Prognostic role of the cancer stem cell marker CD44 in ovarian cancer: a meta-analysis

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Genet. Mol. Res. 15 (3): gmr.15038325
Received December 22, 2015
Accepted February 16, 2016
Published August 12, 2016
DOI http://dx.doi.org/10.4238/gmr.15038325

ABSTRACT. This meta-analysis study aimed to investigate the correlation between CD44-positive cancer stem cells (CSCs) and clinicopathological features and its effect on the survival of ovarian cancer patients. A comprehensive literature search in the electronic databases, including PubMed, EMBASE, and Wanfang (up to December 1, 2015), was conducted. Publications assessing the clinical or prognostic significance of CD44 expression in ovarian cancer were identified and reviewed until December 1, 2015. A meta-analysis was then performed to examine the association between CD44 expression and clinical outcomes of ovarian cancer. A total of 8 publications comprising 957 cases satisfied the criteria and were included for this meta-analysis. Our results show that CD44 expression was not significantly associated with the tumor grade (OR = 2.31, 95%CI =

0.61-8.73, P = 0.22), age of the patients (OR = 0.89, 95%CI = 0.32-2.53, P = 0.83), residual tumor size (OR = 1.01, 95%CI = 0.30-3.40, P = 0.99), or response to chemotherapy (OR = 3.49, 95%CI = 0.51-23.65, P = 0.20). However, our meta-analyses of the data from the identified studies demonstrate that CD44 expression was significantly correlated with tumor lymphatic metastasis (OR = 2.66, 95%CI = 1.36-5.22, P = 0.004), tumor TNM stage (OR = 2.34, 95%CI = 1.76-3.12, P < 0.00001), and decreased overall survival for ovarian cancer patients (RR = 1.47, 95%CI = 1.23-1.74, P < 0.0001). In conclusion, our findings show that CD44-positive ovarian cancer patients exhibit worse prognosis, which was associated with common clinicopathological features and poor prognostic factors.

Key words: Ovarian cancer; Cancer stem cells; CD44; Outcome; Meta-analysis

INTRODUCTION

Ovarian cancer usually occurs in women over age 50 and is the leading cause of death from gynecological malignancy worldwide (Holschneider and Berek, 2000). The frequent recurrence and quick metastasis of ovarian cancer throughout the peritoneal cavity result in extremely low 5-year survival for patients with ovarian cancer (Jin et al., 2010). Thus, it is necessary to further explore the biology of this disease to improve the treatment efficacy of current therapeutic approaches and to develop novel and more effective therapies against ovarian cancer. Intriguingly, cancer stem cells (CSCs), a subset of cells within cancer tissues, have been implicated in the initiation, progression, metastasis, and recurrence of human cancer. CSCs exhibit the distinct capacity of self-renewal and can generate all the heterogeneous lineages of cancer cells that constitute the bulk of the tumor mass (Clevers, 2011). Therefore, it is very important to investigate the role of ovarian cancer CSCs in cancer progression to improve the clinical outcomes of this highly aggressive cancer.

CD44 is a receptor for hyaluronic acid (HA) and the binding of HA to CD44 leads to the activation of a variety of biological processes, including tumor progression, metastasis and proliferation (Savani et al., 2001). Importantly, it has been reported that CD44 plays a critical role in multiple steps that regulate cell migration (Nagano and Saya, 2004). Once activated, the cytoplasmic tail of CD44 interacts with the actin cytoskeleton, resulting in the translocation of CD44 to the leading edge of migrating cells (Ponta et al., 2003). These findings highlight the important role of CD44 in regulating cancer progression and metastasis. Interestingly, in recent years, it has been found that CD44 can be reliably used as a marker to identify CSCs and serve as a prognostic factor for a variety of human cancers (Ricardo et al., 2011; Wakamatsu et al., 2012; de Beça et al., 2013). However, the correlations between CD44 and the clinicopothological features and prognosis of ovarian cancer are still not clear (Sillanpää et al., 2003; Chen et al., 2009a; Gao et al., 2015; Wang et al., 2015). In this study, we performed a systematic review of the published literature and conducted a meta-analysis of the included studies to examine the association of CD44 expression with the clinicopathological features and the prognosis of ovarian cancer patients. Our findings may provide novel insights into the potential cellular origin of ovarian cancer and offer important guidelines for the prognostic stratification of ovarian cancer patients that need adjuvant therapy.

