Arch Gynecol Obstet (2016) 294:1019–1029
DOI 10.1007/s00404-016-4137-3

GYNECOLOGIC ONCOLOGY

The prognostic value and clinicopathological significance of CD44 expression in ovarian cancer: a meta-analysis

Luyang Zhao[1] · Chenglei Gu[1,2] · Ke Huang[1] · Zhe Zhang[1] · Mingxia Ye[1] · Wensheng Fan[1] · Weidong Han[3] · Yuanguang Meng[1]

Received: 24 April 2016 / Accepted: 7 June 2016 / Published online: 14 June 2016

Abstract
Purpose The prognostic value and clinicopathological significance of CD44 in ovarian cancer (OC) remain unclear. This meta-analysis, therefore, aims to evaluate the correlation between CD44 expression and OC.
Methods Studies published until March 2016 were searched in PubMed, EMBASE, and ISI Web of Knowledge databases. The odds ratio (OR) and the hazard ratio (HR) with 95 % confidence interval (CI) were used to assess the effects.
Results Twenty-four studies that include 2267 OC patients were identified for the final analysis. Sixteen studies investigated the expression difference of CD44 standard (CD44s) in 1848 patients. Results showed that high CD44s expression is associated with chemoresistance (OR 5.94, 95 % CI 1.91–18.47) and short disease-free survival (DFS) time (HR 2.57, 95 % CI 1.34–4.91). In addition, CD44s expression is not associated with tumor differentiation grade, residual mass, lymphoid nodal metastasis, and overall survival (OS). Ten studies investigated the expression difference of CD44v6 in 724 patients. Results showed that the CD44v6 expression is not correlated with FIGO stage, tumor differentiation grade, lymphoid nodal metastasis, and OS.
Conclusion High CD44s expression possibly indicates poor prognosis in OC patients given that high CD44s expression initiates chemotherapy resistance, although this expression pattern is not an independent predictive factor for OS. Meanwhile, high CD44s expression may be related to poor DFS of OC, but this relationship must be further confirmed. In addition, the result in which CD44v6 is not associated with OS of OC patients should be interpreted with caution.

Keywords Ovarian cancer · CD44 · Meta-analysis · Prognosis

Introduction

Ovarian cancer (OC) is the most lethal gynecological malignancy and the fifth leading cause of cancer deaths in women [1]. Given the lack of specific symptoms and methods for early screening, more than 70 % of patients were diagnosed with advanced stage and their 5-year survival rate was only 25 % [2]. Several independent prognostic factors, such as disease stage, age, and residual tumor bulk, were identified in the previous investigations, and these factors are useful in choosing the optimal treatment [3, 4]. However, prognoses of patients showing similar condition and receiving similar treatments obviously vary [5]. In addition, the potential factors and the underlying mechanism remain unclear [6]. Continuing

L. Zhao and C. Gu contributed equally to this work.

Electronic supplementary material The online version of this article (doi:10.1007/s00404-016-4137-3) contains supplementary material, which is available to authorized users.

✉ Yuanguang Meng
meng6512@vip.sina.com

[1] Department of Gynecology and Obstetrics, PLA Medical School, Chinese People's Liberation Army General Hospital, Beijing, China

[2] Department of Gynecology and Obstetrics, The 309th Hospital of Chinese People's Liberation Army, Beijing, China

[3] Department of Molecular Biology, Institute of Basic Medicine, PLA Medical School, Chinese People's Liberation Army General Hospital, Beijing, China

exploration for new molecular biological prognostic indicators in OC patients is, therefore, necessary.

The cancer stem cell (CSC) hypothesis has been recently formulated to explain tumor occurrence and recurrence [7]. Increasing evidence has indicated that CSCs possess self-renewal ability and are responsible for tumor progression, metastasis, and therapeutic resistance [8, 9]. CD44, being an important CSC marker, is a highly heterogeneous transmembrane glycoprotein with different isoforms [10]. The CD44 gene is located on the short arm of chromosome 11 and contains 20 exons, 10 of which are non-variable exons and expressed in standard form. Alternative splicing of the remaining 10 exons produces multiple variant isoforms (CD44v1–v10) [11]. The CD44 standard (CD44s) and CD44 variants possibly play essential roles in tumor occurrence, progression, and metastasis [12, 13], and they demonstrate prognostic value in various cancers [14–17].

Until now, the correlation between CD44 expression and OC has been widely studied, but the results are controversial. Several studies have suggested that the high expression of CD44 is related to improved survival rate, whereas some studies have indicated that high CD44 expression is associated with unfavorable prognosis. In addition, some studies did not reveal any relationship between CD44 and outcome of OC. These discrepancies are possibly caused by small sample size and multiple factors. Therefore, to achieve a better understanding of the relationship between CD44 expression and OC, we performed a meta-analysis to further evaluate the prognostic value of CD44 and to analyze its correlation with other clinicopathological factors of OC.

