Original Paper

Clinicopathological and Prognostic Significance of Cancer Stem Cell Markers in Ovarian Cancer Patients: Evidence from 52 Studies

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Key Words

Aldh1 • CD117 • CD133 • CD44 • Ovarian cancer • Meta-analysis

Abstract

Background/Aims: Relevant markers of cancer stem cells (CSCs) may serve as commonly used biomarkers of ovarian cancer (OC). However, their actual clinicopathological and prognostic significance remains inconclusive. Thus, we conducted a meta-analysis to quantitatively evaluate the association between the expression of CSC-relevant markers (ALDH1, CD117, CD133, and CD44) and OC. **Methods:** We used an odds ratio (OR) and a hazard ratio (HR) with a 95% confidence interval (CI) to estimate the effects by analyzing 52 studies from a literature search. Heterogeneity and sensitivity were evaluated, as well. Publication bias was assessed using funnel plots and Egger tests. **Results:** ALDH1 expression was statistically associated with FIGO stage (OR=1.872, 95%CI=1.14-3.076, P=0.013) and lymph invasion (OR=2.78, 95%CI=1.08-7.152, P=0.034). CD117 expression was significantly associated with FIGO stage (OR=2.01, 95%CI=1.35-2.98, P=0.001). CD133 expression was correlated with FIGO stage (OR=3.410, 95%CI=2.196-5.294, P<0.001) and differentiation grade (OR=2.672, 95%CI=1.354-5.272, P=0.005). CD44s was related to chemotherapy resistance (OR=3.218, 95%CI=1.148-9.016, P=0.026). Furthermore, overexpression of ALDH1 (HR=1.494, 95%CI=1.207-1.849, P<0.001), CD117 (HR=1.395, 95%CI=1.025-1.898, P=0.034) or CD44s (HR=1.725, 95%CI=1.135-2.623, P=0.011) was associated with poor OS. Further, overexpression of both ALDH1 (HR=1.524, 95%CI=1.158-2.007, P=0.003) and CD44s (HR=2.12, 95%CI=1.692-2.657, P<0.001) was correlated with worse DFS. **Conclusion:** CSC markers are useful predictive or prognostic biomarkers for OC in clinical assessments. Combined detection of CSC marker expression may be a powerful tool for prognostic predictions in clinical practice for patients with OC.

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Introduction

Ovarian cancer (OC) is the fifth leading cause of cancer deaths in women and the most lethal gynecological malignancy [1]. Although most OC patients initially exhibit a sensitive response to platinum-based chemotherapy, the overall survival (OS) of patients with OC is relatively low because 70% of patients are diagnosed with an advanced stage of OC and will ultimately succumb to chemo-resistant disease [2-4]. More seriously, the five-year survival rate of OS remains poor [2]. Therefore, there is a great need for identification of molecular biological prognostic markers to predict patient outcomes, which could also be beneficial in developing strategies and improving survival rates for OC.

Cancer stem cells (CSCs) are a distinct subpopulation of cancer cells characterized by their ability to generate heterogeneity and sustain tumor self-renewal [5-7]. Recently, accumulated evidence has identified CSCs as a pivotal players in OC progression and prognosis [8-10]. Several stem cell markers have been described for OC, including CD44, CD133, ALDH1, CD117, CD24, BMP and EpCAM [11-16]. Furthermore, it has been reported that CSC markers, such as ALDH1, CD117, CD133 and CD44, may serve as valuable prognostic indicators for OC [17-20]. However, due to the diversity of research methods employed, differences in cut-off value, study sample size, and variance in race, the prognostic value of ALDH1, CD117, CD133 and CD44 expression in OC remains controversial [18, 21-27].

No study has compared the relationship between these 4 common CSC markers and OC, including clinicopathological features of the disease, and the effect of the markers on survival. Therefore, we collected the available literature and conducted this meta-analysis to determine whether ALDH1, CD117, CD133 or CD44 overexpression would correlate with OC clinicopathology and prognosis and to explain which of these markers would have more clinical value based on meta-analysis evidence.

