Medicine®

OPEN

CD44v6: A metastasis-associated biomarker in patients with gastric cancer?

A comprehensive meta-analysis with heterogeneity analysis

Li Lu, MD, Fei Huang, MS, Zhicheng Zhao, PhD, Chuan Li, PhD, Tong Liu, MD, PhD, Weidong Li, MD, PhD*, Weihua Fu, PhD*

Abstract

Background: The diagnostic and prognostic value of CD44v6 in patients with gastric cancer remains unclear. Therefore, a quantitative meta-analysis was conducted to determine the clinical value of CD44v6 in patients with gastric cancer.

Methods: Sixteen studies with 2177 patients were included. Pooled odds ratios (ORs) and hazard ratio (HR) with 95% confidence intervals (CIs) were calculated to estimate the impact of CD44v6 in patients with gastric cancer on clinicopathological features and 5-year overall survival (OS). Sensitivity analysis, subgroup analysis, and regression analysis were introduced to evaluate the heterogeneity across the studies. Publication bias was also explored among the studies.

Results: The meta-analysis showed that the upregulated CD44v6 was associated with lymph node metastasis (OR 1.91, 95% CI 1.19–3.08; $P = 0.007$), distant metastasis (OR 3.41, 95% CI 2.01–5.78; $P = 0.000$), high TNM stage (OR 2.29, 95% CI 1.10–4.75; $P = 0.026$), lymphatic vessel invasion (OR 1.59, 95% CI 1.21–2.09; $P = 0.001$), and vascular invasion (OR 1.57, 95% CI 1.19–2.07; $P = 0.001$). When excluded 1 study based on sensitivity analysis, pooled HR indicated that CD44v6 positive expression was correlated poor 5-year OS (OR 1.76, 95% CI 1.30–2.39; $P = 0.000$), meanwhile, heterogeneity was eliminated. The heterogeneity of Lauren type mainly existed in the big sample size subgroup. Different region and publication year might contribute to the heterogeneity of differentiation type. While the heterogeneity of lymph node mainly existed in Asian and big sample size group. Publication bias was observed among 12 studies on lymph node metastasis ($P_{\text{publication bias}} = 0.041$), and 5 studies on TNM stage ($P_{\text{publication bias}} = 0.026$).

Conclusion: Taken together, CD44v6 overexpression might be correlated to the characteristics of tumor metastasis in gastric cancer, consisting with many mechanism studies. Therefore, CD44v6 might present a metastasis-associated biomarker in patients with gastric cancer.

Abbreviations: CSC = cancer stem cell, EMT = epithelial-mesenchymal transition, IHC = immunohistochemistry, LI = lymphatic vessel invasion, LN = lymph node metastasis, NA = not available, NOS = Newcastle-Ottawa Scale, OR = odds ratio, OS = overall survival, VI = vascular invasion.

Keywords: CD44v6, gastric cancer, meta-analysis, metastasis, prognosis

1. Introduction

Nowadays, gastric cancer remains the life-threatening complaint in spite of advanced surgical procedures and other methods, such as radiotherapy and chemotherapy, having improved the overall survival (OS) of patients dramatically. According to the statistics, gastric cancer ranks the 5th most common cancer, meanwhile the third leading cause of patients with cancer.[1,2] To further improve the prognosis of patients with gastric cancer, investigations of mechanisms toward gastric carcinogenesis and invasion appear in full swing.[3,4] Hereinto, several genetic biomarkers have been regarded as prognostic factors of gastric cancer and might be helpful to individual treatment.

Accumulating evidence has suggested that cancer is hierarchically organized, with only a rare subpopulation of cancer cells termed cancer stem cells (CSCs).[5] CSC has been identified to play vital roles in the initial, dissemination, and recurrence of numerous solid tumors, including gastric cancer.[4,5] CD44, one of the CSC markers, has attracted increasing interest since about 2 decades ago. Certain CD44 variant isoforms are generated by alternative splicing of at least 10 out of 20 exons (v1–v10).[6,7] Particularly, CD44v6 has been extensively studied in many tumors and its prognostic value has been

reported. Moreover, the monoclonal antibodies of CD44v6 have been identified their target-therapy potential.[8,9] Despite, several clinical studies were carried out to evaluate the relationship of CD44v6 expression with clinicopathology and prognosis of gastric cancer, no consistent finding was available. Hence, a meta-analysis of published data was performed to systematically elucidate whether CD44v6 overexpression would have correlations with the diagnostic and prognostic value in patients with gastric cancer. Particularly, it is also a very important task to investigate the sources of heterogeneity in meta-analysis. Lots of approaches were introduced to explore the sources than other meta-analyses in our study. These methods guaranteed the integrity of our study.

2. Methods

2.1. Literature search

There is no available review protocol. The electronic databases, including PubMed, Embase, and the Cochrane Library, were thoroughly searched for available literature before January 10, 2016, without any limitations of origin and languages. The search terms included "gastric cancer" or "gastric carcinoma" or "gastric tumor" or "gastric neoplasm" "CD44v6" or "CD44 variant 6." Additional relevant search was performed by manually searching the references of eligible studies or relevant reviews.

2.2. Study selection criteria

Studies were considered eligible and selected in this meta-analysis if they fulfilled the following requirements: the study was published in English with the full text available, the study could be either randomized controlled study or observational study (case–control or cohort), the diagnosis of gastric cancer was confirmed using pathological examination, CD44v6 expression was evaluated by immunohistochemistry (IHC) and based on primary gastric cancer tissue (neither serum nor any other kinds of specimen), and the study could provide sufficient information on the OS or clinicopathological indicators of patients related to the CD44v6 expression. Reviews, case reports, letters, or animal studies were excluded. Two observers separately selected the eligible studies, and disagreements were disposed by consensus.

2.3. Data extraction and quality assessment

Two observers carried out the data extraction independently, and disagreements were disposed by a 3rd observer. The following data were extracted from the eligible studies: first author, publication year, country, number of cases, study methods, cut-off value of CD44v6, positive percentage, clinicopathological features, and the related survival data. Hazard ratio (HR) and 95% confidence interval (CI) of 5-year OS from univariate analysis were taken to count pooled HR. Calculation method was applied to extract HR and 95% CI where HR was not reported. Kaplan–Meier curves of those studies were read by Engauge Digitizer (version 4.1, http://digitizer.sourceforge.net/) and the methods introduced by Tierney et al[10] and Parmar et al.[11]

The quality of included studies was assessed by the Newcastle-Ottawa Scale (NOS) criteria, and the study with NOS score of was 6 or higher was defined as a high-quality study, while the study with 5 or less score was considered as low-quality study.

