Original Paper

CD44 and CD44v6 are Correlated with Gastric Cancer Progression and Poor Patient Prognosis: Evidence from 42 Studies

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Key Words

CD44 • CD44v6 • Gastric cancer • Prognosis • Meta-analysis

Abstract

Background/Aims: The prognostic power of the levels of total CD44 and its isoform CD44v6 for patients with gastric cancer (GC) remains controversial. Therefore, our study aims to generalize the clinicopathological and prognostic significance of these two proteins in GC.

Methods: A literature search of the PubMed, Web of Science and Embase databases was conducted to identify eligible studies. The odds ratio (OR) with a 95% confidence interval (CI) was used to assess the effects. **Results:** In all, 42 studies including 6,229 patients were included in this analysis. Total CD44 was mentioned in 21 papers, and the results showed that CD44 was positively correlated with the T category, the N category, distant metastasis, lymphatic invasion and TNM stage. Moreover, patients with CD44 overexpression had a lower 5-year overall survival (OS) rate (OR = 3.35, 95%CI = 1.83-6.13). CD44v6 was mentioned in 24 studies, with results that were similar to those for total CD44. However, total CD44 or CD44v6 expression was not correlated with tumor size and histological grade. **Conclusion:** High CD44 or CD44v6 expression levels were correlated with cancer progression and poor prognosis in patients with GC. Both CD44 and CD44v6 may be useful diagnostic or prognostic biomarkers for GC.

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Introduction

Although the incidence of gastric cancer (GC) has substantially declined in recent years, the disease still ranks fourth in incidence among all cancers and remains the second most deadly cancer worldwide [1]. The diagnosis and treatment of GC have improved significantly, though 5-year overall survival (OS) rate of patients with middle- and late- stage disease is M. Fang and JR Wu contributed equally to this work.

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only ~20% due to local recurrence as well as metastatic disease and drug resistance [2, 3]. Recent research has suggested that cancer stem cells (CSCs), which represent a rare sub-population of cancer cells with the main properties of self-renewal and multipotency, may account for tumor initiation, metastasis, relapse, and resistance to chemotherapy or radiotherapy [4].

Several molecules have been reported to be useful markers in GC, such as CD44, CD133 and Hsa-miR-21, and high expression of these markers is usually indicative of a poor prognosis in patients with GC [5, 6]. Among these, the CD44 family is one of the most consistently reported markers in GC. The CD44 proteins, which are expressed in many cell types, belong to a polymorphic family of cell surface glycoproteins that were originally described as lymphocyte homing receptors [7]. However, the CD44 family plays critical roles in tumor occurrence, progression, and metastasis [8, 9], and members of this family have prognostic value in a wide variety of tumor types [10-13]. CD44 has recently been revealed as a target of the Wnt pathway, which is acknowledged as a key pathway for the maintenance of stemness in CSCs [14, 15]. CD44v6 is the most frequently reported splicing variant member of the CD44 family in GC and studies have shown that increased expression of total CD44 or CD44v6 is related to GC invasion, lymph node metastasis and poor outcomes [5, 16, 17].

The paradigm of CD44 or CD44v6 as a biomarker of CSCs has provoked numerous studies that exploring the prognostic significance of their expression in patients with GC. Despite these numerous independent studies, the prognostic value of CD44/CD44v6 in GC remains controversial. For example, Mayer et al. and Okayama et al. demonstrated high CD44 and CD44v6 expression in GC was to be correlated with poor clinical outcomes of GC patients [18, 19]. Conversely, Kim et al. and Fanelli et al. found no obvious correlation between CD44/CD44v6 and clinical outcomes of patients with GC [20, 21], and Songun et al. reported a poor prognosis for CD44v6-negative patients [22]. To address these discrepancies, we performed a meta-analysis to examine the relationship of expression of total CD44 and its isoform CD44v6 with clinicopathological features of GC and to determine their value as diagnostic or prognostic markers for GC.

Materials and Methods

Literature search and eligibility criteria

We collected data until May 25, 2016, using a comprehensive literature search of the PubMed, Web of Science and Embase databases. The following search terms were used: “gastric carcinoma” or “gastric cancer” or “stomach neoplasms” and “CD44”.