Materials and Methods

Literature search strategy

Literature searches without any linguistic limitations were conducted up to May 3, 2017, in the following electronic databases: PubMed and Web of Science. The search strategy included the following words and phrases: ("Aldehyde Dehydrogenase" or "ALDH") and ("Ovarian Neoplasm," "Ovarian Carcinoma," "Ovarian Cancer," and "Ovarian Tumor"); ("CD117," "c-kit" or "tyrosine-protein kinase Kit") and ("Ovarian Neoplasm," "Ovarian Carcinoma," "Ovarian Cancer," and "Ovarian Tumor"); ("CD133," "Prom-1" or "AC133") and ("Ovarian Neoplasm," "Ovarian Carcinoma," "Ovarian Cancer," and "Ovarian Tumor"); and ("CD44") and ("Ovarian Neoplasm," "Ovarian Carcinoma," "Ovarian Cancer" and "Ovarian Tumor"). Additionally, the reference lists of relevant articles were screened to identify additional relevant studies.

Study selection

Two reviewers (Yifeng Tao and Meiqin Li) selected the eligible studies independently, and disagreements were resolved by discussion. The eligible studies for this meta-analysis had to meet the following inclusion criteria: 1) the expression of OC CSC-relevant markers (ALDH1, CD117, CD133 and CD44) was detected in cancer tissue rather than in the serum or any other kinds of specimens; 2) the expression of these markers was detected using an immunohistochemistry (IHC) method; 3) the relationship between the expression of these markers and clinicopathological characteristics of OC was evaluated; 4) the expression of these markers with OS/disease-free survival (DFS) for OC was reported; and 5) HRs and 95% CIs were provided in the text, or sufficient data was provided for the calculation of HRs and 95% CIs. Non-research articles, studies that were focused on animal or human cell lines, or papers lacking information on OC prognosis were excluded.

In addition, due to a lack of adequate information for CD44v3, CD44v7-8, CD44v9, and CD44v10, we analyzed only two CD44 isoforms, namely, CD44s and CD44v6.

Data extraction

Two investigators (Rongyong Huang and Meiqin Li) carried out the data extraction independently, and disagreements were resolved by a 3rd party (Yifeng Tao). The following information was extracted: first author's surname, year of publication, country of origin, sample size, cut-off values for ALDH1/CD117/CD133/CD44, FIGO stage, and survival data.

Qualitative assessment

The quality of each of the eligible studies was assessed independently by 2 investigators using the Newcastle-Ottawa Quality Assessment Scale (NOS) for cohort studies. Briefly, the scale uses a star system to indicate the quality of each study. Studies that receive a score of ≥ 7 stars are considered to be of high quality.

Statistical Analysis

The odds ratio (OR) and 95% confidence interval (CI) were used to assess the correlation between the expression of these OC CSC markers and clinicopathological parameters, including FIGO stage (III/IV versus I/II), tumor differentiation grade (poor versus well/moderate), lymph nodal metastasis (positive versus negative), pathological type (serous type versus other type) and response to chemotherapy (resistant versus sensitive). In the present study, an $OR > 1$ indicated a higher probability of tumor progression in OC patients overexpressing these CSC markers. Hazard ratios (HR) were used as a summary statistic for survival outcomes, as described by Parmar et al. An HR greater than 1 represented poor prognosis in OC. Heterogeneity among primary studies was assessed with the Q-test and the I^2 statistic. A Q test P value < 0.10 and/or an I^2 statistic $> 50\%$ indicated significant heterogeneity between studies, and we used the random effects model to calculate the pooled OR/HR and 95% CI [28]. Otherwise, the fixed effects model was used [29]. Begg's funnel plots were used to evaluate publication bias. Sensitivity analysis was introduced to evaluate the influence of a single study on the overall estimate. All statistical analyses were performed using Stata 12.0 statistical software (Stata Corporation, College Station, TX, USA). A two-tailed $P < 0.05$ was considered to be statistically significant.

