Original Article
Role of cancer stem cell marker CD44 in gastric cancer: a meta-analysis

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Abstract: Cluster of differentiation 44 (CD44), a principal cell surface receptor for hyaluronic acid, has been implicated in tumorigenesis and metastasis. However, the relationship between CD44 expression and the patients with gastric cancer remains controversial. A meta-analysis was performed to quantitatively review the correlation of CD44 expression with the clinicopathological data of the patients with gastric cancer. We conducted a final analysis of the patients from 18 studies. Combined odds ratios (OR) suggested that CD44 expression was related with stage, tumor size, and LN metastasis of gastric cancer, and CD44v6 was related with LN metastasis, lymphatic invasion, and venous invasion. Our results suggested that CD44 and CD44v6 expression could be used to predict the metastasis of gastric cancer.

Keywords: CD44, gastric cancer, metastasis, tumorigenesis, invasion

Introduction

Gastric cancer is one of the leading causes of cancer-related mortality worldwide. A total of 989,600 new stomach cancer cases and 738,000 deaths are estimated to have occurred in 2008 [1]. In China, gastric cancer currently ranks third among the most common cancers, and will remain a significant cancer burden during the next decade [2].

Cluster of differentiation 44 (CD44) is a principal cell surface receptor for hyaluronic acid, a major component of extracellular matrices [3]. The CD44 gene is located on chromosome 11p13 and contains 20 exons, 10 of which are expressed in the standard form (CD44s) [4]. CD44 isoforms, containing variant exon 6 (CD44v6), are generated by alternative splicing of at least 12 exons [5]. CD44 has been reported to play important roles in adherence to the extracellular matrices, motility, matrix degradation, proliferation and cell survival [6, 7]. CD-44v6 also plays an important biological role in the invasion and metastasis of tumor [8]. Previous studies showed that increased expression of CD44 or CD44v6 was found in gastrointestinal tumors and was associated with tumor

invasion, lymph node metastasis and patients' survival [9-12].

The association between CD44 or CD44v6 and the clinicopathological parameters of gastric cancer patients has been studied for many years. A single study may fail to completely demonstrate this complicated relationship because of a small sample size. Therefore, we performed a meta-analysis in an attempt to resolve this issue.

Materials and methods

Search strategy

Science Direct, EMBASE, and PubMed were searched to identify potentially relevant published literature. The following criteria were used to search English language articles and abstracts: 'CD44' and 'gastric carcinoma' or 'gastric cancer' or 'stomach neoplasms'. Existing systematic reviews and reference lists were also checked for any potentially relevant additional studies.

Selection criteria

The studies included in this meta-analysis could be either randomized controlled studies (RCTs)
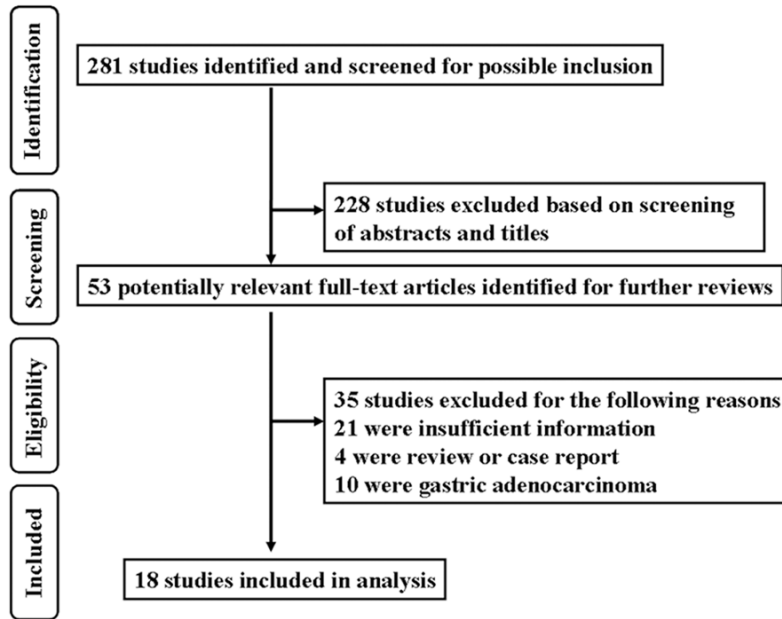
Figure 1. Flow diagram of identifying potential studies in our meta-analysis.