The studies included in the meta-analysis were randomized controlled studies or observational research (case control or cohort studies) evaluating the association between CD44/CD44v6 expression and GC clinicopathological features or prognosis. Eligible studies were included when the following criteria were met: (1) expression of the CD44 or CD44v6 protein was evaluated in cancer tissues by immunohistochemistry (IHC) rather than by serum analysis or any other type of detection method; (2) the relationship between the CD44/CD44v6 expression and clinicopathological characteristics of GC was evaluated, and the association of these markers with the 5-year OS rate was also evaluated; (3) the data for the 5-year OS rate were adequate and published; (4) the full-text articles were published in English or Chinese. Moreover, the references listed in the articles were also searched to avoid omission related studies.

Data extraction

All data from the eligible studies were extracted by two independent reviewers (Fang M and Wu JR) using predefined criteria. For every eligible study, the following data were assessed: the first author's name, publication year, country where the study was conducted, TNM stage of the patients, antibody used and cutoff scores for the definition of positive staining and the 5-year OS rate. We primarily examined the associations between total CD44/CD44v6 expression and certain clinicopathological parameters, including the T category, the N category, distant metastasis, histological grade, lymphatic invasion, vascular invasion, tumor size and TNM stage. More importantly, we investigated the association between CD44/CD44v6

expression and the 5-year OS rate. For those articles that contained only a plotted Kaplan-Meier curve, the software GetData Graph Digitizer 2.24 was used to directly digitize the 5-year OS rate.

Sensitivity analysis

A sensitivity analysis was performed through via sequential omission of individual studies. With the exception of the 5-year OS rate for the CD44v6 analysis, it was found that no single study could alter the results demonstrating that the results of our meta-analysis were quite stable.

Publication bias

We utilized Begg's and Egger's tests to identify potential publication bias,. In general, the funnel plots did not show obvious evidence of asymmetry and the P-value of Egger's test was greater than 0.05. This indicated no publication bias in most of the pooled studies, except those studies for which the N category was included in the CD44v6 analysis. The detailed results of the P-value derived from Egger's test are summarized in Tables 3-4.

Statistical analysis

The strength of the association between total CD44/CD44v6 expression and clinicopathological features of GC or the 5-year OS rate was assessed by the OR with the corresponding 95%CI. In the present study, an $OR > 1$ indicated a higher probability of tumor progression and poorer prognosis in patients with GC with CD44/CD44v6 overexpression. In the course of data pooling, statistical heterogeneity was evaluated by the Q-test. The I^2 value indicates the degree of heterogeneity. A P-value < 0.10 and/or $I^2 > 50\%$ signified significant heterogeneity, which necessitated the use of a random-effect model. Otherwise, a fixed-effect model was used[23]. Furthermore, we evaluated potential publication bias by funnel plot and Egger's linear regression test. The funnel plots were visually symmetrical, and the P-value of Egger's test was greater than 0.05, which indicated no deviation among the studies. All statistical tests in this meta-analysis were two-tailed and P-values ≤ 0.05 were considered statistically significant. The statistical analyses were performed with STATA software version 12.0.

Results

Included studies and characteristics

A total of 1618 articles were initially retrieved based on the search strategy described above. After the titles and abstracts were reviewed, 1502 of the articles were excluded because they included non-human experiments or non-gastric-related studies or were non-original articles (e.g., review, letter). After evaluating the remaining articles, 72 records were excluded because they did not provide appropriate data. Eventually, 42 studies were included in the analysis. The flow diagram of the selection of eligible studies is shown in Fig. 1.

Description of eligible literature

Of the 42 studies that were used for the meta-analysis, total CD44 was reported in 21 papers [5, 16, 18,

Fig. 1. Flowchart of the eligible study selection process.