Results

Study characteristics

The literature selection process for choosing eligible studies is shown in Fig. 1. In accordance with the inclusion criteria, a total of 52 articles were eligible for the meta-analysis [13, 17-27, 30-69]. The basic characteristics of each eligible study are summarized in Table 1. The eligible studies were published between 1997 and 2017. The sample sizes of the included studies ranged from 11 to 440. Among these studies, 15 studies involved patients with ALDH1 [19, 22, 26, 30-41], 8 studies involved patients with CD133 [18, 23, 25, 30, 31, 37, 42, 43], 25 studies involved patients

Fig. 1. The flowchart of the study selection process.

with CD44 [17, 21, 24, 44-64, 66], 7 studies involved patients with CD117 [13, 20, 26, 27, 67-69], 3 studies involved patients with ALDH1 and CD133 [30, 31, 37], and 1 study involved patients with ALDH1 and CD117 [26]. According to the NOS quality assessment, 52 of the studies were categorized as high quality.

Correlation of ALDH1/CD117/CD133 /CD44 with clinicopathological features of OC patients

Table 2 shows the results for correlation between ALDH1/CD117/CD133/CD44 and the clinicopathological parameters. Overall analyses demonstrated that ALDH1 expression was statistically associated with FIGO stage (OR=1.872, 95% CI = 1.14 - 3.076, P=0.013) and lymph invasion (OR=2.78, 95% CI = 1.08 - 7.152, P=0.034) (Fig. 2A-B). CD117 expression was significantly associated with FIGO stage (OR=2.01, 95%CI=1.35-2.98, P=0.001) (Fig. 2E). CD133 expression was correlated with FIGO stage (OR=3.41, 95%CI=2.196-5.294, P<0.001), which was consistent with the results for ALDH1 (Fig. 2C). Moreover, CD133 expression was statistically associated with differentiation grade (OR=2.672, 95%CI=1.354-5.272, P=0.005) (Fig. 2D), suggesting that CD133 might be involved in the malignant progression of OC. It is worth noting that overexpression of CD44s is associated with chemotherapy resistance (OR=3.218, 95%CI=1.148-9.016, P=0.026) (Fig. 2F), indicating that high CD44s expression possibly contributes to a high risk of chemotherapy resistance in OC patients. However, no clear correlation was found between CD44s/CD44v6 expression and FIGO stage, lymph invasion, differentiation grade, and pathological type (all P>0.05).

Table 1. Association between CSCs expression and ovarian cancer prognosis. Abbreviations: OS, overall survival; DFS, disease-free survival; Pbias, the p-value of Egger linear regression test for evaluating publication bias