Materials and methods

Literature search

We systematically searched the relevant studies published before March 2016 in PubMed, EMBASE, and ISI Web of Knowledge databases. No restrictions on population or sample size were set in this meta-analysis. Studies were searched using the combinations of the following keywords: "CD44," "ovarian or ovary," "cancer, carcinoma, tumor, or neoplasms." Additional relevant literatures were obtained from the reference lists of the prospective articles.

Inclusion and exclusion criteria

Studies were included in this meta-analysis based on the following criteria: (1) English or Chinese articles; (2) OC was diagnosed through pathological examination; (3) full text is available and data on clinical features and/or prognosis of OC are sufficient; and (4) CD44 expression was detected by immunohistochemistry (IHC) assay. Studies were excluded based on the following criteria: (1) letters, reviews, or case reports without original data and (2) studies using cell lines, ascites cells, and animal models. For studies published by the same authors, only those that include the largest sample sizes or the most recently published were selected to avoid data overlap.

Data extraction

Two investigators (LZ and CG) independently extracted useful data from each study. Discrepancies were resolved through discussion. The following information was extracted from each included study: name of the first author, publication year, country, ethnicity, number of patients, age, tumor pathological type, antibody and dilution, cut-off score, staining pattern, International Federation of Gynecology and Obstetrics (FIGO) stage, tumor differentiation grade, lymphoid nodal metastasis, residual tumor mass, chemotherapy sensitivity, follow-up period, and survival data. The primary endpoint was overall survival (OS). Studies using disease-free survival (DFS) were also analyzed.

Statistical analysis

All analyses were performed using Stata 12.0 (Stata Corporation, College Station, TX, USA). The odds ratio (OR) and 95 % confidence interval (CI) were used to assess the correlation between CD44 expression and clinicopathological parameters, including FIGO stage (I–II versus III–IV), tumor differentiation grade (G1–G2 versus G3), lymphoid nodal metastasis (positive versus negative), residual tumor mass ($\leq$1 cm versus $>$1 cm), and chemotherapy sensitivity (resistant versus sensitive). The hazard ratio (HR) and 95 % CI of OS and DFS were extracted from eligible studies. Some studies did not clearly provide these data, so we estimated these data using the method reported by Tierney et al. [18]. By convention, HR and 95 % CI not overlapped with one would be considered statistically significant. Heterogeneity among studies was assessed by Q test, and its effect was quantified by the I^2 test. When heterogeneity existed ($P < 0.05$ or $I^2 > 50$ %), the random-effects model was used; otherwise, the fixed-effects model was employed. Sensitivity analysis was used to assess the quality and stability of the results. Begg's and Egger's tests were conducted to evaluate potential publication bias, with $P < 0.05$ being considered statistically significant.

In addition, because the cut-off score varied in different studies, we defined the high CD44 expression group in accordance with the original article. Considering that CD44v3, CD44v7–8, CD44v9, and CD44v10 were

estimated in less than three studies and that the parameters were not overlapped, we only analyzed two CD44 isoforms, namely, CD44s and CD44v6.

Result

Characteristics of the included studies

As shown in Fig. 1, a total of 651 citations were identified in the initial search. After removing the duplicates, 288 articles were left. After screening the title and abstract, we screened the full texts of the remaining 59 articles. Twelve investigations were excluded for insufficient information. Eight articles were excluded for abstract only. Six articles were excluded, because they used cell-line or animal or ascites models, and five articles were excluded, because they used immunofluorescence or RT-PCR assays. Four papers were removed as they are case reports or reviews. Finally, 24 studies [19–42] that include 2267 patients with a median of 68 patients (11–483) were included in this meta-analysis (Table 1). The publication year of the