Table 1. Main characteristics of the included studies on total CD44. Abbreviations: NA, not available

Author	Year	Country	Cohort	Tumor stage	Antibody	Cut-off	5 Year OS rate	
							CD44+	CD44-
Chen Jian Hui[41]	2016	China	101	I -III	Novus	5 Score	26.90%	73.60%
Liang Cao[38]	2014	China	203	NA	BD	0	NA	NA
Yue Qiu[16]	2014	China	243	I -IV	Abcam	4 Score	NA	NA
Lu Guoyu[36]	2013	China	145	I -IV	NA	3 Score	15.90%	59.20%
Woon Yong Jung[35]	2013	Korea	430	I -IV	Novocastra	NA	NA	NA
Shi Chen[34]	2013	China	152	I -IV	Abcam	65.00%	29.50%	54.10%
Jian Wei Xie[37]	2013	China	156	I -IV	Abcam	3 Score	NA	NA
Han Suk Ryu[33]	2012	Korea	276	I -IV	BD	5%	NA	NA
Yuta Wakamatsu[5]	2012	Japan	96	I -IV	Novocastra	10%	56.00%	62.10%
Doventas[32]	2012	Turkey	48	I -IV	Zymed	0	NA	NA
T Wang[31]	2011	Singapore	106	I -IV	Bender	5 score	27.70%	66.70%
S.Dhingra[30]	2011	USA	138	I -IV	Novocastra	NA	NA	NA
Jung Yeon Kim[20]	2009	Korea	210	I -IV	Zymed	10%	46.00%	46.90%
Kamran. G[29]	2008	Iran	100	I -IV	Dako	5%	0.00%	31.00%
A.Nosrati[40]	2004	Iran	95	NA	Abcam	5%	NA	NA
Mee Joo[28]	2003	Korea	99	I -IV	Biogenex	10%	NA	NA
Chang Hak Yoo[27]	1999	Korea	261	II -III	Novocastra	5%	43.20%	63.40%
H.Isozaki[26]	1998	Japan	108	I -IV	Dako	10%	NA	NA
Ja Mun Chong[25]	1997	Japan	104	NA	R&D	NA	NA	NA
Ruey Long Hong[24]	1995	China	103	NA	Sigma	0	NA	NA
Barbara Mayer[18]	1993	Germany	61	NA	NA	NA	NA	NA

Table 2. Main characteristics of the included studies on CD44v6. Abbreviations: NA, not available

Author	Year	Country	Cohort	Tumor stage	Antibody	Cut-off	5 Year OS rate	
							CD44+	CD44-
Jian Wei Xie[17]	2015	China	208	I -IV	Abcam	4 Score	38.80%	73.60%
Xueyuan Cao[39]	2014	China	290	I -IV	Thermo	30 Sore	50.50%	58.50%
Jian Wei Xie[37]	2013	China	156	I -IV	Abcam	3 Score	NA	NA
Liang Yi Zhi[42]	2012	China	59	NA	NA	0 Score	NA	NA
Marcello.F[21]	2012	Brazil	137	I -IV	Novocastra	0	46.40%	58.60%
Cristiana.B[43]	2010	Portugal	43	NA	Invitrogen	25%	NA	NA
H.Okayama[19]	2009	Japan	135	I -III	R&D	5%	NA	NA
Donghui Zhou[44]	2007	China	103	I -IV	NA	10%	NA	NA
I Songun[22]	2005	Netherlands	285	NA	Bender	25%	48.00%	28.00%
ZQ Zhao[45]	2005	China	40	I -IV	NA	5%	NA	NA
Wojciech P.P[46]	2004	Poland	19	II -IV	Bender	10%	NA	NA
JQ Chen[47]	2004	China	43	I -IV	Maixin	10%	NA	NA
Mee Joo[28]	2003	Korea	99	I -IV	Biogenex	10%	NA	NA
Yong Li[48]	2003	China	110	I -IV	Santa Cruz	5%	NA	NA
A.Yamaguchi[49]	2002	Japan	201	I -IV	NA	0%	63.30%	33.40%
Yan Xin[50]	2001	Ireland	155	I -IV	R&D	5%	NA	NA
Yan Xin[51]	2000	Ireland	170	NA	NA	NA	NA	NA
Hong Li[52]	2000	China	74	NA	Oncogene	NA	NA	NA
K.Kurozumi[53]	1998	Japan	98	I -III	Bender	30%	NA	NA
H. Saito[54]	1998	Japan	118	I -IV	Bender	5%	41.90%	75.60%
W.Muller[55]	1997	Germany	418	NA	Bender Corp	5%	NA	NA
Hideki Ura[56]	1996	Japan	110	NA	R&D	10%	NA	NA
R.L Hong[24]	1995	China	103	NA	NA	0%	NA	NA
J.Mirecka[57]	1995	Poland	105	NA	Bender	NA	NA	NA

