEA level, or CA19-9 level.

In multivariate analysis adjusted for age, Lauren classification, lymphatic invasion, and N stage (which were statistically significant in univariate analysis), older age (≥60 years) was independently associated with CD44 expression (adjusted OR, 2.27; 95% CI, 1.09 to 4.70; P=0.028) (Table 3).

3. Association between CD44 expression and prognosis

We carried out Kaplan-Meier analysis with log-rank tests to evaluate the association of clinicopathologic factors with prognosis (overall, 5-year, and disease-free survival) in the study subjects. The associations between clinicopathologic factors and overall survival of gastric cancer patients are shown in Fig. 3. High expression of CD44 was significantly correlated with poor overall survival (P=0.006) (Fig. 3H). Also, older age (≥60 years), male gender, vascular invasion, lymphatic invasion, perineural invasion, and advanced TNM stage (TNM 2, 3, or 4) were associated with poor overall survival (P=0.003, P=0.017, P<0.001, P<0.001, P<0.001, and P<0.001, respectively) (Fig. 3A–3G). The Lauren classification was not significantly associ-
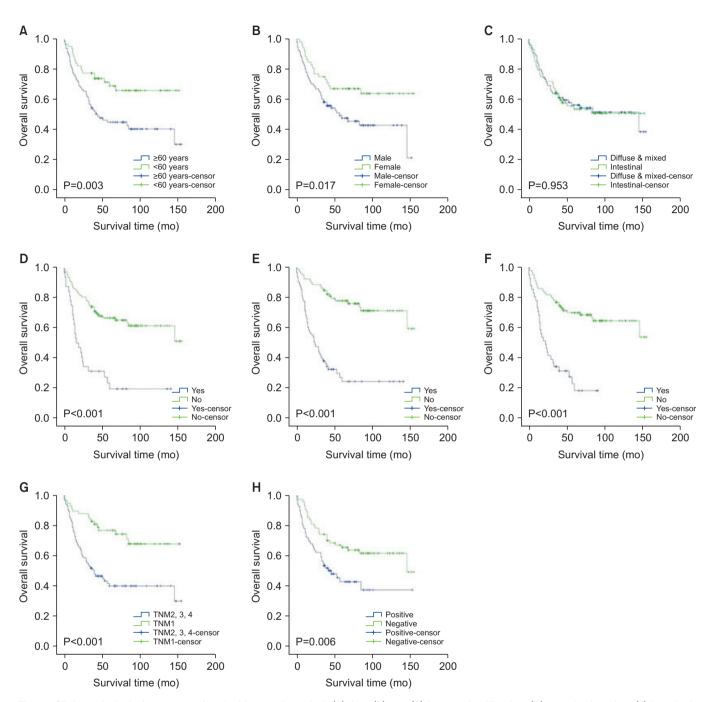
Fig. 3. Clinicopathologic factors associated with overall survival. (A) Age, (B) sex, (C) Lauren classification, (D) vascular invasion, (E) lymphatic invasion, (F) perineural invasion, (G) TNM stage, and (H) CD44 expression.

ated with overall survival (P=0.953). In the multivariate analysis using a Cox regression model, older age (≥60 years) (HR, 2.15; 95% CI, 1.21 to 3.82; P=0.009), male gender (HR, 1.88; 95% CI, 1.04 to 3.41; P=0.038), and lymphatic invasion (HR, 3.11; 95% CI, 1.46 to 6.64; P=0.003) were independent predictors for poor overall survival (Table 4). However, CD44

expression was not associated with poor overall survival in the multivariate analysis (P=0.735).

Kaplan–Meier analysis of 5-year survival revealed that older age (≥60 years), male gender, vascular invasion, lymphatic invasion, perineural invasion, advanced TNM stage, and CD44 expression were associated with poor 5-year survival

Table 4. Clinical factors associated with prognosis

	Hazard ratio (95% CI)	P-value
Overall survival		
Age ≥60 yr	2.15 (1.21–3.82)	0.009
Male	1.88 (1.04–3.41)	0.038
Diffuse & mixed type	0.86 (0.50–1.49)	0.597
Vascular invasion	1.41 (0.73–2.74)	0.308
Lymphatic invasion	3.11 (1.46–6.64)	0.003
Perineural invasion	1.83 (0.94–3.58)	0.078
TNM stage 2,3,4	0.95 (0.44–2.06)	0.892
CD44 expression	1.10 (0.64–1.88)	0.735
5-Year survival		
Age ≥60 yr	2.06 (1.15–3.71)	0.016
Male	1.66 (0.90–3.07)	0.102
Diffuse & mixed type	0.77 (0.44–1.34)	0.353
Vascular invasion	1.39 (0.72–2.71)	0.327
Lymphatic invasion	3.22 (1.47–7.05)	0.004
Perineural invasion	1.88 (0.95–3.69)	0.069
TNM stage 2,3,4	1.09 (0.47–2.48)	0.846
CD44 expression	1.10 (0.63–1.92)	0.729
Disease-free survival		
Age ≥60 yr	1.92 (0.82–4.50)	0.132
Male	1.61 (0.70–3.68)	0.260
Diffuse & mixed type	0.85 (0.39–1.82)	0.668
Vascular invasion	0.90 (0.67–2.21)	0.823
Lymphatic invasion	4.60 (1.68–12.59)	0.003
Perineural invasion	1.58 (0.65–3.82)	0.309
TNM stage 2,3,4	1.87 (0.61–5.72)	0.271
CD44 expression	1.16 (0.55–2.45)	0.693

CI, confidence interval; CD44, cluster of differentiation 44.

