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Review Article

CD44 expression and its clinical significance in pancreatic cancer: a meta-analysis

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Abstract: CD44 is one of the most commonly used markers of pancreatic cancer stem cells (CSCs), which are characterized by their ability for self-renewal and tumorigenicity. The results of numerous clinical trials investigating CD44 expression in pancreatic cancer are inconsistent. In this study, we performed a strategic literature search of PUBMED and EMBASE (updated to 1st May, 2016) for studies that assessed the correlation between CD44 expression and prognosis and clinicopathological features in patients with pancreatic cancer. Data from eligible studies was extracted and analyzed using a fixed or random effect model. Outcomes included overall survival and various clinicopathological features. We performed a final analysis of 557 patients from 7 evaluable studies. Our results showed that the pooled hazard ratio (HR) of increased CD44 for overall survival in pancreatic cancer was 2.10 (95% confidence interval [CI] = 1.68-2.62, $P<0.001$) by univariate analysis and 1.88 (95% CI = 1.31-2.70, $P = 0.007$) by multivariate analysis. With respect to clinicopathological features, CD44 upregulation was closely correlated with lymph node metastasis (OR = 2.43, 95% CI = 1.64-3.61), vascular invasion (OR = 1.75, 95% CI = 1.04-2.95), tumor differentiation (OR = 0.73, 95% CI = 0.45-1.18), tumor location (OR = 0.73, 95% CI = 0.45-1.18, $P = 0.20$ and $I^2 = 44\%$) and tumor size (OR = 1.61, 95% CI = 0.98-2.65) in patients with pancreatic cancer. Our meta-analysis results suggest that CD44 is an efficient prognostic factor in pancreatic cancer. Upregulation of CD44 is significantly associated with lymph node metastasis, vascular invasion, and clinical tumor differentiation.

Keywords: Pancreatic cancer, CD44, meta-analysis, prognosis

Introduction

Pancreatic cancer is one of the most aggressive human malignancies, with high incidence and mortality [1]. Most patients in the early stage have no recognizable symptoms and early detection tests for pancreatic cancer are unavailable. As a result, more than half of the patients are newly diagnosed when metastases have appeared, for whom the overall 5-year survival is less than 5%. Although great improvements have been made in diagnostic and therapeutic treatment for pancreatic cancer in recent years, the prognosis is still poor [2]. Clinicopathological characteristics such as tumor differentiation and clinical stage cannot reliably predict individual clinical prognosis [3]. Thus, identifying molecular markers of prognosis will help us better understand the pathogenesis of cancer and facilitate the rational choice of therapeutic strategies.

CD44 is a cell surface HA-binding glycoprotein that is up regulated to some extent in almost all tumors of epithelial origin and plays an important role in the biological activities of various tumors [4]. It is also reported that some variant subtypes of CD44 are associated with modulated progression and poor prognosis [5]. Studies have shown that CD44 is linked to tumor malignancy and metastatic potential. Moreover, the prognostic value of CD44 for patients with cancers has been reported in various solid tumors, including liver, gastric, pancreatic, and colon cancers [6]. Expression of CD44 is increased in many cancers of the digestive system [7]. However, prognostic outcomes of these studies are contradictory. Therefore, the aim of this meta-analysis was to