MATERIAL AND METHODS

Literature search

A comprehensive literature search (up to December 1, 2015) of electronic database, including PubMed, EMBASE, and Wanfang databases, for the abstracts about studies on human subjects was conducted to identify articles on the prognostic role of CD44 expression in ovarian cancer. The key words that were used for the literature search are: "CD44" as well as "ovarian neoplasms" or "ovary neoplasms" or "ovarian cancer" or "cancer of ovary". All relevant studies were retrieved and, in the meantime, we also checked the bibliographies of those articles for other related reports to identify additional eligible studies.

Two of the authors extracted data from all eligible studies independently based on the following inclusion criteria: 1) articles that reported the expression of CD44 and either prognostic factors or overall survival (OS) of ovarian cancer; 2) studies that contain sufficient data to allow for the estimation of the odds ratio (OR) or relative risk (RR) of OS; 3) articles that were reported in English or Chinese; and 4) studies published as original research. Reviews, comments, duplicated studies, and articles unrelated to our analysis were excluded. Disagreements in data extraction between the two authors were resolved by discussion.

The key information that we extracted from the included papers are listed as follows: author, publication year, patient's country, number of patients, tumor stage, research protocols, and cutoff scores for the definition of positive staining or staining intensity. Two major groups of data were collected based on the purpose of our meta-analysis. One group of data was used to evaluate the association between the expression of CD44 and clinicopathological parameters, including tumor grade, age of the patients, residual tumor size, or response to chemotherapy, tumor lymphatic metastasis and TNM stage. We analyzed another group of data to investigate the association between the expression of CD44 and OS.

Statistical analysis

The meta-analysis was conducted as previously described. ORs with 95% confidence intervals (CI) were calculated to evaluate the association between the stem cell marker (CD44) and clinicopathological features of ovarian cancer, including tumor grade, age of the patients, residual tumor size, or response to chemotherapy, tumor lymphatic metastasis and TNM stage. The RR was employed to assess the correlation of CSC marker CD44 and OS. When RRs were not directly described in certain studies, we then extracted the original data from the articles and calculated RRs based on the methods described by Parmar et al. (1998). *Q* test and P values were used to estimate heterogeneity across studies. ORs and RRs were calculated using a random-effect model when the P values are less than 0.05. Otherwise, a fixed-effect model was used. The Begg and Egger funnel plots were generated to assess publication bias. All statistical analyses were performed using the Review manager software. P values were two-sided and results are considered significant when P values are less than 0.05.

RESULTS

Characteristics of included studies

After the literature search of electronic databases, including the PubMed, EMBASE, and Wanfang, we initially identified a total of 197 articles that are relevant for this meta-analysis. One hundred and eight-two articles were then excluded after reviewing the titles and abstracts and 7 studies were further excluded after examining the full-text of the articles (Figure 1). We excluded those articles from our meta-analysis for the following reasons: a) Non-association studies were performed, b) Researchers in those articles did not carry out histopathologic analysis or intensive clinical and imaging follow-up for at least 6 months, c) There were not association studies for other kinds of diseases d), They were review articles and no original data were reported, e) Data could not be extracted, and/or f) The data in certain articles were repeated from the same or similar population. After extensively reviewing the literature, we eventually identified a total of 8 studies with 957 patients, who met the inclusion criteria for our meta-analysis (Sillanpää et al., 2003; Chen et al., 2009a; Jiang and Chen, 2010; Steffensen et al., 2011; Li et al., 2012; Gao et al., 2015; Wang et al., 2015; Zhu et al., 2015) . Five of these studies reported the correlation of CD44 expression with the OS for ovarian cancer patients, which was assessed using the Kaplan-Meier method. Three of the included studies examined the association between CD44 and the clinicopathological factors without survival analysis. The characteristics of the included studies for this meta-analysis were summarized in Table 1.