Table 1. Characteristics of studies included in the meta-analysis

First author	Year	Country	Cases	Ages (mean)	Type	Method
da Cunha [12]	2010	Portugal	43	not shown	CD44v6	IHC
Okayama [14]	2013	Japan	135	63.4 y	CD44v6	IHC
Horikawa [15]	2013	Japan	147	66 ± 11 y	CD44	RT-PCR
Chen [16]	2013	China	152	55 y	CD44	IHC
Mayer [17]	1993	Germany	60	not shown	CD44	IHC
Müller [18]	1997	Germany	529	64.9 y	CD44v6	IHC
Wakamatsu [19]	2012	Japan	190	not shown	CD44	IHC
Kurozumi [20]	1998	Japan	572	61 ± 11 y	CD44	IHC
Yamaguchi [21]	2002	Japan	201	not shown	CD44v6	WB
Chen [22]	2013	China	43	58.5 ± 13.4 y	CD44v6	RT-PCR
Liang [23]	2012	China	59	61.8 ± 10.5 y	CD44v6	IHC
Kim [24]	1997	Korea	26	not shown	CD44v6	RT-PCR
Chen [25]	2005	China	31	not shown	CD44v6	IHC
Yoo [26]	1999	Korea	261	56 y	CD44	JHC
Xin [27]	2001	China	155	not shown	CD44v6	IHC
Chong [28]	1997	Japan	104	62.8 y	CD44v6	IHC
Cao [29]	2014	China	203	60.5 y	CD44	IHC
Qiu [30]	2014	China	309	60.4 ± 10.4 y	CD44	IHC

RT-PCR, reverse transcription PCR; IHC, immunohistochemistry; WB, Western blot.

Data extraction

Data were independently extracted from each report by two authors (Wei Wang and Ning Zhang), using a data recording form developed for this purpose. Data tables were made to extract all relevant data from texts, tables and figures of each included studies, including author, year, country, patient number, and detection method. Any discrepancies between the two investigators were resolved by discussion and consultation with a third reviewer (Cheng-Hai Zhao).

Statistical analysis

The statistical process was performed according to the guidelines proposed by the Meta-Analysis of Observational Studies in Epidemiology group [13]. Cochrane Review Manager, version 5.2 (Cochrane Library, Oxford, UK) was used to calculate the available data from each investigation. In addition, if sufficient studies were included, we intended to construct a funnel plot of all studies to investigate the likelihood of publication bias.

Results

Search results

Detailed search steps were described in **Figure 1**. Two hundred and eighty-one articles were identified initially using the search strategy above. After titles and abstracts were previewed, 53 identified studies concerning CD44 and gastric cancer were further evaluated. Thirty-five of residual 53 papers were excluded due to nonhuman experiments, review, or letter to editor. Eventually, 18 eligible studies were included in the present meta-analysis and listed in **Table 1**.