20, 24-41] and CD44v6 expression in 24 studies [17, 19, 21, 22, 24, 28, 37, 42-57]. The main characteristics of these studies are summarized in Table 1 and Table 2. However, in addition the antibody and dilution ratio, it is worth noting that no generalization regarding the cutoff value for defining positive CD44/ CD44v6 expression is presented.

Table 3. Analysis of associations of total CD44 expression with clinical features. P_{bias} , the P-value of Egger's linear regression test for evaluating publication bias

	OR	95% CI	P	I ²	P _{bias}
T category	2.14	1.24-3.68	0.006	82.10%	0.083
N category	1.52	1.09-2.10	0.012	71.30%	0.224
TNM stage	1.98	1.18-3.31	0.010	84.60%	0.071
Distant metastasis	2.78	1.29-5.96	0.009	75.00%	0.079
Lymphatic invasion	1.34	1.01-1.77	0.043	0.07%	0.329
Vascular invasion	1.18	0.78-1.76	0.435	0.01%	0.464
Tumor size	1.02	0.60-1.72	0.951	64.20%	0.998
Histological grade	1.32	0.99-1.76	0.055	54.60%	0.747
5 Year OS rate	3.35	1.83-6.13	<0.001	72.80%	0.255

Fig. 2. Associations of total CD44 expression with clinical features. (A) T category; (B) N category; (C) distant metastasis; (D) lymphatic invasion; (E) TNM stage.

Correlation of total CD44 expression with the clinical features and 5-year OS rate of GC patients

The results of correlations between CD44 and the clinicopathological features of GC are presented in Table 3 and Fig. 2. The overall analysis showed that CD44 expression was statistically correlated with the T category (T_{1+2} vs T_{3+4} , OR = 2.14, 95%CI = 1.24-3.68, P=0.006, Fig. 2A), the N category (N_0 vs N_{1+2+3} , OR = 1.52, 95%CI = 1.09-2.10, P = 0.012, Fig. 2B), distant metastasis (NO vs YES, OR = 2.78, 95%CI = 1.29-5.96, P = 0.009, Fig. 2C),

Table 4. Analysis associations of CD44v6 expression with clinical features. P_{bias} , the P-value of Egger's linear regression test for evaluating publication bias. *, Excluding the study of I Songun et al., the correlation between CD44v6 expression and the 5-Year OS rate

	OR	95% CI	P	I ²	P _{bias}
T category	1.94	1.18-3.18	0.009	79.5%	0.294
N category	2.53	1.77-3.63	<0.001	68.7%	0.001
TNM stage	3.64	1.85-7.16	<0.001	82.1%	0.128
Distant metastasis	2.99	1.25-7.17	0.014	0.0%	0.893
Lymphatic invasion	1.68	1.31-2.16	<0.001	0.0%	0.858
Vascular invasion	1.73	1.32-2.27	<0.001	0.0%	0.805
Tumor size	1.09	0.50-2.39	0.833	66.2%	NA
Histological grade	1.15	0.76-1.75	0.501	64.2%	0.935
5 Year OS rate	1.97	0.83-4.70	0.125	87.7%	0.501
5 Year OS rate*	2.84	1.72-3.63	<0.001	50.3%	0.428

Fig. 3. Association between total CD44 expression and the 5-year OS rate. (A) The plot of sensitivity analysis for evaluating the association between total CD44 expression and the 5-year OS rate. (B) Forrest plot of OR for the correlation between total CD44 expression and the 5-year OS rate.

lymphatic invasion (NO vs YES, OR = 1.34, 95%CI = 1.01-1.77, P = 0.043, Fig. 2D) and TNM stage (I/II vs III/IV, OR = 1.98, 95%CI = 1.11-3.31, P = 0.010, Fig. 2E). Strictly speaking, higher expression of total CD44 indicated a higher T category, N category and TNM stage, as well as greater possibility of distant metastasis and lymphatic invasion. However, no clear correlation was found between total CD44 expression and tumor size (OR = 1.02, 95%CI = 0.60-1.72, P = 0.951), vascular invasion (NO vs YES, OR = 1.18, 95%CI = 0.78-1.76, P = 0.435) or histological grade (OR = 1.324, 95%CI = 0.99-1.76, P = 0.055).