(P=0.004, P=0.035, P<0.001, P<0.001, P<0.001, P<0.001, and P=0.006, respectively) (Fig. 4). The Lauren classification were not related with 5-year survival (P=0.802). The multivariate analysis using a Cox regression model showed that older age (≥60 years) (HR, 2.06; 95% CI, 1.15 to 3.71; P=0.016) and lymphatic invasion (HR, 3.22; 95% CI, 1.47 to 7.05; P=0.004) were significantly correlated with poor 5-year survival (Table 4).

Older age (≥60 years), vascular invasion, lymphatic invasion, perineural invasion, advanced TNM stage, and CD44 expression were factors associated with poor disease-free survival in the Kaplan-Meier analysis (P=0.019, P=0.002, P<0.001,

P<0.001, P<0.001, and P=0.043, respectively) (Fig. 5). Sex and Lauren classification were not related with disease-free survival (P=0.235 and P=0.842, respectively). In the multivariate analysis using a Cox regression model, only lymphatic invasion was identified as an independent factor of poor disease-free survival (HR, 4.60; 95% CI, 1.68 to 12.59; P=0.003) (Table 4).

Discussion

This study evaluated the difference in CD44 expression according to clinicopathologic characteristics in gastric cancer patients who underwent gastrectomy and the relationship between the expression of CD44 and prognosis after surgery. CD44 expression was significantly higher in patients with age ≥60 years, intestinal type gastric cancer, presence of lymphatic invasion, or lymph node metastasis. Interestingly, in the multivariate analysis, older age was the only independent factor associated with CD44 expression. It is difficult to completely explain this result; however, intestinal type gastric cancer was significantly more frequent in older patients (≥60 years) (P=0.021) in our study, which may have had an influence on the association of CD44 expression with old age. Gastric atrophy and intestinal metaplasia progress with age, and intestinal type gastric cancer is known to follow the intestinal metaplasia to adenoma to carcinoma sequence [13]. In addition, Dhingra et al. [21] suggested that CSCs might play an important role in intestinal type gastric carcinogenesis through CD44 and nestin, one of the CSC markers, which were increased in intestinal metaplasia. Khurana et al. [23] showed that CD44 regulates the rate of proliferation of gastric epithelial stem cells through STAT3. ERK signaling, which is an upstream regulator of STAT3, is increased in regions of intestinal metaplasia, indicating that CD44 is a significant marker in intestinal metaplasia [23]. Our results are in accordance with previous studies. Ghaffarzadehgan et al. [24] reported that CD44 positivity was higher in intestinal type than in diffuse type gastric cancer through CD44 antibody immunostaining in 100 patients who underwent total or subtotal gastrectomy due to gastric adenocarcinoma (71% vs. 42%, P=0.002). In a meta-analysis of 4,729 patients enrolled in a total of 26 studies utilizing CD44 immunostaining for gastric cancer tissue, CD44 expression was related with intestinal type gastric cancer [25].
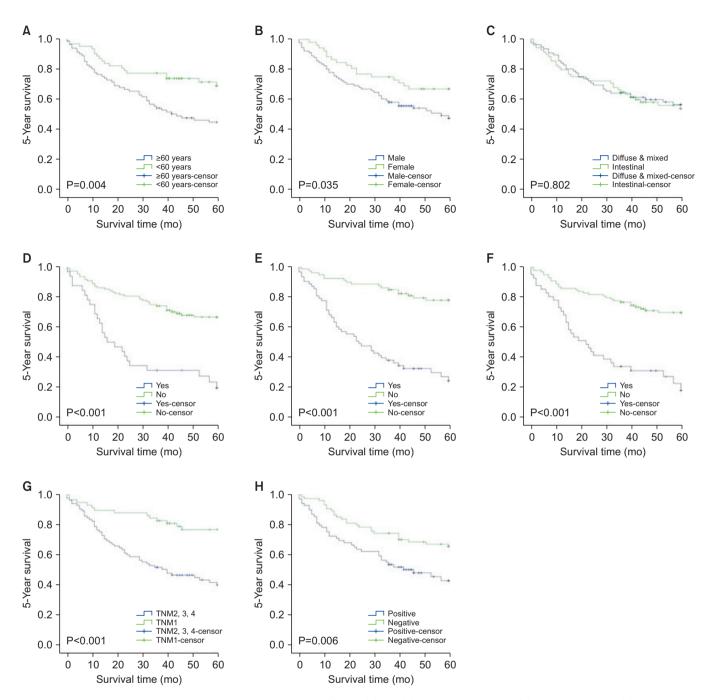
Fig. 4. Clinicopathologic factors associated with 5-year survival. (A) Age, (B) sex, (C) Lauren classification, (D) vascular invasion, (E) lymphatic invasion, (F) perineural invasion, (G) TNM stage, and (H) CD44 expression.