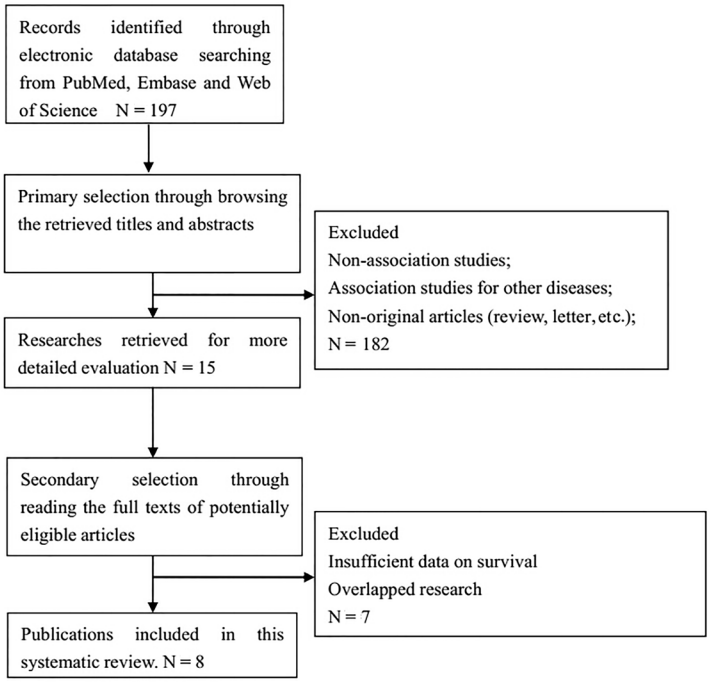

Figure 1. Flow diagram of article selection.

Table 1. Main characteristics and results of the eligible studies.

Study	Patient's country	Ethnicity	Year	Time of collection	Pathological stage	Method	Number of patients	Age in years	Follow-up months	Cutoff for CD44 positive	Survival analysis
Sillanpää et al.	Finland	Caucasian	2003	1976-1992	I-IV	IHC	445	ND	237	>10% staining	OS
Chen et al.	China	Asian	2009a	2001-2007	I-IV	IHC	120	40-70	56	>25% staining	ND
Jiang and Chen	China	Asian	2010	2007-2008	I-IV	IHC	33	33-74	ND	>50% staining	ND
Steffensen et al.	Denmark	Caucasian	2011	ND	I-IV	IHC	109	32-79	40	>20% staining	OS
Li et al.	China	Asian	2012	2007-2008	I-IV	IHC	46	ND	ND	>10% staining	ND
Gao et al.	USA	Caucasian	2015	ND	I-III	IHC	26	ND	150	>25% staining	OS
Wang et al.	China	Asian	2015	2006-2012	I-IV	IHC	86	29-73	94	>50% staining	OS
Zhu et al.	USA	Caucasian	2015	2006-2010	I-IV	IHC	92	24-78	84	>25% staining	OS

IHC: immunohistochemistry; OS: overal survival; ND: not documented.

Correlation of CD44 expression with clinicopathological parameters of ovarian cancer

We first conduced the meta-analysis to evaluate the association between CD44 and several clinicopathological parameters of ovarian cancer as shown in Figure 2. Our results demonstrate that CD44 expression is not significantly correlated with tumor grade (OR = 2.31, 95%CI = 0.61-8.73, P = 0.22; Figure 2A), the age of patients (OR = 0.89, 95%CI = 0.32-2.53, P = 0.83; Figure 2B), residual tumor size (OR = 1.01, 95%CI = 0.30-3.40, P = 0.99; Figure 2C), or response to chemotherapy (OR = 3.49, 95%CI = 0.51-23.65, P = 0.20; Figure 2D). However, there is a significant correlation between CD44 expression and tumor lymphatic metastasis (OR = 2.66, 95%CI = 1.36-5.22, P = 0.004; Figure 2E) and tumor TNM stage (OR = 2.34, 95%CI = 1.76-3.12, P < 0.00001; Figure 2F).