or observational studies (case-control or cohort) that evaluated the association between CD44 expression and gastric cancer. Articles were excluded from the analyses if there was insufficient published data for determining an estimate of RR and a CI, or if the full text couldn't be found. If there were several publications from the same population, only the most recent reports were selected for analysis.
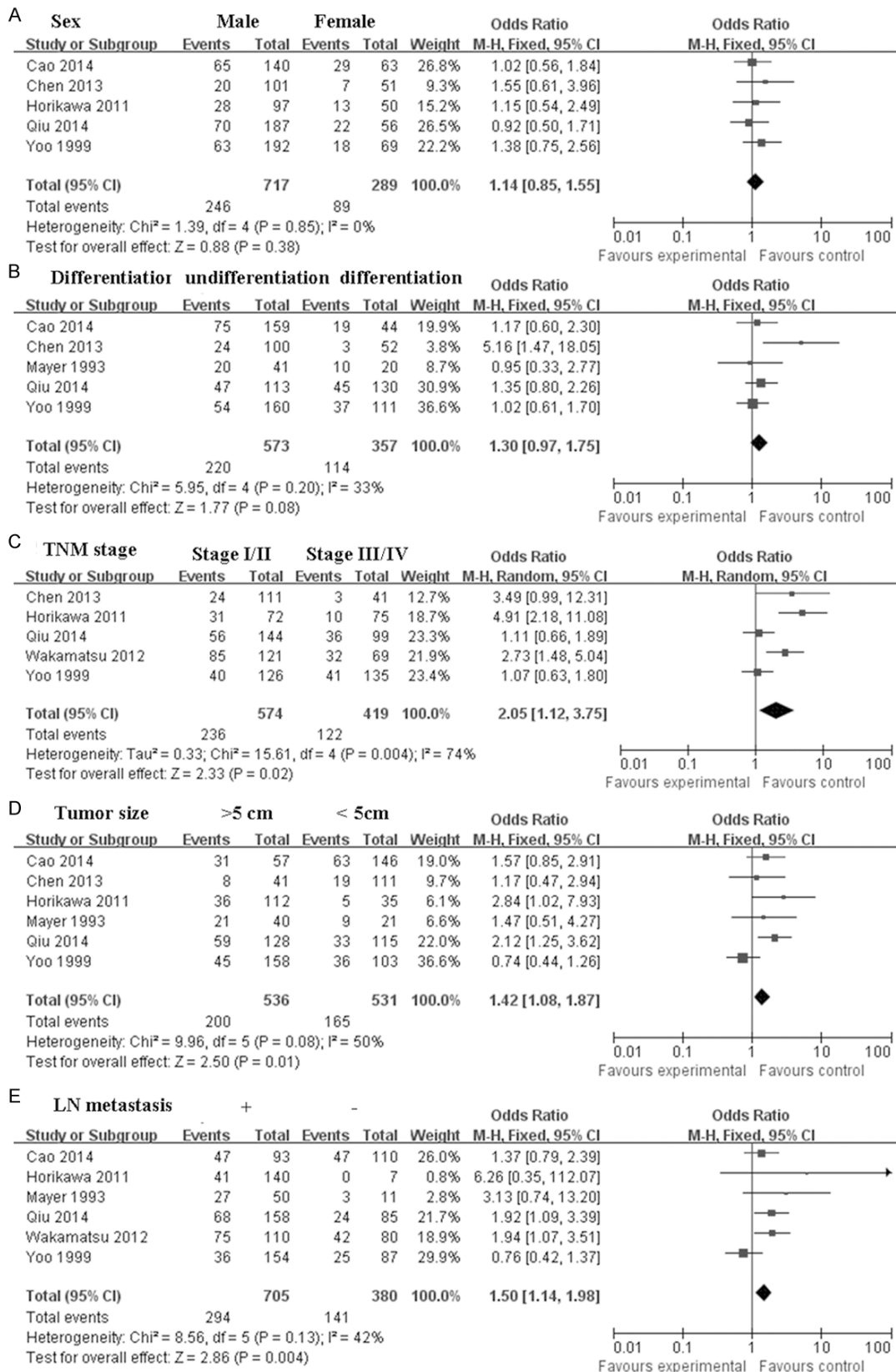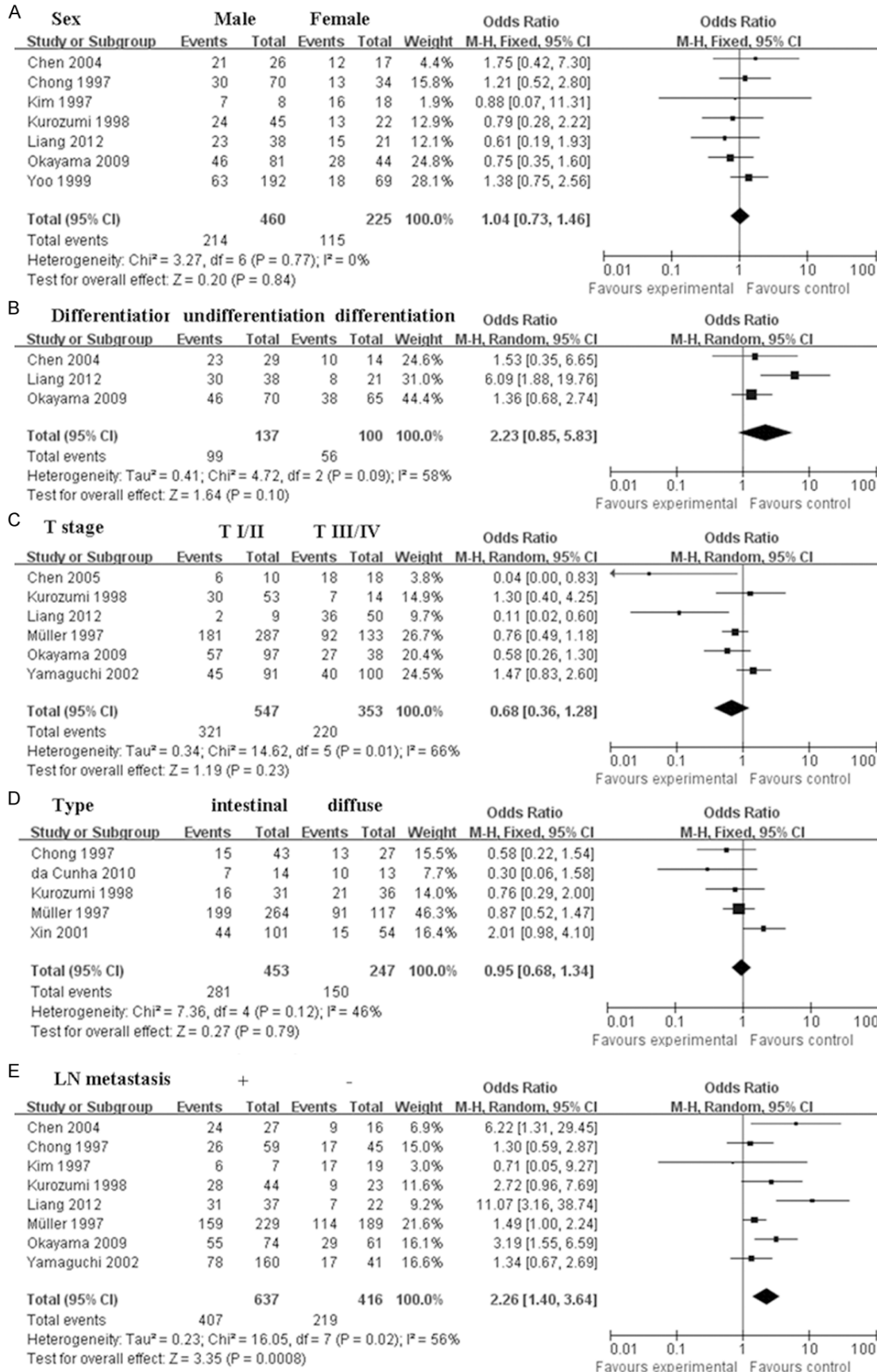
Figure 2. Meta-analysis of CD44 and the clinical characteristics of patients with gastric cancer. A. Sex; B. Differentiation; C. Stage; D. Tumor size; E. LN metastasis.