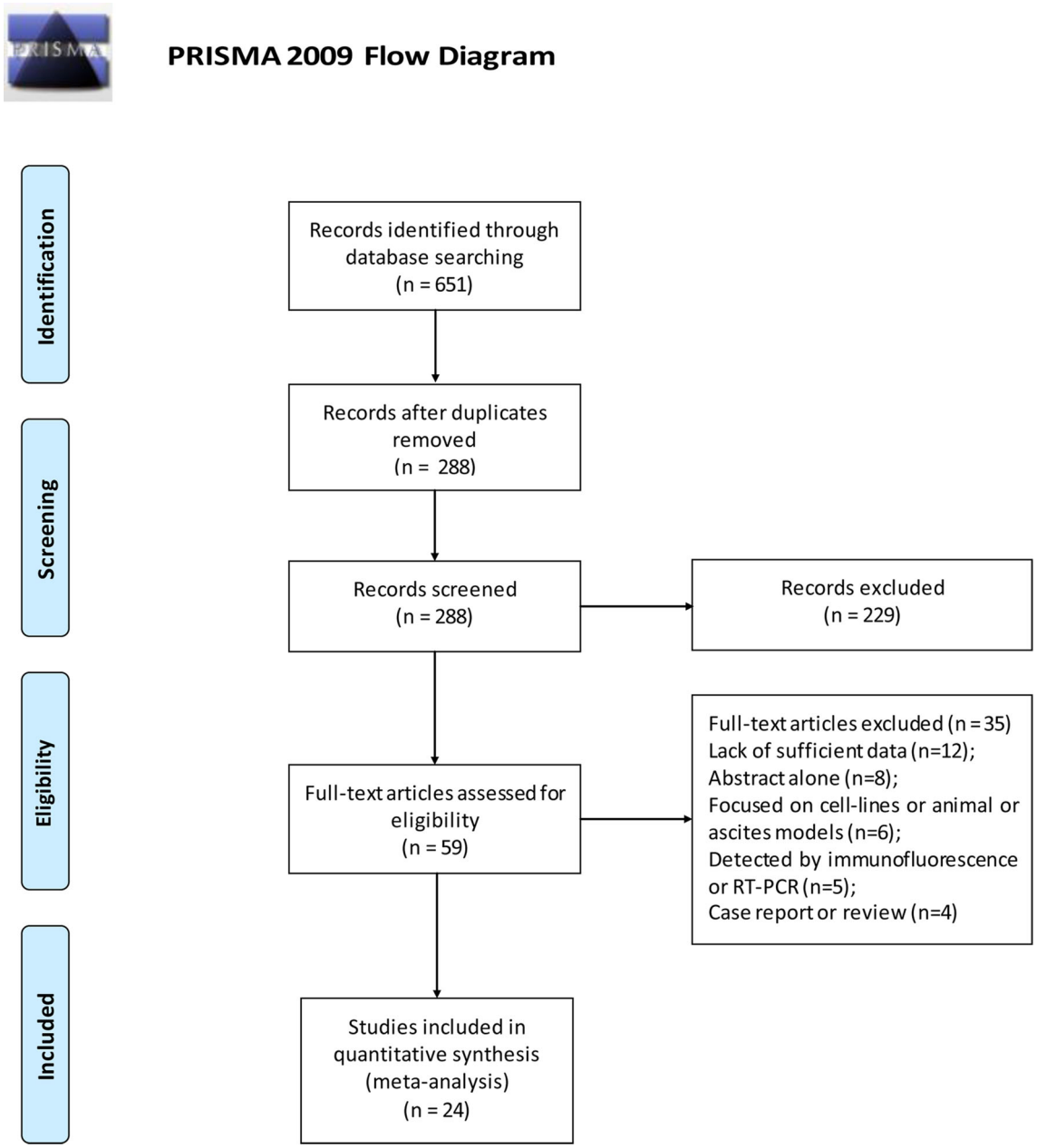

Fig. 1 Flowchart of selection of studies for inclusion in meta-analysis

Table 1 Main characteristics of all eligible studies in the meta-analysis

No.	Author	Year	Country	Patients	Age, years	Pathological type	Antibody	Dilution	Staining pattern	Cut-off score	High/low
1	Yorishima	1997	Japan	11	NA	S, E, C, O	CD44v6	NA	Membrane	0 %	8/3
2	Darai	1998	France	20	53.5	S, M	CD44v6	NA	Membrane	0 %	6/14
3	Kayastha	1999	USA	56	NA	S, M, E, C, D	CD44s	1:50	Membrane	0 %	22/34
4	Saegusa	1999	Japan	106	55.4 (26–83)	S, M, E, C	CD44s	1:100	Membrane	IRS ≥5	43/63
4	Saegusa	1999	Japan	106	55.4 (26–83)	S, M, E, C	CD44v6	1:1000	Membrane	IRS ≥3	35/71
5	Schroder	1999	Germany	50	57 (22–83)	S, M, E, O	CD44v6	1:100	Membrane	IRS ≥2	10/40
6	Afify	2001	USA	28	62 (44–79)	S	CD44s	1:50	Membrane	≥1 %	15/13
7	Ross	2001	USA	64	59.8 (30–85)	S, M, E, C	CD44s	NA	Membrane	0 %	15/49
8	Rodriguez–Rodriguez	2003	Canada	121	44 (24–80)	S, M, E	CD44s	1:1000	Membrane and cytoplasm	0 %	62/59
8	Rodriguez–Rodriguez	2003	Canada	121	44 (24–80)	S, M, E	CD44v6	1:10	Membrane and cytoplasm	0 %	36/85
9	Sillanpaa	2003	Finland	307	NA	S, M, E, C, O	CD44s	1:1200	Membrane	≥10 %	155/152
10	Qu	2004	China	43	NA	S, M, O	CD44s	NA	Membrane and cytoplasm	≥20 %	40/3
11	Zagorianakou	2004	Greece	48	58 (17–85)	S, M, MI	CD44s	1:40	Membrane	≥10 %	24/24
12	Hong	2006	Korea	30	46.5 (22–77)	S	CD44v6	1:50	Membrane	≥10 %	12/18
13	Steffensen	2011	Denmark	117	63.2 (32–79)	S, M, E, C, MI, U	CD44s	1:2000	Cytoplasm	≥20 %	20/97
14	Zhou	2012	China	62	NA	S, M, E, U	CD44v6	1	Membrane	>5 %	41/21
15	Hu	2013	China	92	NA	S, M, E, C	CD44s	1:200	Membrane and cytoplasm	IRS ≥3	62/30
16	Shi	2013	China	90	Median 50	S, M, O	CD44v6	1:500	NA	IRS ≥2	54/36
17	Ryabtseva	2013	Ukraine	72	57 (28–73)	S	CD44s	NA	NA	>10 %	42/30
18	Zhang	2013	USA	483	59 (22–89)	S, E, C, T, O	CD44s	1:50	Membrane	0 %	184/299
19	Wang	2014	China	102	52.1	S	CD44v6	1:100	Membrane	0 %	47/55
20	Wang	2015	China	86	50.3 (29–73)	C	CD44s	1:100	Membrane and cytoplasm	IRS >100	43/43
21	Tjhay	2015	Japan	59	57 (37–82)	S, M, E, C, MI, U	CD44v6	NA	Membrane	>10 %	13/46
22	Zhu	2015	China	92	54.15 (24–78)	S, E, C, U, O	CD44s	1:200	Membrane	IRS >100	44/48
23	Bonneau	2015	France	32	62.4 (±10.1)	S, M, E, U	CD44s	1:50	Membrane	>Mean ratio	24/8
24	Elzarkaa	2016	Germany	96	57 (37–70)	S	CD44s	1:100	Membrane	5 %	47/49