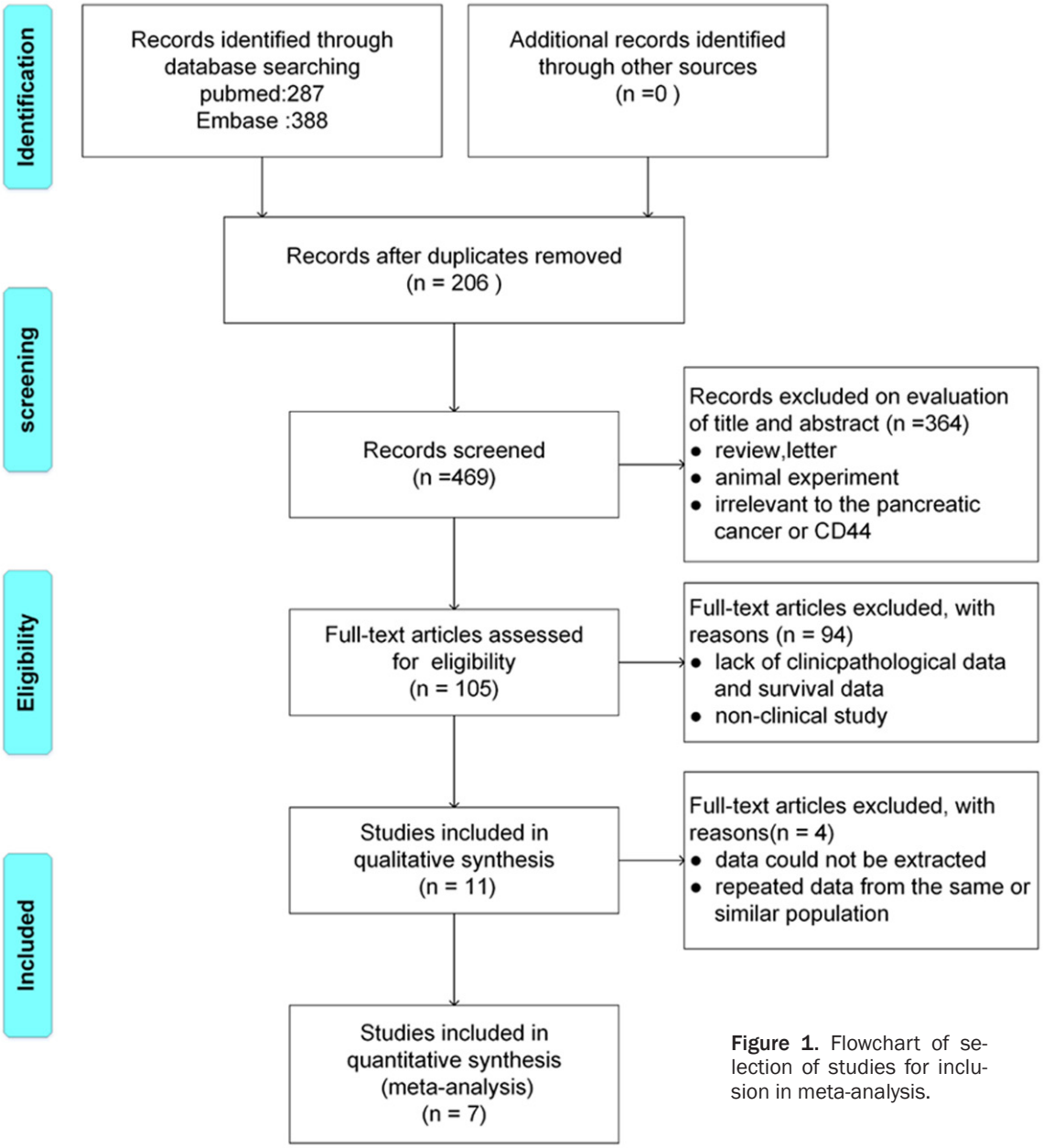

Figure 1. Flowchart of selection of studies for inclusion in meta-analysis.

Table 1. Quality assessment of studies

Author	Year	Selection			Comparability	Outcome assessment			Score
		1	2	3	4	5	6	7	
Takuji G	1998		*	*		*		*	*****
Gang Z	2013	*	*		**	*	*	*	*******
Zhongh L	2014	*	*		*	**	*	*	*******
Ya Chin H	2014		*	*	**	*	*	*	*******
KAI C	2014	*	*	*	**	**	*	*	********
TsannL H	2014	*	*	*	*	*		*	******
Xiao-P Li	2015	*	*	*	*	*	*	*	*******

A study can be awarded a maximum of one star for each numbered item within the Selection and Outcome Assessment. A maximum of two stars can be given for Comparability.

investigate the relationship between CD44 expression and its clinical significance in patients with pancreatic cancer.

Materials and methods

Literature search strategy

A literature search for relevant studies was conducted on PubMed, EMBASE, and Cochrane Library databases from th-

Table 2. Characteristics and results of eligible prognostic studies evaluating survival

Author	Year	Country	CD44 type	Cutt off value	Follow up time	Number of patients			Univariate		Multi-variate	
						Total	Cd44 (+)	Cd44 (-)	HR	95% CI	HR	95% CI
Takuji G	1998	Japanese	CD44v6	NA	3A44v6seria	42	21	21	3.84	1.75-8.41	NA	NA
Gang Z	2013	Chinese	CD44v6	HIGH	36 months	54	24	30	3.9	1.20-7.50	3.7	1.30-7.50
Zhongh L	2014	Chinese	CD44v6	HIGH	4-46 months	101	51	50	1.77	1.17-2.67	1.869	0.60-5.82
Ya Chin H	2014	Chinese	CD44	NA	1.9-12.5 years	96	55	41	NA	NA	0.291	0.09-0.957
KAI C	2014	Chinese	CD44v6	GRADE	3-46 months	109	65	44	1.76	1.17-2.66	0.968	0.28-3.37
TsannL H	2014	Chinese	CD44	SCORE	60 months	86	53	33	2.39	1.30-4.39	2.19	1.27-3.97
Xiao-P Li	2015	Chinese	CD44	> 10%	39 months	67	46	21	2.25	1.30-3.90	3.21	1.29-8.00