2.4. Statistical analysis

STATA version 12.0 was used to conduct all the statistical calculations. Pooled odds ratios (ORs) and its 95% CIs were

calculated to determine the diagnostic significance of CD44v6 expression by evaluating the association between its expression and clinicopathological features (gender [male vs female], age [≤ 60 vs >60], tumor location [antrum vs nonantrum], Lauren type [intestinal type vs nonintestinal type], differentiation type [poor/ undifferentiated vs well/moderate], depth of invasion [T3/T4 vs T1/ T2], lymph node metastasis [yes vs no], distant metastasis [yes vs no], TNM stage [III/IV vs I/II], lymphatic vessel invasion [yes vs no], and vascular invasion [yes vs no]). And pooled HR with 95% CI was calculated to evaluate the prognostic significance of CD44v6 expression. I^2 test and Q test were used to assess study heterogeneity among the studies. If heterogeneity was significant ($P_{bias} < 0.05$), the random-effect model would be used, while fixed-effect model was applied when there was no significant heterogeneity. Sensitivity analysis was introduced to evaluate the influence of a single study on the overall estimate for preliminarily exploring the sources of heterogeneity. Subgroup analysis and regression analysis were also used to explore the potential sources of heterogeneity. The potential publication bias was assessed by the funnel plots as well as Egger tests. Above all, the effects of CD44v6 expression on pathological features and survival were considered as statistically significant if pooled estimates of OR/HR with 95% CI did not overlap the value of 1. P values were 2-sided and difference was considered as statistically significant when $P < 0.05$.

2.5. Ethical statement

All analyses were based on previous published studies, thus no ethical approval and patient consent are required.

3. Results

3.1. Identification of eligible studies

First of all, a total of 242 potential-related studies were selected from the databases as aforementioned earlier. Endnote, the literature manager software, was utilized to exclude nongastric cancer studies, nonoriginal articles (review and letter), and the duplicated studies (n = 169) through reading titles. The remaining 73 articles were further assessed by screening the full texts, among which 47 articles were excluded due to non-CD44v6-related human studies, non-IHC research, not test in tumor tissues. A total of 26 studies were assessed by reading the full texts, and then 10 studies were excluded due to insufficient information and non-English published articles. Eventually, 16 eligible articles[12–27] with 2177 patients with gastric cancer were included in this meta-analysis. Detailed selection process was shown in a flow chart in Fig. 1.
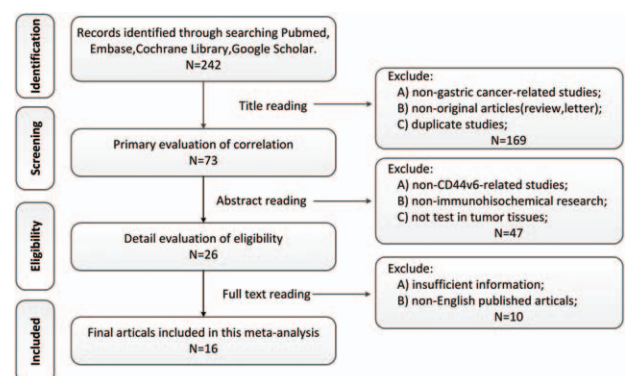

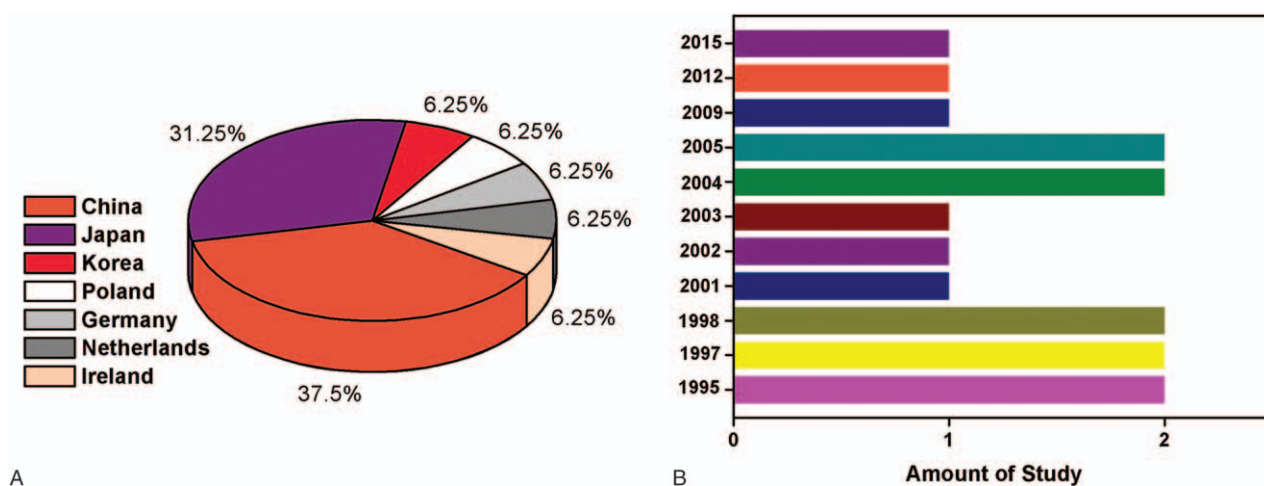**Figure 1.** Flow chart of study selection.

Figure 2. Distribution of studies according to the country (A) and publication year (B).

3.2. Study characteristics and quality assessment

All the eligible studies used the IHC method to evaluate the expression of CD44v6 in gastric tumor tissues. Notably, 12 (75%) involved studies were conducted in Asian countries (6 from China, 5 from Japan, and 1 form Korea), the rest 4 (25%) studies were all conducted in European countries (1 from Poland, 1 from Germany, 1 from the Netherlands, and 1 from Ireland) (Fig. 2A). The publication years of all studies ranged from 1995 to 2015 (Fig. 2B). The sample size ranged from 40 to 418, and the percentage of positive CD44v6 expression varied from 22.5% to 77%. The NOS scores ranged from 7 to 9, which indicated that the quality of all studies was high. Further detailed characteristics were listed in Table 1.

3.3. Association between CD44v6 in gastric cancer and clinicopathological features

To confirm the diagnostic value of CD44v6, the correlation between CD44v6 and numerous clinicopathological features was explored carefully. As seen in Table 2, Figs. 3 to 5, pooled

ORs of 16 eligible studies showed that the upregulated CD44v6 was associated with lymph node metastasis (OR 1.91, 95% CI 1.19–3.08; $P=0.007$), distant metastasis (OR 3.41, 95% CI 2.01–5.78; $P=0.000$), high TNM stage (OR 2.29, 95% CI 1.10–4.75; $P=0.026$), lymphatic vessel invasion (OR 1.59, 95% CI 1.21–2.09; $P=0.001$), and vascular invasion (OR 1.57, 95% CI 1.19–2.07; $P=0.001$). However, no relationship was found between positive CD44v6 and gender (OR 0.95, 95% CI 0.67–1.35; $P=0.784$), age (OR 0.81, 95% CI 0.46–1.44; $P=0.478$), tumor location (OR 1.04, 95% CI 0.67–1.60; $P=0.877$), Lauren type (OR 1.48, 95% CI 0.83–2.63; $P=0.183$), differentiation type (OR 1.09, 95% CI 0.55–2.18; $P=0.802$), and depth of invasion (OR 1.41, 95% CI 0.77–2.59; $P=0.269$).