S serous, *M* mucinous, *E* endometrioid, *C* clear cell, *MI* mixed, *U* undifferentiated, *T* transitional cell, *O* others, *IRS* immunohistochemical score, *NA* not available

included studies ranged from 1997 to 2016. These studies were conducted in Japan, France, United States, Germany, Canada, Finland, China, Greece, Korea, Denmark, and Ukraine. The subjects of 11 studies were of Asian descent, whereas the subjects of the remaining 13 studies were non-Asians. All studies used IHC staining to detect CD44 expression.

Sixteen studies with 1848 patients used anti-CD44s antibody, and the median percentage of CD44s positive expression was 50 % (20.6–93.2 %). Among them, eight studies reported FIGO stage; eight studies reported tumor differentiation grade; five, chemotherapy sensitivity; three, residual mass; two, lymphoid nodal metastasis; and two, recurrence. Survival data were reported in 11 studies (OS in nine studies, DFS in two studies, progression-free survival (PFS) in one study, and recurrence-free survival (RFS) in one study).

Ten studies that include 724 patients used anti-CD44v6 antibody, and the median percentage of positive CD44v6 expression was 33 % (0–75.3 %). Among them, seven studies reported FIGO stage, five, tumor differentiation grade, two, lymphoid nodal metastasis, and six, OS to assess the prognostic value of CD44v6.

CD44 expression and clinicopathological parameters of OC

Table 2 shows the correlation results between CD44s and clinicopathological parameters. Overall analyses demonstrated that CD44s expression is associated with chemotherapy sensitivity (OR 5.94, 95 % CI 1.91–18.47, $P = 0.00$, $I^2 = 77.2$ %, Fig. 2) and postoperative recurrence (OR 0.21, 95 % CI 0.13–0.35, $P = 0.00$, $I^2 = 39.0$ %, Supplementary Fig. 1). The association between CD44s expression and chemotherapy sensitivity of OC was also statistically significant in the following subgroups: Asian (OR 8.08, 95 % CI 4.26–15.32, $P = 0.00$, $I^2 = 0.0$ %), membrane and cytoplasm staining (OR 7.61, 95 % CI 3.26–17.74, $P = 0.00$, $I^2 = 88.6$ %), and large sample size (OR 10.64, 95 % CI 6.13–18.47, $P = 0.00$, $I^2 = 0.0$ %). No associations were observed between CD44s expression and FIGO stage (OR 1.05, 95 % CI 0.37–2.94, $P = 0.93$, $I^2 = 90.8$ %, Supplementary Fig. 1), tumor differentiation grade (OR 0.62, 95 % CI 0.23–1.67, $P = 0.35$, $I^2 = 87.4$ %, Supplementary Fig. 1), residual mass (OR 0.58, 95 % CI 0.14–2.53, $P = 0.47$, $I^2 = 83.9$ %, Supplementary Fig. 1), and lymphoid nodal metastasis (OR 2.11, 95 % CI 0.99–4.48, $P = 0.01$, $I^2 = 0.0$ %, Supplementary Fig. 1). In the subgroup analyses, studies in Asian group (OR 0.44, 95 % CI 0.23–0.85, $P = 0.01$, $I^2 = 0.0$ %) and membrane and cytoplasm staining group (OR 0.31, 95 % CI 0.12–0.84, $P = 0.012$) indicated that high CD44s expression is associated with advanced FIGO stage.