Furthermore, total CD44 expression was highly correlated with a lower 5-year OS rate (OR = 3.35, 95%CI = 1.83-6.13, P < 0.001, Fig. 3A-B). The data indicated that a poorer prognosis for GC patients with CD44⁺ tumors than those with CD44⁻ tumors.

Correlation of CD44v6 expression with the clinical features and 5-year OS rate of GC patients

Table 2 shows the correlations between CD44v6 and the clinical features of GC. The pooled analysis illustrated that CD44v6 expression was significantly associated with the T category (T_{1+2} vs T_{3+4} , OR = 1.94, 95%CI = 1.18-3.18, P = 0.009, Fig. 4A), N category (N_0 vs $N_{1+2+3+4}$, OR = 2.53, 95%CI = 1.77-3.63, P < 0.001, Fig. 4B), distant metastasis (NO vs YES, OR = 2.99, 95%CI = 1.25-7.17, P = 0.002, Fig. 4C), lymphatic invasion (NO vs YES, OR = 1.68, 95%CI = 1.31-2.16, P < 0.001, Fig. 4D), vascular invasion (NO vs YES, OR = 1.73, 95%CI = 1.32-2.27,

Fig. 4. Associations between CD44v6 expression and clinical features. (A) T category; (B) N category; (C) distant metastasis; (D) lymphatic invasion; (E) vascular invasion; (F) TNM stage.

$P < 0.001$, Fig. 4E), and TNM stage (I/II vs III/IV, pooled OR = 3.64, 95%CI = 1.85–7.16, $P < 0.001$, Fig. 4F). In contrast, no associations were observed between CD44v6 expression and tumor size (OR = 1.10, 95%CI = 0.82–1.49, $P = 0.526$) or between CD44v6 expression and histological grade (OR = 0.87, 95%CI = 0.60–1.27, $P = 0.479$), which was consistent with the analysis of total CD44.

The relationship between CD44v6 expression and the 5-year OS rate was evaluated in 6 cohorts. A sensitivity analysis revealed that the study by Songun et al. could alter the results of the analysis. After exclusion of this study, increased CD44v6 expression was found to be significantly associated with a lower 5-year OS rate (OR = 2.84, 95%CI = 1.72–4.68, $P < 0.001$, Fig. 5C) and the heterogeneity was decreased ($I^2 = 50.3\%$) (Fig. 5D). However, when the study by Songun et al. was included, no correlation was observed between CD44v6 expression and the 5-year OS rate (OR = 1.97, 95%CI = 0.83–4.70, $P = 0.125$), and significant heterogeneity ($I^2 = 87.7\%$) was observed (Fig. 5A–B and Table 3).

Fig. 5. Association between CD44v6 expression and the 5-year OS rate. When including I Songun et al. study: (A) sensitivity analysis plot for evaluating the association between CD44v6 expression and the 5-year OS rate; (B) Forrest plot of OR for the correlation between CD44v6 expression and the 5-year OS rate. After excluding the I Songun et al. study: (C) sensitivity analysis plot for evaluating the association between CD44v6 expression and the 5-year OS rate; (D) Forrest plot of OR for the correlation between CD44v6 expression and the 5-year OS rate.

Discussion

Although advances in diagnostic techniques and treatment methods have improved overall outcomes of patients with GC, the 5-year OS rate of these patients remains low [1], which may be a consequence of the occurrence of distant metastases, as evaluated by lymph node metastasis or vascular invasion [2, 3]. Recently, some studies have suggested that CSCs are closely related to tumor progression, metastasis, and processes of treatment resistance in GC [58]. Notably, recent research has demonstrated that CD44-positive tumor cells possess CSC properties, such as self-renewal and tumorigenicity, and the presence of CSCs is typically regarded as a marker of poor prognosis in patients with GC [59].