CD44 and gastric cancer

A

Sex	Male		Female			Odds Ratio	Odds Ratio
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Fixed, 95% CI	M-H, Fixed, 95% CI
Chen 2004	21	26	12	17	4.4%	1.75 [0.42, 7.30]	
Chong 1997	30	70	13	34	15.8%	1.21 [0.52, 2.80]	
Kim 1997	7	8	16	18	1.9%	0.88 [0.07, 11.31]	
Kurozumi 1998	24	45	13	22	12.9%	0.79 [0.28, 2.22]	
Liang 2012	23	38	15	21	12.1%	0.61 [0.19, 1.93]	
Okayama 2009	46	81	28	44	24.8%	0.75 [0.35, 1.60]	
Yoo 1999	63	192	18	69	28.1%	1.38 [0.75, 2.56]	
Total (95% CI)		460		225	100.0%	1.04 [0.73, 1.46]	
Total events	214		115				

Heterogeneity: Chi² = 3.27, df = 6 (P = 0.77); I² = 0%
Test for overall effect: Z = 0.20 (P = 0.84)

0.01 0.1 1 10 100
Favours experimental Favours control

B

Differentiation	undifferentiation		differentiation			Odds Ratio	Odds Ratio
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Random, 95% CI	M-H, Random, 95% CI
Chen 2004	23	29	10	14	24.6%	1.53 [0.35, 6.65]	
Liang 2012	30	38	8	21	31.0%	6.09 [1.88, 19.76]	
Okayama 2009	46	70	38	65	44.4%	1.36 [0.68, 2.74]	
Total (95% CI)		137		100	100.0%	2.23 [0.85, 5.83]	
Total events	99		56				

Heterogeneity: Tau² = 0.41; Chi² = 4.72, df = 2 (P = 0.09); I² = 58%
Test for overall effect: Z = 1.64 (P = 0.10)

0.01 0.1 1 10 100
Favours experimental Favours control

C

T stage	T I/II		T III/IV			Odds Ratio	Odds Ratio
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Random, 95% CI	M-H, Random, 95% CI
Chen 2005	6	10	18	18	3.8%	0.04 [0.00, 0.83]	
Kurozumi 1998	30	53	7	14	14.9%	1.30 [0.40, 4.25]	
Liang 2012	2	9	36	50	9.7%	0.11 [0.02, 0.60]	
Müller 1997	181	287	92	133	26.7%	0.76 [0.49, 1.18]	
Okayama 2009	57	97	27	38	20.4%	0.58 [0.26, 1.30]	
Yamaguchi 2002	45	91	40	100	24.5%	1.47 [0.83, 2.60]	
Total (95% CI)		547		353	100.0%	0.68 [0.36, 1.28]	
Total events	321		220				

Heterogeneity: Tau² = 0.34; Chi² = 14.62, df = 5 (P = 0.01); I² = 66%
Test for overall effect: Z = 1.19 (P = 0.23)

0.01 0.1 1 10 100
Favours experimental Favours control

D

Type	intestinal		diffuse			Odds Ratio	Odds Ratio
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Fixed, 95% CI	M-H, Fixed, 95% CI
Chong 1997	15	43	13	27	15.5%	0.58 [0.22, 1.54]	
da Cunha 2010	7	14	10	13	7.7%	0.30 [0.06, 1.58]	
Kurozumi 1998	16	31	21	36	14.0%	0.76 [0.29, 2.00]	
Müller 1997	199	264	91	117	46.3%	0.87 [0.52, 1.47]	
Xin 2001	44	101	15	54	16.4%	2.01 [0.98, 4.10]	
Total (95% CI)		453		247	100.0%	0.95 [0.68, 1.34]	
Total events	281		150				

Heterogeneity: Chi² = 7.36, df = 4 (P = 0.12); I² = 46%
Test for overall effect: Z = 0.27 (P = 0.79)