3.4. Association between CD44v6 in gastric cancer and 5-year OS

Five studies including 913 patients were assessed for the correlation between CD44v6 and 5-year OS. The result (Table 2; Fig. 6) indicated that a positive CD44v6 expression was not

Table 1

Characteristics of included studies.

No. of studies	Author	Year	Country	Cases, n	Method	Antibody	Antibody dilution	Cut-off value	Positive percentage	Quality score
1	Xie[12]	2015	China	208	IHC	NA	NA	4 score	59.1	8
2	Liang[13]	2012	China	59	IHC	VFF-7	1:30	0 score	64.4	9
3	Okayama[14]	2009	Japan	135	IHC	2F10	1:100	5%	62.2	8
4	Liu[15]	2005	China	40	IHC	NA	NA	50%	63.3	7
5	Songun[16]	2005	The Netherlands	284	IHC	VFF-7	NA	1 score	22.5	8
6	Polkowski[17]	2004	Poland	49	IHC	BMS125	NA	10%	73.4	7
7	Chen[18]	2004	China	43	IHC	NA	NA	10%	76.7	9
8	Joo[19]	2003	Korea	99	IHC	2F10	NA	10%	68.7	7
9	Yamaguchi[20]	2002	Japan	201	IHC	mAb 44-2V	NA	NA	47.3	8
10	Xin[21]	2001	Ireland	155	IHC	NA	1:200	5%	40.6	7
11	Kurozumi[22]	1998	Japan	98	IHC	VFF-18	NA	30%	69.4	7
12	Saito[23]	1998	Japan	117	IHC	VFF-7	NA	5%	26.5	8
13	Müller[24]	1997	Germany	418	IHC	VFF-18	1:100	5%	77.0	7
14	Chong[25]	1997	Japan	104	IHC	2F10	NA	Grade 0	41.3	8
15	Hong[26]	1995	China	103	IHC	NA	NA	0%	55.3	8
16	Harn[27]	1995	China	64	IHC	VFF-7	NA	NA	26.6	8

IHC = immunohistochemistry, NA = not available.

Table 2

Main results for meta-analysis between CD44v6 and clinicopathological features/OS and publication bias (Egger test).

| Association between CD44v6 and clinical features/OS	No. of studies	Overall OR/HR (95% CI)	z, $P_{OR/HR}$	Heterogeneity test (I^2, P_{bias})	Publication bias (Egger test) (t, $P_{publication\ bias}$)
Gender (male vs female)	1, 2, 3, 7, 11, 14	0.95 (0.67–1.35)	0.27, 0.784	0.0%, 0.852	0.37, 0.727
Age (≤60 vs >60)	1, 2, 4	0.81 (0.46–1.44)	0.71, 0.478	0.0%, 0.650	−0.16, 0.896
Tumor location (antrum vs nonantrum)	1, 7, 14	1.04 (0.67–1.60)	0.15, 0.877	9.3%, 0.332	1.07, 0.478
Lauren type (intestinal type vs nonintestianl type)	1, 6, 8, 9, 10, 11, 13, 14, 15	1.48 (0.83–2.63)	1.33, 0.183	81.0%, 0.000	0.63, 0.551
Differentiation type (poor/undifferentiated vs well/moderate)	1, 2, 3, 6, 7, 15, 16	1.09 (0.55–2.18)	0.25, 0.802	72.3%, 0.001	0.10, 0.926
Depth of invasion (T3/T4 vs T1/T2)	1, 2, 3, 9, 11, 13, 15	1.41 (0.77–2.59)	1.11, 0.269	78.1%, 0.000	0.69, 0.522
LN metastasis (yes vs no)	1, 2, 3, 4, 7, 8, 10, 11, 12, 13, 14, 15	1.91 (1.19–3.08)	2.68, 0.007	74.0%, 0.000	2.35, 0.041
Distant metastasis (yes vs no)	7, 9, 10, 12, 15	3.41 (2.01–5.78)	4.54, 0.000	0.0%, 0.540	−1.43, 0.248
TNM (III/IV vs I/II)	1, 3, 4, 7, 8	2.29 (1.10–4.75)	2.22, 0.026	62.5%, 0.031	4.09, 0.026
LI (yes vs no)	3, 8, 9, 11, 12, 13, 14, 15	1.59 (1.21–2.09)	3.31, 0.001	0.0%, 0.494	0.45, 0.667
VI (yes vs no)	3, 8, 9, 11, 12, 13, 14	1.57 (1.19–2.07)	3.91, 0.001	0.0%, 0.479	−0.18, 0.865
5-y OS	1, 5, 9, 12, 15	1.41 (0.80–2.49)	1.18, 0.236	82.1%, 0.000	2.54, 0.085

CI = confidence interval, HR = hazard ratio, LI = lymphatic vessel invasion, LN = lymph node metastasis, OR = odds ratio, OS = overall survival, VI = vascular invasion.

predictive of a poor prognosis in patients with gastric cancer (OR 1.41, 95% CI 0.80–2.49; P = 0.263).