Mayer et al. and Jian-Hui et al. indicated that higher expression of total CD44 is associated with a greater possibility of distant metastasis and higher TNM stages [18, 41]. CD44v6, one of the major variants of CD44, plays an important biological role in tumor invasion and metastasis. Indeed, Yamaguchi et al. demonstrated that positive CD44v6 expression of cancers is more frequently correlated with liver and hematogenous metastases [49]. Despite numerous studies, the validity of CD44 and CD44v6 as diagnostic or prognostic indicators in GC has not been fully investigated and some findings are controversial. Therefore, we performed a meta-analysis to reveal the relationship of CD44 and its isoform CD44v6 with the prognosis of patients with GC. Importantly, heterogeneity and sensitivity analyses were also performed to ensure the epidemiological credibility of this meta-analysis. Here, we report high expression of total CD44 or CD44v6 to be positively associated with the T

category, the N category, distant metastasis, lymphatic invasion and TNM stage. Moreover, CD44/CD44v6 overexpression predicts a low 5-year OS rate indicating that CD44 or CD44v6 could be used as a marker of poor prognosis in GC patients.

We first conducted a comprehensive meta-analysis to examine the relationship between total CD44 expression and the clinicopathological features of GC. CD44 positivity was found to be associated with certain clinicopathological features, such as the T category, the N category, distant metastasis, lymphatic invasion and TNM stage. We then assessed the relationship between CD44 expression and its prognostic significance in GC. The pooled data indicated that high total CD44 expression significantly predicted a lower 5-year OS rate. The results of our meta-analysis suggest that CD44 overexpression might contribute to the malignant progression of GC, which often leads to a poor prognosis.

We then investigated the pooled association between CD44v6 expression and the clinical features of GC, and the results were similar to those for CD44. However, no correlation was observed between CD44v6 expression and the 5-year OS rate, and the heterogeneity was significant. An additional sensitivity analysis revealed that the study by I Songun was the primary cause of this heterogeneity. After the study by I Songun was eliminated, the 5-year OS rate was significantly lower in GC patients with CD44-positive tumors than in those patients with CD44-negative tumors, and the heterogeneity was decreased.

It was notable that expression CD44 or CD44v6 was determined to have prognostic significance in GC according to our meta-analysis, which suggests that CD44/CD44v6 may be developed as potential prognostic factors for GC.

Several studies have shown that expression of CSC-related factors, including CD133, Lgr5, ALDH1 and Sox2, is associated with the progression of GC [30–32]. Future studies may reveal that co-expression of CSC markers associated with the prognosis of GC patients are valuable for clinical application.

Although we conducted a systematic and comprehensive analysis, certain limitations exist, and some results need to be carefully interpreted. First, we estimated the 5-year OS based on Kaplan–Meier curves derived from some of the original studies. These data might be less reliable than data from a direct analysis from the original paper. Second, despite an adjustment for heterogeneity through the use of random-effect model and sensitivity analyses, a high level of heterogeneity remained in some analyses. Third, a subset of the selected studies had a limited sample size, and it was therefore difficult to draw a definite conclusion on regarding prognostic value of CD44 among GC patients. Fourth, we did not find any significant deviation when assessing publication bias, but it is worth noting that positive results are likely to be published; thus, the association between high CD44 or CD44v6 expression and poor outcome of patients with GC might have surpassed our estimation. Furthermore, different antibodies and different cut-off values for determining positive CD44 or CD44v6 expression were used in the studies, which resulted in inconsistent conclusions.

Conclusion

In summary, although certain limitations exist, the results of the present study showed that high CD44 or CD44v6 expression is positively correlated with progression of gastric cancer. Additionally, CD44 or CD44v6 overexpression was found to be associated with a poorer prognosis of GC patients. Our results suggest that CD44 and CD44v6 may be useful diagnostic or prognostic biomarkers for GC and may be beneficial for novel targeted therapeutic strategies in the future. However, a future larger scale multicenter study may be needed to verify our results.

Disclosure Statement

The authors declare no competing financial interests.

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