Correlation results between CD44v6 and clinicopathological parameters are shown in Table 3. Neither the overall analysis nor the subgroup analysis showed a statistically significant association between CD44v6 expression and FIGO stage (OR 0.84, 95 % CI 0.52–1.36, $P = 0.47$, $I^2 = 48.9$ %, Supplementary Fig. 2), tumor differentiation grade (OR 0.45, 95 % CI 0.16–1.28, $P = 0.14$, $I^2 = 53.1$ %, Supplementary Fig. 2), and lymphoid nodal metastasis (OR 1.50, 95 % CI 0.06–32.79, $P = 0.80$, $I^2 = 72.2$ %, Supplementary Fig. 2).

CD44 expression and prognosis of OC

Table 4 summarizes the main data on survival. The combined HR suggests that CD44s expression does not affect the OS of patients (HR 0.83, 95 % CI 0.46–1.49, $I^2 = 73.0$ %, Supplementary Fig. 3), but it is possibly correlated with short DFS time (HR 2.57, 95 % CI 1.34–4.91, $I^2 = 0.0$ %, Fig. 3). In addition, the overall analysis did not reveal association between CD44v6 expression and OS (HR 1.33, 95 % CI 0.66–2.70, $I^2 = 73.0$ %, Supplementary Fig. 3). When stratified according to geographic area, staining pattern, sample size, and analysis type, statistically significant associations were observed in the Asian group (HR 1.61, 95 % CI 1.07–2.41, $I^2 = 0.0$ %), membrane alone group (HR 1.75, 95 % CI 1.21–2.51, $I^2 = 0.0$ %), and small sample size (<68) group (HR 2.35, 95 % CI 1.42–3.89, $I^2 = 0.0$ %).

Publication bias

To identify potential publication bias, we performed the Begg's and Egger's tests. No evidence of publication bias was observed in the pooled studies (Fig. 4, Supplementary Table).

Sensitivity analysis

In sensitivity analysis, we sequentially removed the eligible studies to assess the influence of each individual study on the pooled ORs or HRs. Most results were not qualitatively changed after omitting any single study at a given time, except the result of OS and CD44v6 expression. After removing the study conducted by Rodriguez–Rodriguez et al. [26], we again pooled the remaining five studies and observed the statistically significant effect of CD44v6 expression on OS of OC patients. In addition, the heterogeneity dramatically decreased from 79.6 to 0 % (Fig. 5). The association between CD44v6 and OS remained stable after excluding the study of Rodriguez–Rodriguez et al. and any other study from the sensitivity analysis.

Table 2 Main results of the pooled data in association of CD44s with clinicopathological parameters of ovarian cancer patients

Groups	FIGO stage (I–II vs. III–IV)			Tumor grade (G1–2 vs. G3)			Chemotherapy (resistant vs. sensitive)			Chemotherapy (resistant vs. sensitive)			Lymphoid nodal metastasis (positive vs. negative)			Recurrence (positive vs. negative)		
	N	OR [95 % CI]	I^2 (%)	*N*	OR [95 % CI]	I^2 (%)	*N*	OR [95 % CI]	I^2 (%)	*N*	OR [95 % CI]	I^2 (%)	*N*	OR [95 % CI]	I^2 (%)	*N*	OR [95 % CI]	I^2 (%)
Overall	8	1.05 [0.37, 2.94]	90.8	8	0.62 [0.23, 1.67]	87.4	5	5.94 [1.91, 18.47]	77.2	3	0.58 [0.14, 2.53]	83.9	2	2.11 [0.99, 4.48]	0.0	2	0.21 [0.13, 0.35]	39.0
Geographic area																		
Asian	2	0.44 [0.23, 0.85]	0.0	2	0.63 [0.18, 2.20]	0.0	3	8.08 [4.26, 15.32]	0.0	1	0.57 [0.25, 1.28]	NA	2	2.11 [0.99, 4.48]	0.0	0	NA	NA
Non-Asian	6	1.42 [0.41, 4.97]	91.4	6	0.55 [0.25, 1.24]	90.6	2	2.74 [0.03, 218.44]	94.3	2	0.57 [0.03, 9.83]	91.8	0	NA	NA	2	0.21 [0.13, 0.35]	39.0
Staining pattern																		
Membrane	6	1.30 [0.36, 4.72]	91.1	5	0.62 [0.16, 2.33]	91.5	3	4.41 [0.54, 18.47]	88.6	3	0.58 [0.14, 2.53]	83.9	1	1.89 [0.77, 4.61]	NA	2	0.21 [0.13, 0.35]	39.0
Cytoplasm	0	NA	NA	1	1.82 [0.63, 5.21]	NA	0	NA	NA	0	NA	NA	0	NA	NA	0	NA	NA
Membrane and cytoplasm	1	0.31 [0.12, 0.84]	NA	1	0.61 [0.05, 7.30]	NA	2	7.61 [3.26, 17.74]	0.0	0	NA	NA	1	2.75 [0.66, 11.44]	NA	0	NA	NA
Sample size																		
<68	3	1.27 [0.19, 8.46]	85.4	2	0.87 [0.28, 2.70]	0.0	1	0.28 [0.05, 1.69]	NA	0	NA	NA	0	NA	NA	0	NA	NA
≥68	5	0.94 [0.24, 3.75]	93.6	6	0.58 [0.23, 1.67]	91.0	4	10.64 [6.13, 18.47]	0.0	3	0.58 [0.14, 2.53]	83.9	2	2.11 [0.99, 4.48]	0.0	2	0.21 [0.13, 0.35]	39.0