Reference	Year	Country	Case	Cutoff value	FIGO stage	Outcome
ALDH1						
Yu[6]	2017	China	207	Scores≥3	I -IV	OS
Ruscito[31]	2017	Germany	112	Scores≥2	I -IV	OS,DFS
Jing[30]	2016	China	62	Scores≥4	I -IV	NA
Huang[32]	2015	China	248	Scores≥1	I -IV	OS,DFS
Sun[33]	2015	China	100	Scores≥9	I -IV	DFS
Ayub[26]	2015	Germany	55	NA	III-IV	OS,DFS
Chen[34]	2015	China	80	≥10%	I -IV	OS
Kuroda[36]	2013	Japan	99	> mean ratio	I -IV	OS,DFS
Liebscher[35]	2013	Germany	131	Scores≥4	I -IV	OS
Ricci[37]	2013	Italy	47	NA	I -IV	OS,DFS
Wang[38]	2012	China	84	>50%	I -IV	OS,DFS
Rahadiani[39]	2011	Japan	98	>10%	NA	OS,DFS
Deng[40]	2010	USA	439	≥10%	NA	OS,DFS
Landen[41]	2010	USA	65	>1%	III-IV	OS,DFS
Chang[22]	2009	USA	440	Scores≥2	I -IV	OS,DFS
CD117						
Conic[67]	2015	Serbia	240	>25%	I -IV	OS
Ayub[26]	2015	Germany	55	NA	III-IV	OS,DFS
Huang[13]	2014	Norway	242	>10%	I -IV	OS,DFS
Brustmann[20]	2005	Austria	41	NA	I -III	OS
Khalifeh[69]	2005	USA	118	NA	III-IV	OS
Lassus[68]	2004	Finland	516	NA	I -IV	OS
Tonary[27]	2000	Canada	35	NA	I -IV	OS,DFS
CD133						
Ruscito[31]	2017	Germany	112	>10%	I -IV	OS,DFS
Jing[30]	2016	China	64	Scores≥4	I -IV	NA
Liang[25]	2016	China	120	Scores>0	I -IV	OS
Kim[42]	2014	Korea	59	Scores≥1.5	I -IV	NA
Francesca[37]	2013	Italy	91	Scores>0	I -IV	OS,DFS
Qin[43]	2012	China	123	≥10%	III-IV	OS
Zhang[18]	2012	USA	400	Scores>0	I -IV	OS,DFS
Ferrandina[23]	2009	Italy	160	Scores>0	III-IV	OS,DFS
CD44s						
Elzarkaa[24]	2016	Egypt	96	Scores≥3	III-IV	OS,DFS
Bonneau[44]	2015	France	32	> mean ratio	III-IV	OS,DFS
Wang[45]	2015	China	86	Scores≥5	I -IV	OS
Gao[46]	2015	USA	26	Scores>0	I -IV	OS,DFS
Zhu[47]	2015	USA	92	Scores>100	I -IV	OS,DFS
Jing[48]	2013	USA	483	>0%	I -IV	OS,DFS
Hu[50]	2013	China	92	Scores≥3	I -IV	OS
Ryabtseva[51]	2013	Ukraine	72	>10%	I -III	OS
Liu[52]	2012	USA	33	NA	I -IV	OS,DFS
Steffensen[53]	2011	Denmark	117	≥20%	I -IV	DFS
Chen[54]	2009	China	120	>25%	I -IV	NA
Cho[17]	2006	Korea	158	>10%	I -IV	NA
Sari[49]	2003	Finland	307	>10%	I -IV	OS,DFS
Rodriguez[21]	2003	Canada	121	>0%	I -IV	OS
Ross[55]	2001	USA	64	>0%	I -IV	OS
Kayastha[56]	1999	USA	56	>0%	NA	OS
CD44v6						
Motohara[61]	2016	Japan	186	>10%	I -III	OS,DFS
Tjhay[58]	2015	Japan	59	>10%	III-IV	OS,DFS
Wang[66]	2014	China	102	Scores≥2	I -IV	OS,DFS
Shi[57]	2013	China	90	Scores≥2	I -III	NA
Gun[60]	2012	Turkey	47	Scores>0	I -IV	NA
Zhou[62]	2012	China	62	>5%	I -IV	OS
Hong[59]	2006	Korea	25	≥10%	I -IV	NA
Cho[17]	2006	Korea	158	>10%	I -IV	NA
Rodriguez[21]	2003	Canada	121	>0%	I -IV	OS,DFS
Darai[64]	1998	France	36	>0%	I -III	NA
Yorishima[63]	1997	Japan	11	>0%	I -IV	NA

Impact of ALDH1/CD117/CD133/CD44 expression on survival of OC patients

To further investigate the relationship between ALDH1/CD117/CD133/CD44 and the prognosis of OC patients, survival analysis of the HR for OS and DFS was conducted. The results are shown in Fig. 3 and Table 3.

The data for this analysis indicated that overexpression of ALDH1 (HR=1.494, 95%CI=1.207–1.849, $P<0.001$), CD117 (HR=1.395, 95%CI=1.025–1.898, $P=0.034$) or CD44s (HR=1.725, 95%CI=1.135–2.623, $P=0.011$) is associated with poor OS (Fig. 3A, C). Furthermore, overexpression of both ALDH1 (HR=1.524, 95%CI=1.158–2.007, $P=0.003$) and CD44s (HR=2.12, 95%CI=1.692–2.657, $P<0.001$) is correlated with worse DFS (Fig. 3B,D). However, data analysis showed that there is no significant relationship between the overexpression of CD117 or CD133 and DFS. Additionally, a relationship between CD44v6 overexpression and poor OS rate was not found, and there was lack of sufficient information to study the relationship between CD44v6 expression and DFS.