3.5. Sensitivity analysis

Sensitivity analysis was conducted to evaluate whether individual studies influenced pooled ORs or HR by excluding 1 study by turns. As shown in Fig. 7A, the study of Songun significantly influenced pooled HR, which contribute to the source of heterogeneity for 5-year OS (I^2 = 82.1%, P_{bias} = 0.000). After omitting Songun's study, pooled HR indicated that CD44v6 positive expression was correlated poor 5-year OS (OR 1.76, 95% CI 1.30–2.39; P = 0.000); meanwhile, heterogeneity was eliminated (I^2 = 0.0%, P_{bias} = 0.548) (Fig. 7B). For other
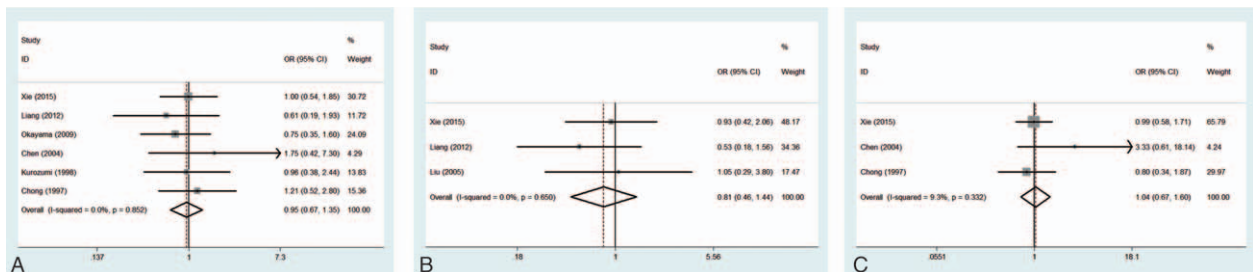
Figure 3. (A) Forest plot of studies evaluating the relationship between CD44v6 expression and gender. (B) Forest plot of studies evaluating the relationship between CD44v6 expression and age. (C) Forest plot of studies evaluating the relationship between CD44v6 expression and tumor location.
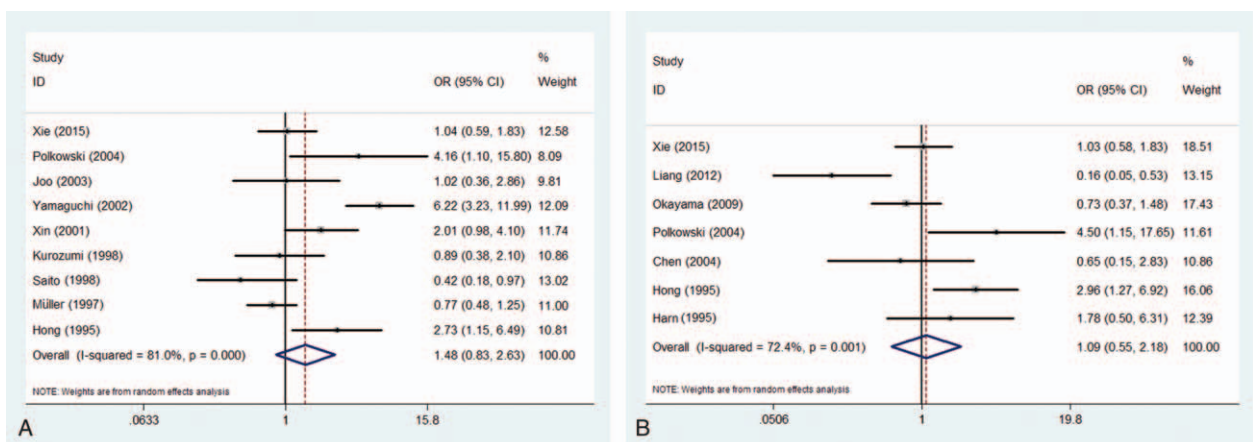
Figure 4. (A) Forest plot of studies evaluating the relationship between CD44v6 expression and Lauren type. (B) Forest plot of studies evaluating the relationship between CD44v6 expression and differentiation type.
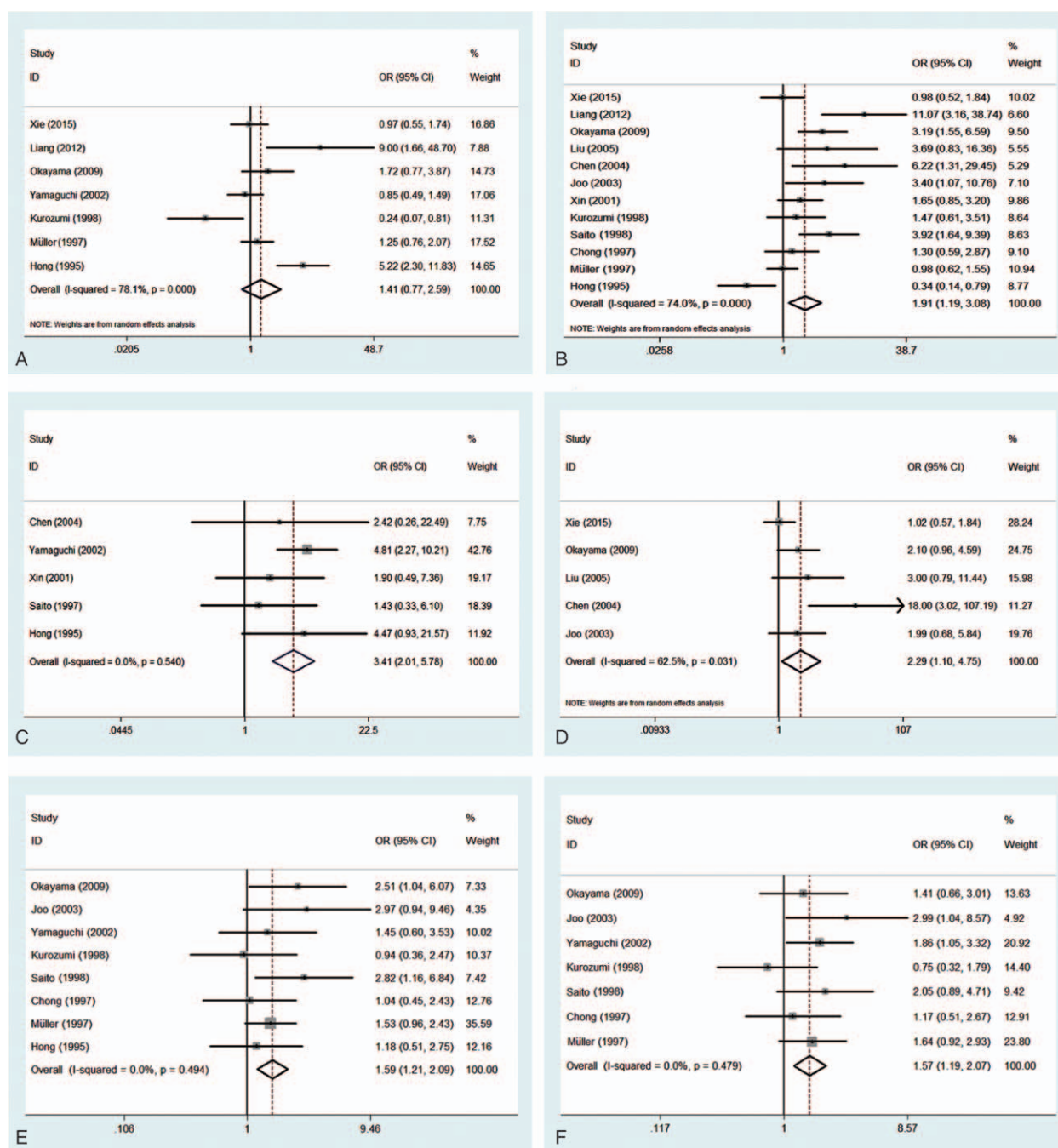
Figure 5. (A) Forest plot of studies evaluating the relationship between CD44v6 expression and depth of invasion. (B) Forest plot of studies evaluating the relationship between CD44v6 expression and lymph node metastasis. (C) Forest plot of studies evaluating the relationship between CD44v6 expression and distant metastasis. (D) Forest plot of studies evaluating the relationship between CD44v6 expression and TNM. (E) Forest plot of studies evaluating the relationship between CD44v6 expression and lymphatic vessel invasion. (F) Forest plot of studies evaluating the relationship between CD44v6 expression and vascular invasion.

meta-analyses, the pooled ORs were similar after excluding each study, which confirmed the stability of meta-analyses.