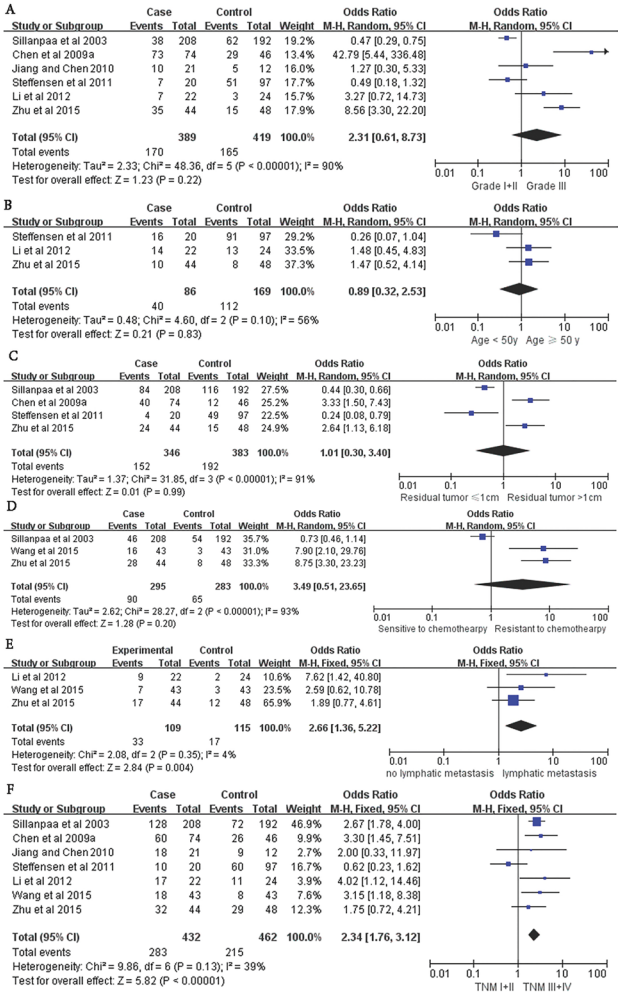

Figure 2. Forest plot of odds ratio was assessed for association between stem cell markers and clinical pathologic features, such as tumor grade (**A**), age of the patients (**B**), residual tumor size (**C**), response to chemotherapy (**D**), tumor lymphatic metastasis (**E**) and TNM stage (**F**).

Association of CD44 expression with the 5-year survival for ovarian cancer patients

Using the methods described previously, we next performed a meta-analysis based on the DerSimonian-Laird fixed-effect model to analyze the correlation of CD44 expression with the OS of 758 patients in five studies as presented in Figure 3. The results from our meta-analysis of the five studies indicate that CD44 expression was significantly associated with a decreased OS rate (RR: 1.47, 95%CI = 1.23-1.74, P < 0.0001; Figure 3). Nevertheless, no significant heterogeneity was observed among the five studies (I^2 = 8%, P_h = 0.36).

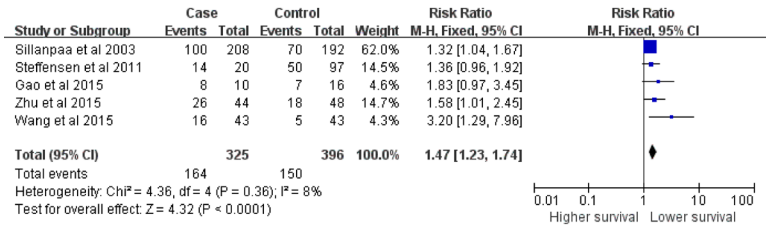

Figure 3. Analysis of CD44 expression and survival of ovarian cancer patients. Forest plot of relative risk for overall survival among included studies.

Sensitivity analysis

In order to rule out possible bias resulting from the low numbers of eligible studies for our meta-analysis, we then performed a sensitivity analysis. For this purpose, an individual study that was identified in this meta-analysis was omitted during each round of meta-analysis to evaluate the effect of the individual dataset of the particular study on the pooled ORs. We find that the pooled ORs as we presented above were not significantly affected by the subtraction of individual studies (data not shown), indicating that our findings are statistically robust.

Publication bias

The nearly symmetrical shape of the Begg's funnel plots also rules out the possibility of publication bias regarding our meta-analyses of the correlation of CD44 expression with the clinicopathological parameters and 5-year OS for ovarian cancer patients. Consistently, our results from the Egger test further demonstrate that there is no significant publication bias concerning our meta-analyses (Table 2).