N number, *P* *P* value, *NA* not available

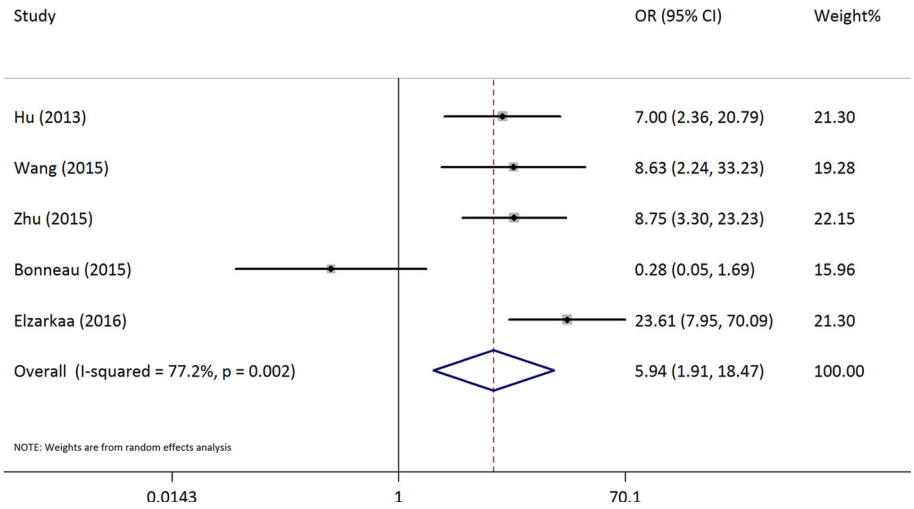

Fig. 2 Forest plot showing the association between CD44s expression and chemotherapy sensitivity of patients with ovarian cancer

Table 3 Main results of the pooled data in correlation with CD44v6 expression and clinicopathological parameters of ovarian cancer patients

Groups	FIGO stage (I–II vs. III–IV)			Tumor differentiation grade (G1–2 vs. G3)			Lymphoid nodal metastasis (positive vs. negative)		
	N	OR [95 % CI]	I^2 (%)	*N*	OR [95 % CI]	I^2 (%)	*N*	OR [95 % CI]	I^2 (%)
Overall	7	0.84 [0.52, 1.36]	48.9	5	0.45 [0.16, 1.28]	53.1	2	1.50 [0.06, 32.79]	72.2
Geographic area									
Asian	5	0.61 [0.23, 1.64]	57.8	3	0.24 [0.11, 0.53]	0.00	2	1.50 [0.06, 32.79]	72.2
Non-Asian	2	1.91 [0.39, 5.96]	0.0	2	1.87 [0.23, 15.04]	54.3	0	NA	NA
Staining pattern									
Membrane	6	1.11 [0.65, 1.90]	23.8	4	0.52 [0.12, 2.34]	64.7	2	1.50 [0.06, 32.79]	72.2
Membrane and cytoplasm	1	0.21 [0.05, 0.82]	NA	0	NA	NA	0	NA	NA
Sample size									
<68	4	0.77 [0.29, 2.02]	27.9	2	0.54 [0.10, 2.83]	33.2	1	11.00 [0.43, 284.31]	NA
≥68	3	0.92 [0.20, 4.11]	72.6	3	0.41 [0.08, 1.99]	71.8	1	0.45 [0.19, 1.03]	NA

N number, *P P* value, *NA* not available

Discussion

CD44, a transmembrane glycoprotein, is primarily recognized as a receptor for hyaluronan (HA), which is involved in cell adhesion, extravasation, and migration [11, 43]. HA is a major component of extracellular matrix, and its binding with CD44 influences the activities of CD44 [44] and induces upregulation of adhesion molecules and thus promotes cell adhesion [45]. In endothelial cells, CD44 expression and its binding force with HA are stimulated by pro-inflammatory cytokines, which can promote the capture of hematopoietic cells and tumor cells [46]. The interactions of the cytoplasmic tail of CD44 with the actin cytoskeleton can be induced by CD44–HA binding; thus, CD44 promotes tumor cell migration [47]. In addition, although the mechanism remains unclear, CD44 and HA are both correlated with drug resistance [10]. Overall, CD44 is a potential prognostic marker for various diseases [48–50].

The clinical significance and prognostic value of CD44s expression in OC remain controversial. The present meta-analysis results, which include 1848 patients, showed that CD44s expression is not associated with tumor differentiation grade, residual mass, lymphoid nodal metastasis, and OS. However, a positive finding was observed in chemotherapy sensitivity analysis (OR 5.94, 95 % CI 1.91–18.47), suggesting that high CD44s expression possibly contributes to a high risk of chemotherapy resistance in OC patients. In accordance with the National Comprehensive Cancer Network guidelines, the chemotherapy sensitive group included patients with clinical remission over 12 months post-chemotherapy, whereas the resistant group achieved clinical remission but showed recurrence during the late stage of chemotherapy or within 12 months.