0.01 0.1 1 10 100
Favours experimental Favours control

E

LN metastasis	+		-			Odds Ratio	Odds Ratio
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Random, 95% CI	M-H, Random, 95% CI
Chen 2004	24	27	9	16	6.9%	6.22 [1.31, 29.45]	
Chong 1997	26	59	17	45	15.0%	1.30 [0.59, 2.87]	
Kim 1997	6	7	17	19	3.0%	0.71 [0.05, 9.27]	
Kurozumi 1998	28	44	9	23	11.6%	2.72 [0.96, 7.69]	
Liang 2012	31	37	7	22	9.2%	11.07 [3.16, 38.74]	
Müller 1997	159	229	114	189	21.6%	1.49 [1.00, 2.24]	
Okayama 2009	55	74	29	61	16.1%	3.19 [1.55, 6.59]	
Yamaguchi 2002	78	160	17	41	16.6%	1.34 [0.67, 2.69]	
Total (95% CI)		637		416	100.0%	2.26 [1.40, 3.64]	
Total events	407		219				

Heterogeneity: Tau² = 0.23; Chi² = 16.05, df = 7 (P = 0.02); I² = 56%
Test for overall effect: Z = 3.35 (P = 0.0008)
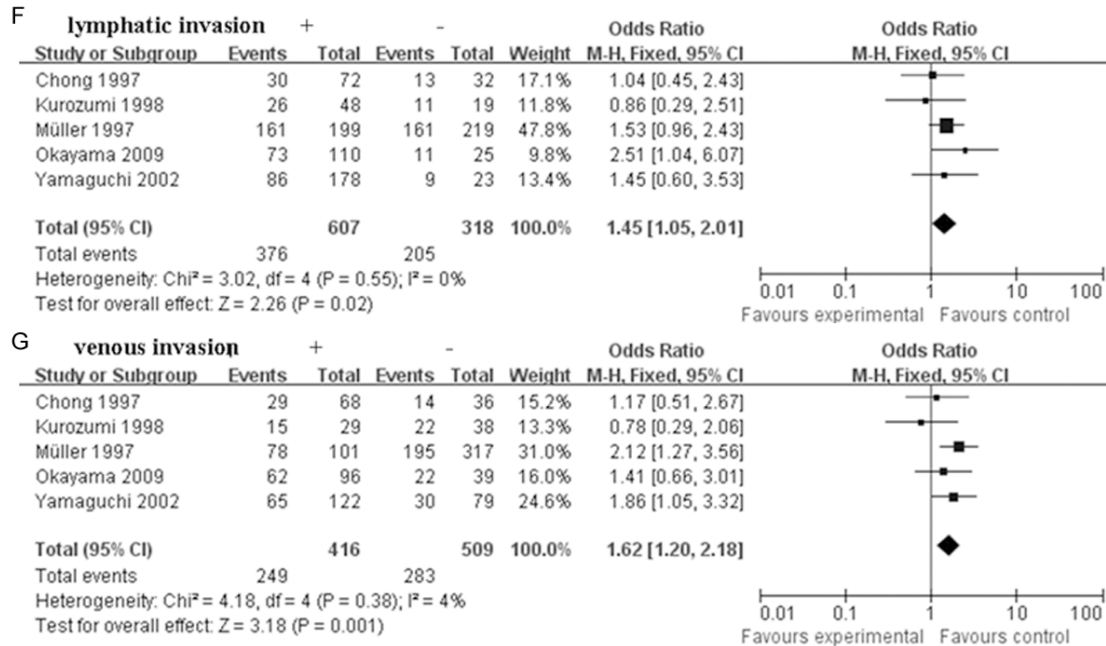
0.01 0.1 1 10 100
Favours experimental Favours control

Figure 3. Meta-analysis of CD44v6 and the clinical characteristics of patients with gastric cancer. A. Sex; B. Differentiation; C. Stage; D. Type; E. LN metastasis; F. Lymphatic invasion; G. Venous invasion.