Table 2. Egger test of funnel plot asymmetry.

Clinicopathological parameters	*t* value	d.f.	P value
Tumor differentiation	0.97	3	0.573
TNM stage	1.78	7	0.453
Primary residual tumor	0.66	3	0.997
Response to chemotherapy	3.67	3	0.602
Age	10.62	3	0.117
Lymphatic metastasis	2.62	2	0.296
Overall survival	0.18	5	0.142

d.f.: degrees of freedom.

DISCUSSION

To our knowledge, our meta-analysis is the first study to systematically examine the association between stem cell marker CD44 and the overall survival for ovarian cancer patients. The importance of stem cells in ovarian cancer remains very controversial in the literature, highlighting the necessity to perform a quantitative meta-analysis study to estimate the overall effect of CD44 expression on the survival of ovarian cancer patients. Our results show that the stem cell marker CD44 is significantly associated with tumor lymphatic metastasis and TNM stage, as well as poor prognosis for patients with ovarian cancer. These findings indicate that CD44 may serve as a marker to guide the clinical management of ovarian cancer patients in the future.

CD44 belongs to a multifunctional family of type I transmembrane proteins and is expressed as multiple isoforms in a variety of human cells (Marhaba and Zöller, 2004). Previous studies have demonstrated that CD44-positive tumor cells possess CSC phenotype, contributing to the self-renewal and tumorigenic capabilities of human cancers (Chen et al., 2009b). Overexpression of CD44 promotes cell proliferation and is associated with increased resistance to chemotherapeutic agents (Hessman et al., 2012). Consistently with these findings, our data indicated that increased CD44 expression correlates with poor OS for ovarian cancer patients. However, Diaz et al. (2005) reported that CD44 expression correlates with favorable prognosis in breast cancer. Additionally, Hsu et al. (2007) demonstrated that gastrointestinal stromal tumor patients with higher levels of CD44 expression exhibit longer overall survival and lower recurrence rates. These conflicting results suggest an elusive role of CD44 in cancer progression and metastasis. Thus, future studies with larger patient population are needed to draw a definite conclusion.

For future studies, it may turn out to be more practical to examine the potential clinical application of a panel of ovarian CSC markers in predicting the overall survival of ovarian cancer patients. Several studies have shown that CSC-related factors, including ALDH1, CD133, and Bmi-1, are involved in promoting ovarian cancer progression (Chang et al., 2009; Yang et al., 2010; Silva et al., 2011). In addition, CSCs exhibit remarkable phenotypic and functional heterogeneities, which may help to distinguish them from non-stem cancer cells and provide a potential therapeutic target for the development of anticancer therapies to improve clinical outcomes (Tang, 2012).

However, it is worth noting that there are some limitations concerning our meta-analysis. First, the number of included studies is relatively small. There are varying preoperative TNM stages and histologic types for ovarian cancer patients and we were unable to evaluate the potential confounding factors in the individual studies. Second, it is very important to follow a standard threshold to determine the expression levels of biomarkers like CD44. Although immunohistochemistry was the only method used for measuring CD44 expression levels, differences in cutoff values for positive CD44 expression may have contributed to the heterogeneity that we observed in our meta-analysis. Third, OS was determined from unadjusted RRs in the published papers and RRs that we calculated from the survival curves might be less reliable than those obtained from direct analysis of variance. Ideally, parameters should be directly extracted from the statistical data in published papers and then adjusted by using other prognostic factors.

In summary, in this meta-analysis, we show that CD44 expression was associated with tumor lymphatic metastasis and TNM stage in ovarian cancer. Moreover, ovarian cancer patients with positive CD44 expression exhibit a worse clinical outcome than those with

negative CD44 expression, indicating that CD44 may be an independent factor associated with reduced survival for ovarian cancer patients. The relative simplicity in the methodology for using CD44 expression in the identification of CSCs indicates that this biomarker should be further evaluated for their potential application as a marker for ovarian cancer stem cells in clinical practice.

Conflicts of interest

The authors declare no conflict of interest

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