With respect to the association of CD44 expression with lymph node metastasis, Okayama et al. [26] performed immunostaining for 135 gastric adenocarcinoma patients to investigate the relationships between biomarkers, such as CD44 variant 6 (CD44v6), cluster of differentiation 54, caudal-related homeobox 2, and matrix metalloproteinase-7, and lymph node metastasis in gastric cancer. They found that CD44v6 and matrix metalloproteinase-7 expression was related with lymph node metastasis. Using CD44v6 immunohistochemical staining of specimens from 98 patients who underwent gastrectomy and systemic lymph node dissection due to gastric adenocarcinoma, CD44v6-positive gastric cancer was significantly associated
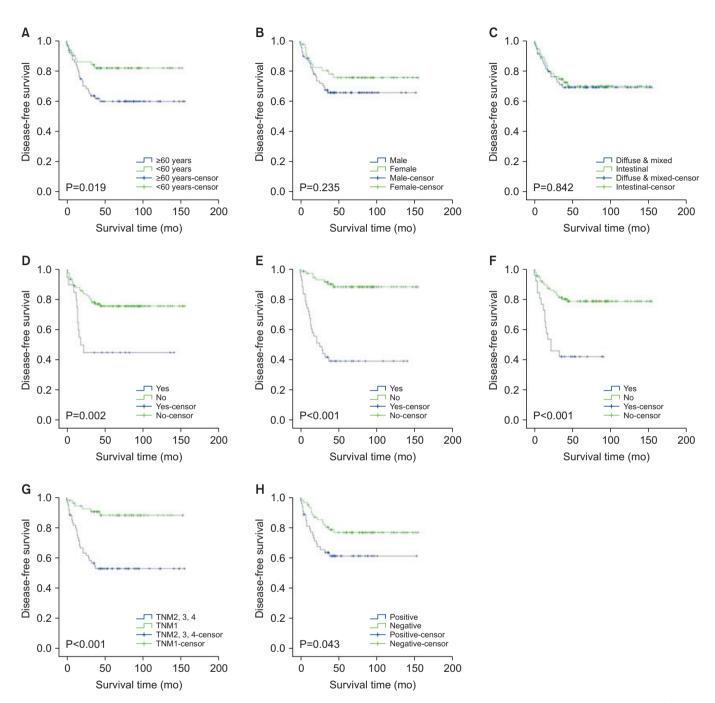
Fig. 5. Clinicopathologic factors associated with disease-free survival. (A) Age, (B) sex, (C) Lauren classification, (D) vascular invasion, (E) lymphatic invasion, (F) perineural invasion, (G) TNM stage, and (H) CD44 expression.

with lymph node metastasis, which is in agreement with our result [27]. One meta-analysis also showed that CD44 expression was related with stage, tumor size, and lymph node metastasis [28].

Moreover, this study showed that CD44 expression was significantly associated with poor overall survival, 5-year survival, and disease-free survival in gastric cancer patients using the Kaplan-Meier method with a log-rank test. However, CD44 expression was not identified as an independent factor of overall survival, 5-year survival, or disease-free survival in the multivariate analysis. In that analysis, age, sex, and lymphatic invasion were significant factors of overall survival. Age and

lymphatic invasion were independent factors of 5-year survival, and lymphatic invasion was the only independent factor of poor disease-free survival. In a study of 430 patients who underwent gastrectomy due to gastric adenocarcinoma, Jung et al. [29] showed that CD44 was an independent predictor of overall survival. In a meta-analysis of the associations between CD44-family proteins and clinicopathologic features of gastric cancer, standard CD44 expression was related to reduced overall survival, poor disease-free survival, lymph node metastasis, and distant metastasis [30]. Other studies have also shown that CD44 expression was an independent risk factor for poor outcome in patients with gastric cancer [31,32]. In the present study, although not significant in the multivariate analysis, CD44 expression was associated with poor overall survival, 5-year survival, and disease-free survival in the univariate analysis. It is thought that CD44 expression is related to poor outcome in gastric cancer patients due to the characteristics of CSCs such as chemoresistance, increased tumorigenesis, and potential for metastasis [8].

There were some limitations in this study. First, the reliability of the scoring may have been low because only one pathologist reviewed the slides. To overcome this, the positivity of the immunohistochemical staining was assessed in three hot spots per specimen. Also, to reduce intraobserver error, we calculated the average of the intensity and proportion scores. Second, the medical records of some patients were not available because of follow-up loss, and their prognosis was obtained from data registered at the National Cancer Center. Therefore, if the cause of death was not cancer, the exact cause of death was unknown. Third, it may be difficult to generalize the results of our study because the study subjects were limited to Koreans at a single center. Further studies are needed to determine whether CD44 expression is associated with intestinal metaplasia and adenoma prior to intestinal type gastric cancer.

In conclusion, older age (≥60 years) was independently associated with CD44 expression in gastric cancer patients. In addition, CD44 expression is predictive of poor prognosis in gastric cancer.

Acknowledgments

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