3.6. Subgroup analysis and regression analysis

Subgroup analysis was mainly performed on region, sample size, and publication year to explore the potential sources of heterogeneity. Due to the limited number of included studies, subgroup analysis on sample size was used to explore the

potential sources of heterogeneity of TNM stage. Regression analysis was also used to explore the potential sources of heterogeneity.

As shown in Table 3, region, sample size, and publication year did not influence the relationship between CD44v6 expression and Lauren type (P_{OR} all ≥ 0.05). However, the heterogeneity of Lauren type mainly existed in the big sample size subgroup (n > 100: $I^2 = 86.9\%$, $P_{bias} = 0.000$), while relatively low heterogeneity in small size group (n $\leq$ 100: $I^2 = 48.4\%$, $P_{bias} = 0.144$).

Figure 6. Forest plot of studies evaluating the relationship between CD44v6 expression and 5-year overall survival.

3.79, 95% CI 1.78–8.05, $P_{OR} = 0.001$; OR 2.85, 95% CI 1.54–5.26, $P_{OR} = 0.001$; respectively). And subgroup analysis also showed that the heterogeneity of lymph node metastasis mainly existed in Asian group and big sample size group ($I^2 = 76.0\%$, $P_{bias} = 0.000$ and $I^2 = 75.5\%$, $P_{bias} = 0.000$, respectively) (Table 3). However, regression analysis did not show similar results (all $P > 0.05$) (Table 4).

When divided by sample size, subgroup analysis indicated that CD44v6 expression was related to high TNM stage in small sample size group, but not in big sample size group (OR 4.00, 95% CI 1.25–12.80, $P_{OR} = 0.020$ and OR 1.40, 95% CI 0.69–2.82, $P_{OR} = 0.349$, respectively) (Table 3). Either subgroup analysis or regression analysis was not able to show the sources of heterogeneity.

3.7. Publication bias

In our meta-analysis, funnel plots as well as Egger tests were introduced to examine potential publication bias. The results showed in Table 2 and Fig. 8 demonstrated that publication bias was observed among 12 studies on lymph node metastasis ($P_{publication\ bias} = 0.041$), and 5 studies on TNM stage ($P_{publication\ bias} = 0.026$), while studies on other clinicopathological features and 5-year OS did not reveal any bias ($P_{publication\ bias}$ all > 0.05).

4. Discussion

The main characteristics of tumors are their ability of invasion and metastasis, which are also the main cause of death for patients with cancer.[28] Therefore, lots of efforts have been taken to avoid such serious events, while the treatments were constantly ineffective. For this reason, several biological biomarkers have drawn researchers' attention for their roles in the progression of cancer. Accurate detection of these biomarkers would affect both short-term and long-term treatment strategies in clinical, which forms crucial part of the personalized medicine.

CD44, a member of cell adhesion molecules,[29] was found to be also a crucial biomarker for recognizing CSCs in the preceding decades. Several studies demonstrated that CD44v6, a CD44 variant, was detectable in all types of adenocarcinoma, while it was rarely expressed on nonepithelial tumors.[8] Notably, some studies suggested a higher prevalence of CD44v6 in metastatic lesions of patients with breast cancer than in their primary

Regression analysis showed region, sample size, or publication year were not the source of heterogeneity of Lauren type (Table 4) (P_{OR} all > 0.05).

Subgroup analysis by region explored that high CD44v6 expression status was related to poor differentiation type in non-Asian group (OR 4.50, 95% CI 1.15–17.65, $P_{OR} = 0.031$), but not in Asian group (OR 0.91, 95% CI 0.45–1.83, $P_{OR} = 0.794$). When divided by publication year, high CD44v6 expression status was also related to poor differentiation type in before 2000 group (OR 2.53, 95% CI 1.25–5.12, $P_{OR} = 0.010$), while not in after 2000 group (Table 3) ($P_{OR} > 0.05$). In addition, there was no heterogeneity in after 2000 group (Table 3) ($I^2 = 0.0\%$, $P_{bias} = 0.512$). As shown in Table 4, regression analysis showed region and publication year might be the sources of heterogeneity; however, region did not have statistical difference ($P = 0.050$ and $P = 0.048$, respectively).

As shown in Table 3, region, sample size, and publication year did not influence the relationship between CD44v6 expression and depth of invasion (P_{OR} all ≥ 0.05). Either subgroup analysis or regression analysis failed to show the sources of heterogeneity.

Upregulated CD44v6 was related to positive lymph node metastasis in Asian group, small sample size group, as well as after 2000 group (OR 2.19, 95% CI 1.19–4.02, $P_{OR} = 0.011$; OR

Figure 7. Sensitivity analysis indicates that 1 study affects heterogeneity and pooled OR/HR. (A) Sensitivity analysis of CD44v6 and 5-year overall survival.[16] (B) Forest plot of studies evaluating the relationship between CD44v6 expression and 5-year overall survival whe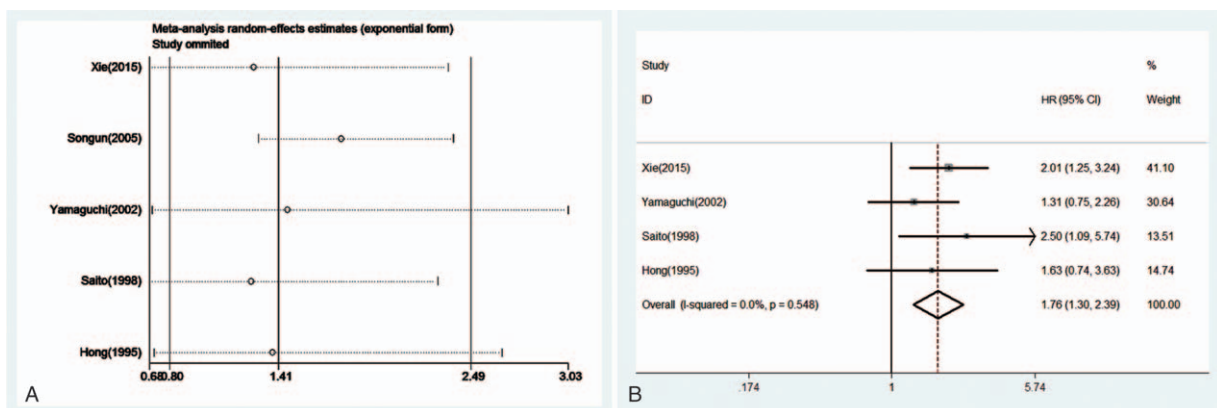n excluded Songun's study (OR 1.76, 95% CI 1.30–2.39; z = 3.63, $P_{OR} = 0.000$; $I^2 = 0.0\%$, $P_{bias} = 0.548$). CI = confidence interval, HR = hazard ratio, OR = odds ratio.