Sensitivity analysis and Publication Bias

Sensitivity analysis indicated that most studies did not substantially influence the pooled OR/HR, except for the results related to OS and CD44v6

Table 2. Overall analysis of CSCs expression association with clinical features. Abbreviations: P_{bias} , the p-value of Egger linear regression test for evaluating publication bias

	OR	95%CI	P	I ²	P _{bias}
ALDH1					
FIGO stage(III/IV vs I / II)	1.872	1.14-3.076	0.013	62.10%	0.581
Differentiation(poor vs well/moderate)	0.916	0.498-1.687	0.779	72.80%	0.278
Lymph invasion (yes vs no)	2.78	1.08-7.152	0.034	79.40%	0.573
Pathological type(serous type vs other type)	0.802	0.545-1.181	0.265	0.41%	0.409
Response to chemotherapy(resistant vs sensitive)	0.845	0.231-3.865	0.937	84.80%	0.306
CD117					
FIGO stage(III/IV vs I / II)	2.01	1.35-2.98	0.001	15.30%	0.524
Differentiation(poor vs well/moderate)	1.698	0.819-3.519	0.154	62.10%	0.627
Pathological type(serous type vs other type)	3.166	1.777-5.642	<0.001	0.00%	0.145
CD133					
FIGO stage(III/IV vs I / II)	3.41	2.196-5.294	<0.001	48.40%	0.405
Differentiation(poor vs well/moderate)	2.672	1.354-5.272	0.005	50.30%	0.404
Pathological type(serous type vs other type)	1.305	0.830-2.052	0.248	0.00%	0.051
Response to chemotherapy(resistant vs sensitive)	1.063	0.504-2.243	0.872	75.00%	0.938
CD44s					
FIGO stage(III/IV vs I / II)	1.055	0.424-2.62	0.909	90.40%	0.106
Differentiation(poor vs well/moderate)	1.609	0.561-4.615	0.377	90.50%	0.055
Lymph invasion (yes vs no)	1.241	0.762-2.022	0.385	43.60%	0.292
Pathological type(serous type vs other type)	0.668	0.401-1.113	0.121	34.30%	0.545
Response to chemotherapy(resistant vs sensitive)	3.218	1.148-9.016	0.026	90.90%	0.053
CD44v6					
FIGO stage(III/IV vs I / II)	0.99	0.496-1.974	0.976	55.40%	0.269
Differentiation(poor vs well/moderate)	1.596	0.848-3.003	0.148	48.90%	0.415
Lymph invasion (yes vs no)	0.927	0.606-1.418	0.725	17.30%	0.266
Pathological type(serous type vs other type)	0.661	0.400-1.091	0.105	44.90%	0.236

Fig. 2. Association of CSCs expression with clinical features.(A-B) association of ALDH1 expression with FIGO stage (A) and lymph invasion (B); (C-D) association of CD133 expression with FIGO stage (C) and differentiation grade (D); (E) association of CD117 expression with FIGO stage; (F) association of CD44s expression with chemotherapy resistant.

expression. Publication bias analysis of the studies indicated there was no obvious publication bias in most studies, except for the results related to OS and ALDH1 expression, which suggests that a publication bias possibly existed for OS results (Table 2).

Discussion

Whereas advanced ovarian cancer is generally responsive to conventional combinations of primary cytoreductive surgery and paclitaxel-platinum chemotherapy initially, most ovarian cancer patients will inevitably relapse with metastasis, recurrence, and drug resistance [2, 3]. Although deadly, ovarian cancer is one of the more chemosensitive solid malignancies. One model by which to explore ovarian cancer tumor heterogeneity is the cancer stem cell hypothesis. This idea proposes that within a heterogeneous ovarian tumor, there are small cell populations with increased tumorigenicity and differentiating capacity compared to other tumor cells that are responsible for ovarian tumor initiation, recurrence, and metastasis [70, 71].

The clinical significance of the most frequently used CSC markers OC, ALDH1, CD117, CD133 and CD44 remains contradictory and inconclusive. Based on these controversial studies, a meta-analysis was conducted to evaluate the precise impact of ALDH1, CD117, CD133 and CD44 on the clinicopathological features and prognosis of OC.

ALDH1 plays a role in retinoic acid formation by oxidizing the all-trans-retinal and 9-cis-retinal involved in retinoid signaling, which has been linked to the stemness of CSCs [72,

Fig. 3. Association between CSCs expression and ovarian cancer prognosis. (A-B) association of ALDH1 expression with overall survival (A) and disease-free survival (B); (C-D) association of CD44s expression with overall survival (C) and disease-free survival (D).