Association of cancer stem cell marker CD44 with the clinicopathological parameters of the patients with gastric cancer

There was no clear correlation between CD44 expression and sex (pooled OR = 1.14, 95% CI: 0.85-1.55, *P* = 0.38) (**Figure 2A**), and differentiation of gastric cancer (pooled OR = 1.30, 95% CI: 0.97-1.75, *P* = 0.08) (**Figure 2B**). However, CD44 expression was associated with stage (pooled OR = 2.05, 95% CI: 1.12-3.75, *P* = 0.02) (**Figure 2C**), tumor size (pooled OR = 1.42, 95% CI: 1.08-1.87, *P* = 0.01) (**Figure 2D**), and LN metastasis (pooled OR = 1.50, 95% CI: 1.14-1.98, *P* = 0.004) (**Figure 2E**). Furthermore, we found that CD44v6 was related with LN metastasis (pooled OR = 2.26, 95% CI: 1.40-3.64, *P* = 0.0008) (**Figure 3E**), lymphatic invasion (pooled OR = 1.45, 95% CI: 1.05-2.01, *P* = 0.02) (**Figure 3F**), and venous invasion (pooled OR = 1.62, 95% CI: 1.20-2.18, *P* = 0.001) (**Figure 3G**), but not with sex (pooled OR = 1.04, 95% CI: 0.73-1.46, *P* = 0.84) (**Figure 3A**), differentiation of gastric cancer (pooled OR = 2.23, 95% CI: 0.85-5.83, *P* = 0.10) (**Figure 3B**), stage (pooled OR = 0.68, 95% CI: 0.36-1.28, *P* = 0.23) (**Figure 3C**), and tumor type (pooled OR = 0.95, 95% CI: 0.68-1.34, *P* = 0.79) (**Figure 3D**). No obvious publication bias was observed in these studies (**Figure 4**).

Discussion

The role of CD44 expression in gastric cancer has been explored for nearly thirty years. CD44 was identified as a surface glycoprotein and a lymphocyte homing receptor found on lymphoid and epithelial cells in 1982 [31]. Its main function on lymphocytes is mediating interaction with the endothelium [32]. CD44v6, one of the major variants of CD44, could alter the conjugation of CD44s and hyaluronic acid (HA), or enhance the metastasis of tumor by conjugation with HA [23]. Despite there being many studies, the validity of CD44 and CD44v6 as a therapeutic or diagnostic target in gastric cancer has not been fully investigated and some findings are still controversial. In this meta-analysis, we found that CD44 could influence stage, tumor size, and LN metastasis. And CD44v6 was related with LN metastasis, lymphatic invasion, and venous invasion. Günthert et al. [33] demonstrated a significant relationship between CD44v6 expression and lymph node metastasis, lymphatic invasion when they transfected plasmids expressing CD44 or CD44v6 into nonmetastatic rat pancreatic carcinoma cells.