Table 3

Subgroup analysis of Lauren type, differentiation type, depth of invasion, LN metastasis, and TNM.

| Subgroups	Studies	OR (95% CI)	z	P_{OR}	I^2, %	P_{bias}
Lauren type						
Region						
Asian	6	1.39 (0.62, 3.13)	0.81	0.420	84.6	0.000
Non-Asian	3	1.61 (0.64, 4.06)	1.02	0.310	76.5	0.014
Sample size						
n > 100	6	1.50 (0.71, 3.15)	1.06	0.290	86.9	0.000
n ≤ 100	3	1.38 (0.59, 3.23)	0.74	0.458	48.4	0.144
Publication year						
After 2000	5	2.18 (1.00, 4.78)	1.96	0.050	79.4	0.001
Before 2000	4	0.92 (0.47, 1.81)	0.24	0.812	69.5	0.020
Differentiation type						
Region						
Asian	6	0.91 (0.45, 1.83)	0.26	0.794	70.9	0.004
Non-Asian	1	4.50 (1.15, 7.65)	2.16	0.031	—	—
Sample size						
n > 100	3	1.25 (0.61, 2.59)	0.61	0.543	69.2	0.039
n ≤ 100	4	0.94 (0.22, 4.03)	0.08	0.939	79.5	0.002
Publication year						
After 2000	5	0.80 (0.36, 1.79)	0.54	0.590	71.3	0.007
Before 2000	2	2.53 (1.25, 5.12)	0.58	0.010	0.0	0.512
Depth of invasion						
Region						
Asian	6	1.47 (0.67, 3.23)	0.96	0.336	81.7	0.000
Non-Asian	1	1.25 (0.76, 2.07)	0.88	0.377	—	—
Sample size						
n > 100	5	1.47 (0.86, 2.54)	1.40	0.162	72.9	0.005
n ≤ 100	2	1.41 (0.04, 0.11)	0.19	0.850	91.6	0.001
Publication year						
After 2000	4	1.37 (0.72, 2.59)	0.96	0.337	63.0	0.044
Before 2000	3	1.25 (0.31, 5.05)	0.31	0.759	89.1	0.000
LN metastasis						
Region						
Asian	10	2.19 (1.19, 4.02)	2.54	0.011	76.0	0.000
Non-Asian	2	1.20 (0.73, 1.99)	0.72	0.473	38.9	0.201
Sample size						
n > 100	7	1.35 (0.80, 2.28)	1.12	0.264	75.5	0.000
n ≤ 100	5	3.79 (1.78, 8.05)	3.46	0.001	47.1	0.109
Publication year						
After 2000	7	2.85 (1.54, 5.26)	3.34	0.001	65.2	0.009
Before 2000	5	1.18 (0.60, 2.32)	0.48	0.628	75.6	0.003
TNM						
Sample size						
n > 100	2	1.40 (0.69, 2.82)	0.94	0.349	52.2	0.148
n ≤ 100	3	4.00 (1.25, 2.80)	2.33	0.020	53.9	0.114

CI = confidence interval, n = number of sample size, LN = lymph node metastasis, OR = odds ratio.

Table 4

Regression analysis of Lauren type, differentiation type, depth of invasion, LN metastasis, and TNM.

Covariates	Coef.	t	P	95% CI
Lauren type				
Region	0.083	0.12	0.907	−1.647, 1.812
Sample size	−0.128	−0.18	0.861	−1.916, 1.659
Publication year	−0.860	−1.35	0.235	−2.499, 0.777
Differentiation type				
Region	2.608	3.18	0.050	−0.003, 5.219
Sample size	−0.912	−2.19	0.117	−2.361, 0.428
Publication year	1.368	3.23	0.048	0.019, 2.717
Depth of invasion				
Region	−0.018	−0.01	0.994	−6.552, 6.516
Sample size	−0.125	−0.08	0.939	−4.894, 4.644
Publication year	−0.326	−0.22	0.837	−4.958, 4.306
LN metastasis				
Region	−0.141	−0.22	0.828	−1.591, 1.309
Sample size	0.804	1.42	0.193	−0.501, 2.108
Publication year	−0.669	−1.34	0.216	−1.819, 0.481
TNM				
Sample size	0.972	1.32	0.277	−1.364, 3.308

CI = confidence interval, LN = lymph node metastasis.

lesions.[30,31] Wang et al[32] indicated that CD44v6 was positively correlated with the expression levels of β-catenin and tumor growth factor-β on an ovarian cancer cell line, which suggested the expression of CD44v6 was correlated to aggression, invasion, and migration of the ovarian cancer cells. Zhou et al[33] demonstrated that down-regulation of Notch1 decreased the migration and invasion capacities of hepatocellular carcinoma cells by regulating CD44v6, E-cadherin, etc. via the ERK1/2 and COX-2 pathways. Briefly, CD44v6 expression was associated with several factors, which induce epithelial-mesenchymal transition (EMT) in various solid tumors.[34,35] It is generally known that the process of EMT is an essential event in the initial step of the metastatic cascade,[35] such as lymph node metastasis and distant metastasis. In other words, the above mechanism researches hypothesized that overexpressed CD44v6 would correlated to tumor metastasis. However, the relationship between CD44v6 upregulation and metastasis-associated characteristics of gastric cancer remains inconsistent. Herein, a rigorous meta-analysis was performed to clarify the impact of CD44v6 expression on clinicopathological features (especially, metastasis-associated features) and OS in patients with gastric cancer.
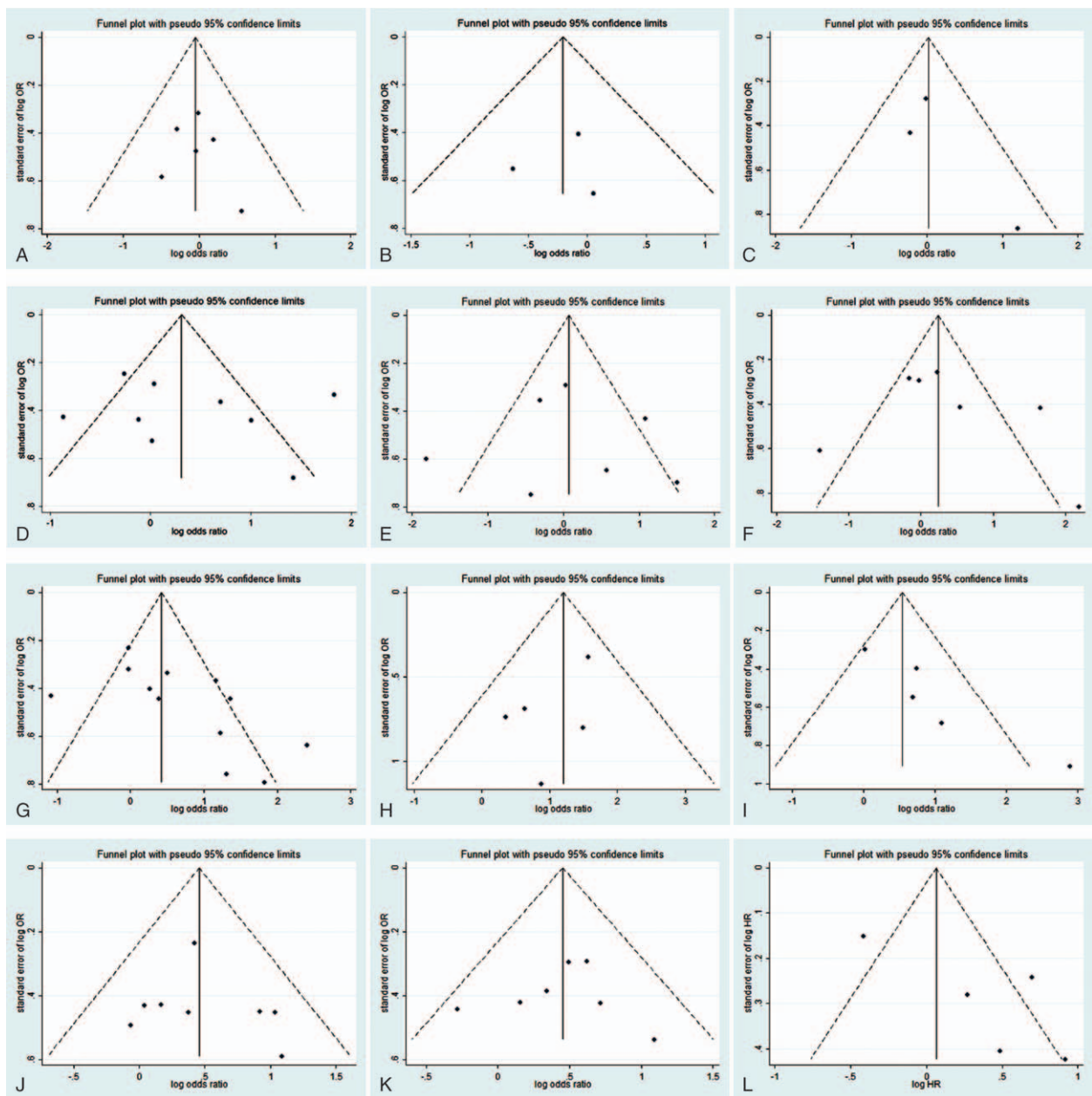
Figure 8. Funnel plot for publication bias test of CD44v6-related studies. (A) Gender; (B) age; (C) tumor location; (D) Lauren type; (E) differentiation type; (F) depth of invasion; (G) lymph node metastasis; (H) distant metastasis; (I) TNM; (J) lymphatic vessel invasion; (K) vascular invasion; and (L) 5-year overall survival.