Table 3. Association between CSCs expression and ovarian cancer prognosis. Abbreviations: OS, overall survival; DFS, disease-free survival; Pbias, the p-value of Egger linear regression test for evaluating publication bias

	HR	95% CI	P	I ²	P _{bias}
ALDH1					
OS	1.494	1.207-1.849	<0.001	75.10%	0.001
DFS	1.524	1.158-2.007	0.003	81.20%	0.063
CD117					
OS	1.395	1.025-1.898	0.034	69.20%	0.438
DFS	1.304	0.789-2.167	0.298	70.48%	0.674
CD133					
OS	1.109	0.989-1.243	0.076	73.60%	0.122
DFS	1.542	0.863-2.756	0.144	87.20%	0.116
CD44s					
OS	1.725	1.135-2.623	0.011	89.00%	0.051
DFS	2.12	1.692-2.657	<0.001	48%	0.066
CD44v6					
OS	1.363	0.270-6.89	0.708	90.60%	0.416

73]. Moreover, ALDH1 plays a key role in the maintenance of ovarian cancer stem cell-like properties and might mediate carboplatin resistance by altering regulation of the cell cycle and DNA repair networks [72].

CD117 (c-kit), a well-known proto-oncoprotein, is normally activated by binding to its ligand (stem cell factor), which activates cell-signaling cascades that are important in the regulation of cell proliferation, apoptosis, adhesion, and differentiation [74, 75].

CD133, a pentaspan membrane glycoprotein, has been identified as a CSC marker for various cancers. The biological function of CD133 remains unclear, but it may be involved in primitive cell differentiation and epithelial-mesenchymal interactions [76].

CD44 is a receptor for the extracellular matrix component hyaluronic acid and other extracellular matrix components that enable CSCs to sense environmental changes and mediate signal transduction to regulate CSC stemness properties [77]. CD44-hyaluronan complex activates Nanog, an embryonic stem cell transcription factor important in regulating CSC survival, self-renewal, maintenance, and chemoresistance [78].

Our results indicate that positive ALDH1, CD117, CD133 and CD44s expression can effectively predict several clinicopathological features and poor outcomes in patients with OC. Because the related features do not overlap, combined detection of ALDH1, CD117, CD133 and CD44s expression may be an especially effective tool for diagnosis of patients with OC. However, because publication bias was observed in the pooled HR for the OS and DFS of ALDH1 expression, our results should be cautiously interpreted.

Several potential limitations should be taken into consideration and additional results should also be interpreted cautiously. First, diverse antibody clones and antibody concentrations were used in detecting ALDH1, CD117, CD133 and CD44 expression, which could cause inconsistent results. Second, lack of a universal standard for defining high and low expression of CSC markers may impact the results of this meta-analysis. Third, relatively large heterogeneity was found in the majority of the analyses. Fourth, expression of these markers was detected using an IHC method, which suffers from severe limitations as no functional test, non-quantitative and non-canonical analysis. Fifth, we evaluated and calculated the HRs via survival curves, which might reduce the reliability of the results. Finally, publication bias was observed in the pooled HR for the OS and DFS of ALDH1 expression, thus potentially inflating the estimate for the association of CSCs with poor prognosis. Despite these limitations, we have provided a comprehensive analysis of the association between CSC markers and the clinicopathology and prognosis of OC patients.

Conclusion

In conclusion, our meta-analysis revealed the value of ALDH1, CD133 and CD44s as 3 significant clinical indicators for patients with OC. ALDH1 overexpression was related to FIGO stage and lymph invasion. CD133 overexpression was significantly correlated with FIGO stage and tumor differentiation grade. CD44s overexpression was associated with chemotherapy resistance. Moreover, ALDH1, CD117, CD133 and CD44s were associated with worse prognosis. Combined detection of ALDH1, CD117, CD133 and CD44s expression may be a powerful tool in clinical practice for predicting prognosis of patients with OC. However, because of certain limitations, further well-designed studies with large samples, standard cohorts, and long-term follow-up are required for confirmation.

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Yifeng Tao, Meiqin Li and conceived and designed the experiments; Min Fang, Hui Li, Dan Mo and Tian Zeng analyzed data, Yifeng Tao, Hui Li, Min Fang, Rongyong Huang and Meiqin Li wrote the manuscript. All authors reviewed and approved the manuscript.

Disclosure Statement

No conflict of interests exists.

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