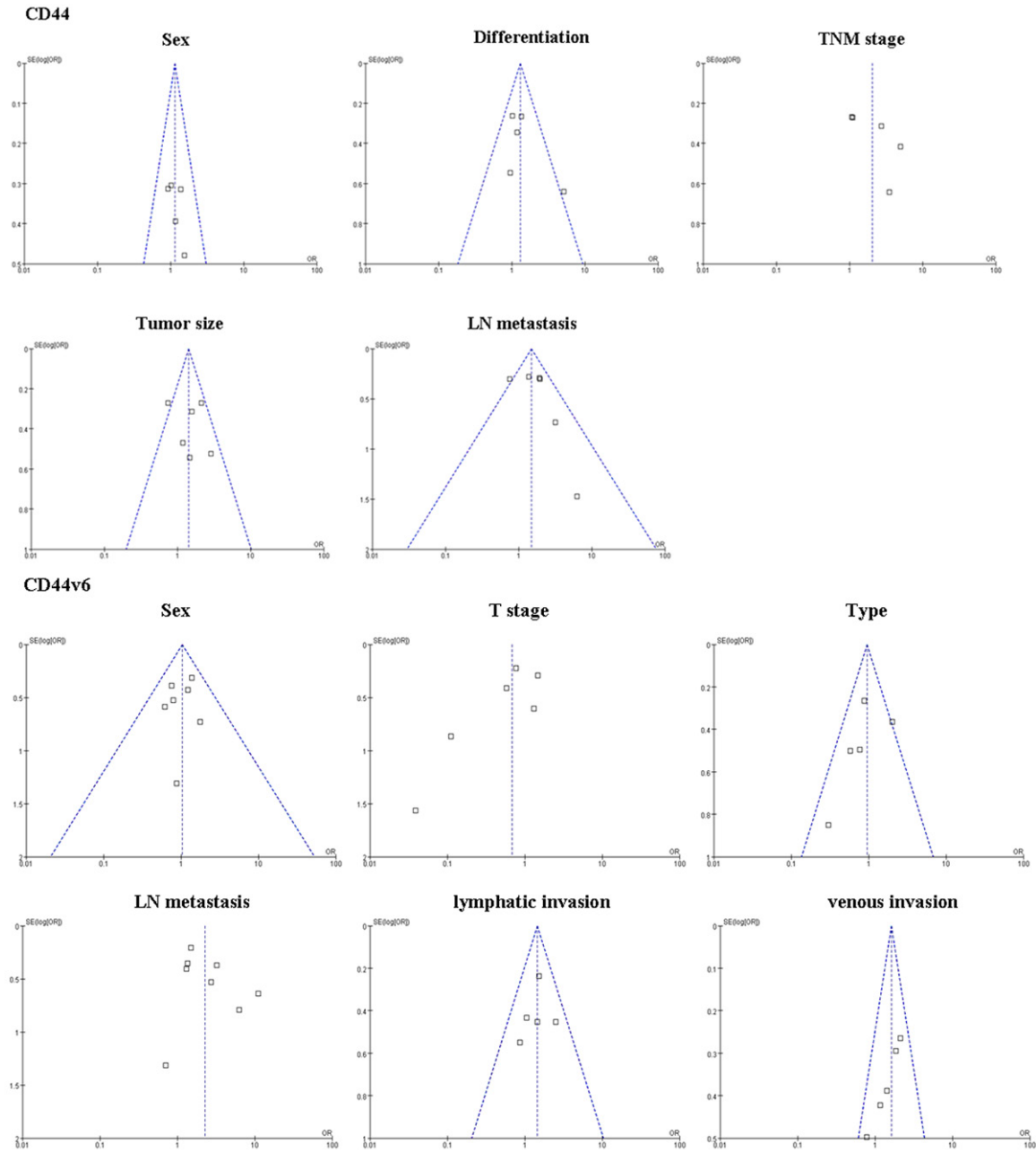Our study provided a more believable result due to a larger size sample, and provides expla-

Figure 4. Funnel plot for publication bias test.

nations for the inconsistencies observed in previous studies. However, some possible limitations of our meta-analysis should be acknowledged and taken into consideration. First, original information was not available in all of the selected studies. Second, the results may be influenced by the lack of observations regarding gene-environment interactions. Third, a meta-analysis is not able to solve problems with confounding factors that could be inherent in the included studies.

In summary, despite the limitations listed above, this present study shows a significant correlation between CD44 expression and stage, tumor size, and LN metastasis of gastric cancer. CD44v6 was related with LN metastasis, lymphatic invasion, and venous invasion. The value of the current meta-analysis compensates for the individual lack of precision of most studies, a problem alleviated by pooling. Further studies are required to evaluate their potential use in predicting patients' outcome.

Disclosure of conflict of interest

None.

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