Sixteen eligible studies were summarized quantitatively based on our inclusion and quality assessment criteria. The meta-analysis indicated that CD44v6 overexpression was correlated to lymph node metastasis (OR 1.91, 95% CI 1.19–3.08; $P = 0.007$), distant metastasis (OR 3.41, 95% CI 2.01–5.78; $P = 0.000$), high TNM stage (OR 2.29, 95% CI 1.10–4.75; $P = 0.026$), lymphatic vessel invasion (OR 1.59, 95% CI 1.21–2.09; $P = 0.001$), and vascular invasion (OR 1.57, 95% CI 1.19–2.07; $P = 0.001$). To sum up, CD44v6 had a significant impact on metastasis-associated features. These results might confirm that CD44v6 presents a metastasis-associated biomarker in patients with gastric cancer, consisting with the above mechanism studies. However, publication bias was observed lymph node metastasis ($P_{\text{publication bias}} = 0.041$) and

TNM stage ($P_{\text{publication bias}} = 0.026$), which might owe to positive outcomes were tended to be reported rather than negative results. The correlation between CD44v6 overexpression and lymph node metastasis as well as the correlation between CD44v6 overexpression and TNM stage both need further scientific data collection and analysis. Whereas, CD44v6 overexpression could not impact on 5-year OS based on 5 related studies. However, there was significant heterogeneity in analysis of CD44v6 and several clinicopathological features, as well as 5-year OS, thus a random-effect model was chosen to determine pooled OR/HR. In additional, sensitivity analysis was conducted to determine the source of heterogeneity, and the result showed that data from Songun significantly influenced pooled HR estimate. After omitting Songun's study,

pooled HR showed CD44v6 positive expression was correlated poor 5-year OS (OR 1.76, 95% CI 1.30–2.39; $P=0.000$); meanwhile, heterogeneity was eliminated ($I^2=0.0\%$, $P_{bias}=0.548$). Diverse ethnic groups might contribute to this result; the Western patients with gastric cancer were included in Songun's study, while other 4 studies were all conducted in the Asian people. As we all know, the well-established risk factors and aggression of gastric cancer differ between eastern and western people.[36] The results in the present meta-analysis are consisting with the hypothesis CD44v6 could present a metastasis-associated biomarker in patients with gastric cancer, as well as a prognostic indicator.

However, sensitivity analysis could not find the other sources of heterogeneity of other clinical features. Therefore, subgroup analysis and regression analysis were combined to explore the sources in the first time on the topic of CD44v6 expression in patients with gastric cancer. The heterogeneity of Lauren type mainly exited in the big sample size subgroup. Regression analysis showed different region and publication year might contribute to the heterogeneity of differentiation type. And subgroup analysis showed that the heterogeneity of lymph node mainly existed in Asian group and big sample size group. Neither subgroup analysis nor regression analysis was able to show the heterogeneity sources of T or TNM stage.

There are several limitations in this study also need to be taken into consideration. Firstly, publication bias was observed on lymph node metastasis and TNM stage, which might influence the positive outcomes in this meta-analysis. Further analysis with more comprehensive literature search and eligible studies is needed in the future. Secondly, in this study, CD44v6 expression in the included studies was measured by IHC method; therefore, different primary antibody or different antibody concentrations could cause inconsistent CD44v6 detection. Thirdly, the varied definition of cut-off values among the studies could also lead to potential bias. However, we were not able to conduct subgroup analysis by diverse antibodies or cut-off values because of small number of studies.

In conclusion, our meta-analysis indicates that CD44v6 overexpression might be correlated to lymph node metastasis, distant metastasis, high TNM stage, lymphatic vessel invasion, vascular invasion, and 5-year OS (when eliminated the heterogeneity). These results consisted with many signal transductions involved in EMT process. Therefore, CD44v6 might present a metastasis-associated biomarker in patients with gastric cancer. However, more well-designed researches with larger samples are needed to further eliminate the existing heterogeneity and publication bias.

References

[1] Torre LA, Bray F, Siegel RL, et al. Global cancer statistics, 2012. CA Cancer J Clin 2015;65:87–108.

[2] Parkin DM, Bray F, Ferlay J, et al. Global cancer statistics, 2002. CA Cancer J Clin 2005;55:74–108.