Table 4 Association between CD44 expression and survival data of ovarian cancer

Variables	*N*	HR [95 % CI]	*P*	I^2 (%)
CD44s				
OS	9	0.83 [0.46, 1.49]	0.53	73.0
Geographic area				
Asian	3	1.43 [0.84, 2.42]	0.19	31.4
Non-Asian	6	0.53 [0.24, 1.21]	0.13	63.8
Staining pattern				
Membrane	7	0.88 [0.61, 1.26]	0.55	27.0
Membrane and cytoplasm	2	0.53 [0.02, 18.11]	0.73	95.1
Sample size				
<68	3	1.05 [0.45, 2.49]	0.91	0.0
≥68	6	0.77 [0.37, 1.62]	0.49	82.4
Analysis type				
Univariate	6	0.89 [0.60, 1.34]	0.58	39.0
Multivariate	3	0.63 [0.05, 8.72]	0.73	90.2
DFS	2	2.57 [1.34, 4.91]	0.00	0.0
CD44v6				
OS	6	1.33 [0.66, 2.70]	0.43	79.6
Geographic area				
Asian	4	1.61 [1.07, 2.41]	0.02	0.0
Non-Asian	2	0.89 [0.12, 6.57]	0.91	93.1
Staining pattern				
Membrane	5	1.75 [1.21, 2.51]	0.03	0.0
Membrane and cytoplasm	1	0.33 [0.18, 0.61]	0.00	NA
Sample size				
<68	3	2.35 [1.42, 3.89]	0.01	0.0
≥68	3	0.81 [0.31, 2.10]	0.66	81.8
Analysis type				
Univariate	4	1.20 [0.42, 3.42]	0.63	84.7
Multivariate	2	1.64 [0.73, 3.70]	0.23	59.2

OS overall survival, *DFS* disease-free survival, *P P* value, *NA* not available

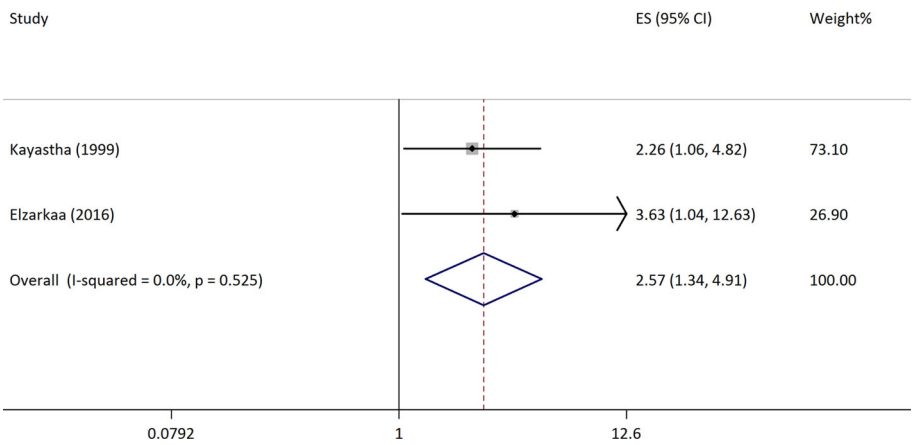

Fig. 3 Forest plot showing the association between CD44s expression and DFS of patients with ovarian cancer

These findings indicate that CD44s may worsen the prognosis by initiating chemotherapy resistance, although CD44s is not an independent predictive factor for OS. The results also suggest that high CD44s expression is a meaningful predictor of poor DFS (HR 2.57, 95 % CI 1.34–4.91). In addition, although no association was

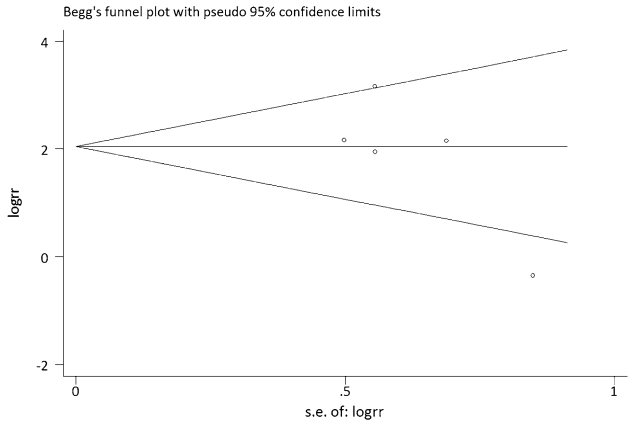

Fig. 4 Funnel plot of the meta-analysis assessing CD44s expression and chemotherapy sensitivity in ovarian cancer

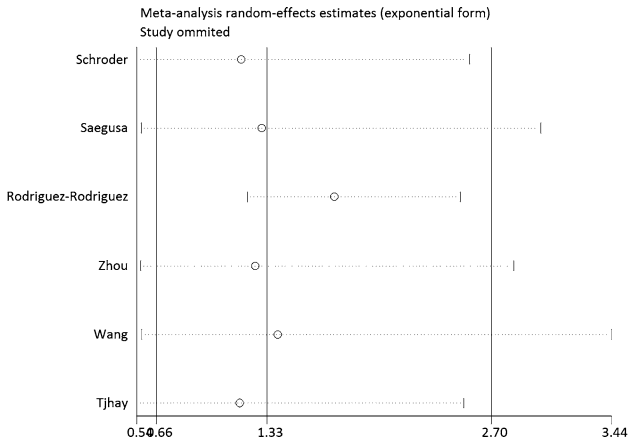

Fig. 5 Sensitivity analysis of the studies assessing CD44v6 expression and OS

observed in the overall analysis, subgroup analyses showed that high CD44s expression is correlated with advanced FIGO stage in Asian subjects (OR 8.08, 95 % CI 4.26–15.32) and membrane and cytoplasm staining pattern (OR 7.61, 95 % CI 3.26–17.74). Considering the limited number of studies, we viewed these results as preliminary and they require validation. Furthermore, OC patients showing high CD44s expression displayed a low risk of recurrence (OR 0.20, 95 % CI 0.10–0.39), inconsistent with the result for chemotherapy sensitivity and DFS. To better interpret this result, we formulated two hypotheses to explain the discrepancy. First, multiple factors, such as FIGO stage and residual mass, could influence the recurrence of cancer, and these potential confounding factors are not counted before analyzed. Second, given that only two published articles with insufficient sample size were included, a small-study bias may have arisen from their results, which may explain the discrepancy.

Similarly, the previous reported relationship between CD44v6 expression and ovarian carcinoma is disputable. The present meta-analysis, which includes 724 patients, did not reveal any association between CD44v6 expression and FIGO stage, tumor stage, lymphoid nodal metastasis, and OS in the overall analyses. However, after excluding the study by Rodriguez–Rodriguez et al. [26], the result of OS was reversed and the heterogeneity was reduced from 79.6 to 0 %, suggesting that this result was not robust and that their study contributes to the heterogeneity. To explore the differences between the study by Rodriguez–Rodriguez et al. and the five other studies, we re-assessed their paper and found several possible reasons to explain why this study caused such heterogeneity First, the study by Rodriguez–Rodriguez et al. included the largest sample size in the analysis of CD44v6 OS and is the only one that has reported that CD44v6 is a good prognostic marker in OC. Second, the IHC methodology may partly explain the inconsistency that has possibly resulted from the differences such as in antibody, cut-off criteria, and staining pattern. Third, no therapy regimens, such as whether the patients received preoperative chemotherapy or not, were described in the manuscript. Given that different therapeutic regimens can modify the effects of CD44 expression [51], it is likely caused the discrepancy. Finally, their study includes a long follow-up time (1–92 months), and their result might have been affected by some losses. Therefore, we cannot draw a definite conclusion regarding the relationships between CD44v6 and OS of OC patients.

To our best knowledge, this meta-analysis is the first to systematically analyze the association between CD44 expression and OC. However, some limitations should be mentioned. First, a relatively large heterogeneity was found in the majority of the analyses. To identify the source of heterogeneity, subgroup analyses according to geographic area of the subjects, staining pattern, sample size, and analysis type were conducted, but none of them could completely explain it. In fact, many other factors, such as age, histological types, cut-off points, antibodies, dilution ratios, surgical interventions, chemotherapy regimens, and study design, may contribute to the heterogeneity. Given that no unified standard exists for some factors and that detailed data are lacking, conducting further analysis of these variables would be difficult. Second, as we mentioned above, the association between OS and CD44v6 expression is unstable. Third, the present meta-analysis did not analyze the relationship between OC and other CD44 variants, such as CD44v3, CD44v5, CD44v7–8, and CD44v9 because of insufficient information. Fourth, several clinicopathological and survival parameters, including distant metastasis, ascites status, microvascular invasion, tumor size, CA125 level, PFS, and RFS were not analyzed because of the lack of overlapped data. Finally, some selection bias may have been caused by the inclusion of studies published only in English and Chinese. Some

potential meaningful data published in other languages were not retrieved. Therefore, future large-scale and well-designed studies are needed to validate our results and to further analyze the associations between different CD44 variants and OC.

In conclusion, this meta-analysis found that high expression of CD44s predicts poor prognosis by initiating chemotherapy resistance of OC, although this expression pattern is not an independent predictive factor for OS. Moreover, high CD44s expression is possibly related to poor DFS in OC patients, although this conclusion requires confirmation. The result that CD44v6 expression is possibly not associated with OS in OC should be interpreted with caution. Further studies must validate and confirm these associations to achieve a more comprehensive understanding of their possible roles in OC.

Acknowledgments This study was funded by the National Natural Science Foundation of China (Grant Number: 81571411).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants performed by any of the authors.

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