[3] Wang ZS, Shen Y, Li X, et al. Significance and prognostic value of Gli-1 and Snail/E-cadherin expression in progressive gastric cancer. Tumour Biol 2014;35:1357–63.

[4] Zhao Y, Feng F, Zhou YN. Stem cells in gastric cancer. World J Gastroenterol 2015;21:112–23.

[5] Singh SR. Gastric cancer stem cells: a novel therapeutic target. Cancer Lett 2013;338:110–9.

[6] Screaton GR, Cáceres JF, Mayeda A, et al. Identification and characterization of three members of the human SR family of pre-mRNA splicing factors. EMBO J 1995;14:4336–49.

[7] Wang W, Dong LP, Zhang N, et al. Role of cancer stem cell marker CD44 in gastric cancer: a meta-analysis. Int J Clin Exp Med 2014;7:5059–66.

[8] Heider KH, Kuthan H, Stehle G, et al. CD44v6: a target for antibody-based cancer therapy. Cancer Immunol Immunother 2004;53:567–79.

[9] Chen Y, Huang K, Li X, et al. Generation of a stable anti-human CD44v6 scFv and analysis of its cancer-targeting ability in vitro. Cancer Immunol Immunother 2010;59:933–42.

[10] Tierney JF, Stewart LA, Ghersi D, et al. Practical methods for incorporating summary time-to-event data into meta-analysis. Trials 2007;8:16.

[11] Parmar MK, Torri V, Stewart L. Extracting summary statistics to perform meta-analyses of the published literature for survival endpoints. Stat Med 1998;17:2815–34.

[12] Xie JW, Chen PC, Zheng CH, et al. Evaluation of the prognostic value and functional roles of CD44v6 in gastric cancer. J Cancer Res Clin Oncol 2015;141:1809–17.

[13] Liang YZ, Fang TY, Xu HG, et al. Expression of CD44v6 and Livin in gastric cancer tissue. Chin Med J (Engl) 2012;125:3161–5.

[14] Okayama H, Kumamoto K, Saitou K, et al. CD44v6, MMP-7 and nuclear Cdx2 are significant biomarkers for prediction of lymph node metastasis in primary gastric cancer. Oncol Rep 2009;22:745–55.

[15] Liu YJ, Yan PS, Li J, et al. Expression and significance of CD44s, CD44v6, and nm23 mRNA in human cancer. World J Gastroenterol 2005;11:6601–6.

[16] Songun I, Litvinov SV, van de Velde CJ, et al. Loss of Ep-CAM (CO17-1A) expression predicts survival in patients with gastric cancer. Br J Cancer 2005;92:1767–72.

[17] Polkowski WP, Skomra DG, Mielko J, et al. E-cadherin expression as predictive marker of proximal resection line involvement for advanced carcinoma of the gastric cardia. Eur J Surg Oncol 2004;30:1084–92.

[18] Chen JQ, Zhan WH, He YL, et al. Expression of heparanase gene, CD44v6, MMP-7 and nm23 protein and their relationship with the invasion and metastasis of gastric carcinomas. World J Gastroenterol 2004;10:776–82.

[19] Joo M, Lee HK, Kang YK. Expression of E-cadherin, beta-catenin, CD44s and CD44v6 in gastric adenocarcinoma: relationship with lymph node metastasis. Anticancer Res 2003;23:1581–8.

[20] Yamaguchi A, Goi T, Yu J, et al. Expression of CD44v6 in advanced gastric cancer and its relationship to hematogenous metastasis and long-term prognosis. J Surg Oncol 2002;79:230–5.

[21] Xin Y, Grace A, Gallagher MM, et al. CD44V6 in gastric carcinoma: a marker of tumor progression. Appl Immunohistochem Mol Morphol 2001;9:138–42.

[22] Kurozumi K, Nishida T, Nakao K, et al. Expression of CD44 variant 6 and lymphatic invasion: importance to lymph node metastasis in gastric cancer. World J Surg 1998;22:853–7.

[23] Saito H, Tsujitani S, Katano K, et al. Serum concentration of CD44 variant 6 and its relation to prognosis in patients with gastric carcinoma. Cancer 1998;83:1094–101.

[24] Müller W, Schneiders A, Heider KH, et al. Expression and prognostic value of the CD44 splicing variants v5 and v6 in gastric cancer. J Pathol 1997;183:222–7.

[25] Chong JM, Fukayama M, Hayashi Y, et al. Expression of CD44 variants in gastric carcinoma with or without Epstein-Barr virus. Int J Cancer 1997;74:450–4.

[26] Hong RL, Lee WJ, Shun CT, et al. Expression of CD44 and its clinical implication in diffuse-type and intestinal-type gastric adenocarcinomas. Oncology 1995;52:334–9.

[27] Harn HJ, Ho LI, Chang JY, et al. Differential expression of the human metastasis adhesion molecule CD44V in normal and carcinomatous stomach mucosa of Chinese subjects. Cancer 1995;75:1065–71.

[28] Hu B, Luo W, Hu RT, et al. Meta-analysis of prognostic and clinical significance of CD44v6 in esophageal cancer. Medicine (Baltimore) 2015;94:e1238.

[29] Han S, Guo J, Liu Y, et al. Knock out CD44 in reprogrammed liver cancer cell C3A increases CSCs stemness and promotes differentiation. Oncotarget 2015;6:44452–65.

[30] Sinn HP, Heider KH, Skroch-Angel P, et al. Human mammary carcinomas express homologues of rat metastasis-associated variants of CD44. Breast Cancer Res Treat 1995;36:307–13.

[31] Dall P, Heider KH, Sinn HP, et al. Comparison of immunohistochemistry and RT-PCR for detection of CD44v-expression: a new prognostic factor in human breast cancer. Int J Cancer 1995;60:471–7.

[32] Wang J, Xiao L, Luo CH, et al. CD44v6 promotes β-catenin and TGF-β expression, inducing aggression in ovarian cancer cells. Mol Med Rep 2015;11:3505–10.

[33] Zhou L, Zhang N, Song W, et al. The significance of Notch1 compared with Notch3 in high metastasis and poor overall survival in hepatocellular carcinoma. PLoS ONE 2013;8:e57382.

[34] Chen D, Li W, Liu S, et al. Interleukin-23 promotes the epithelial-mesenchymal transition of oesophageal carcinoma cells via the Wnt/β-catenin pathway. Sci Rep 2015;5:8604.

[35] Ghosh RD, Ghuwalewala S, Das P, et al. MicroRNA profiling of cisplatin-resistant oral squamous cell carcinoma cell lines enriched with cancer-stem-cell-like and epithelial-mesenchymal transition-type features. Sci Rep 2016;6:23932.

[36] Yiming L, Yunshan G, Bo M, et al. CD133 overexpression correlates with clinicopathological features of gastric cancer patients and its impact on survival: a systematic review and meta-analysis. Oncotarget 2015;6:42019–27.