NA: unknown.

eir inception to 1st May, 2017. Search strings of studies were selected using the following search terms: CD44 and cancer or tumor or carcinoma or neoplasm or carcinogenesis and pancreatic. The references of articles and reviews identified through the search were also manually searched for possible inclusion of additional studies. Manual search of reference lists from potentially relevant articles was also performed to identify other potentially relevant studies. We confined our search to scientific studies published in English.

Selection criteria

The studies included in the meta-analysis were random controlled studies or observational studies that assessed association between expression of CD44 and the prognosis or the clinical significance of pancreatic cancer. To be eligible for inclusion in this systematic review, studies had to satisfy the following criteria: (1) focus on the clinical features or prognosis of pancreatic cancer; (2) investigate the association between CD44 and clinicopathological characteristics; (3) all patients diagnosed with pancreatic cancer were confirmed through histopathologic examinations. If the same category of patients were presented in more than one article, the most complete research was chosen for our study. The major exclusion criteria were (1) letters, communication paper, reviews, and articles published in a book; (2) non-CD44 or PDAC pancreatic cancer; (3) duplication of a previous publication (**Figure 1**).

Methodological assessment

Two reviewers assessed the quality of evidence of each included study independently using the Newcastle-Ottawa Scale (NOS) criteria for assessing the quality of non-randomized studies, which we realigned for pancreatic cancer [8]. The quality assessment and scores are summarized in **Table 1**. The quality of each study was graded as level 1 (0-5 points) or level 2 (6-8 points) and the full score was 9 points. Overall, each study received a total score from zero to nine points, and a study was considered of high quality if it scored more than six stars.

Data extraction

All data were extracted by two independent reviewers. The following relevant data were extracted from each study in a predetermined table: author's name, publication year, patient's country, number of patients, follow-up time, type of CD44, cut-off value and hazard ratio (HR) with 95% confidence intervals (CIs) in **Table 3**. Because some articles presented survival data with a Kaplan-Meier curve, we used GetData Graph Digitizer 2.26 (http://getdatagraph digitizer.com/) to statistics and extract survival data. Ambiguous points were discussed by the authors and resolved by consensus.

Statistical analysis

Data types belong to the queue analysis. Through the extraction of data, which included the study of pathological indicators belonging to the two classification variables, so we selected RR, OR. Survival analysis: the survival time of cancer patients as an important reference index, and then we selected the HR value as our statistical indicators. The intensity of the relationship between the expression level of CD44 and overall survival was regarded as HRs. HRs and the corresponding 95% CIs were reported for individual studies by using Review Manager 5.3. The HR > 1 was associated with poor event. HRs were extracted directly by the reported data or from the Kaplan-Meier curve by an estimated method if they were not provided [9]. Odds ratios (ORs) were assessed for

Table 3. Characteristics of studies included in the meta-analysis

Author	Tumor size		Location		Lymph node metastasis		Vascular invasion		Nerve invasion		Tumor differentiation	
	CD44+	CD44-	CD44+	CD44-	CD44+	CD44-	CD44+	CD44-	CD44+	CD44-	CD44+	CD44-
	<2 cm/≥2 cm	<2 cm/≥2 cm	Head/Body, tail	Head/Body, tail	N/P	N/P	N/P	N/P	N/P	N/P	Well/poorly	Well/poorly
Takuji G	1/20	2/19	15/6	11/10	4/17	4/17	5/16	9/12	NA	NA	16/5	18/3
Gang Z	NA	NA	NA	NA	5/19	16/16	NA	NA	NA	NA	NA	NA
Zhongh L	18/33	21/29	44/7	44/6	23/28	34/16	37/14	36/14	36/15	36/14	36/15	36/14
Ya Chin H	NA	NA	25/16	35/20	22/19	28/27	NA	NA	NA	NA	35/6	43/12
KAI C	15/35	19/40	41/9	53/6	22/28	43/16	28/22	44/15	32/18	38/27	32/18	50/9
TsannL H	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	46/7	25/4
Xiao-P Li	12/37	11/7	31/18	17/1	25/24	16/2	53/6	18/0	8/41	2/16	7/36	9/15

P/N, positive/negative; NA, unknown.

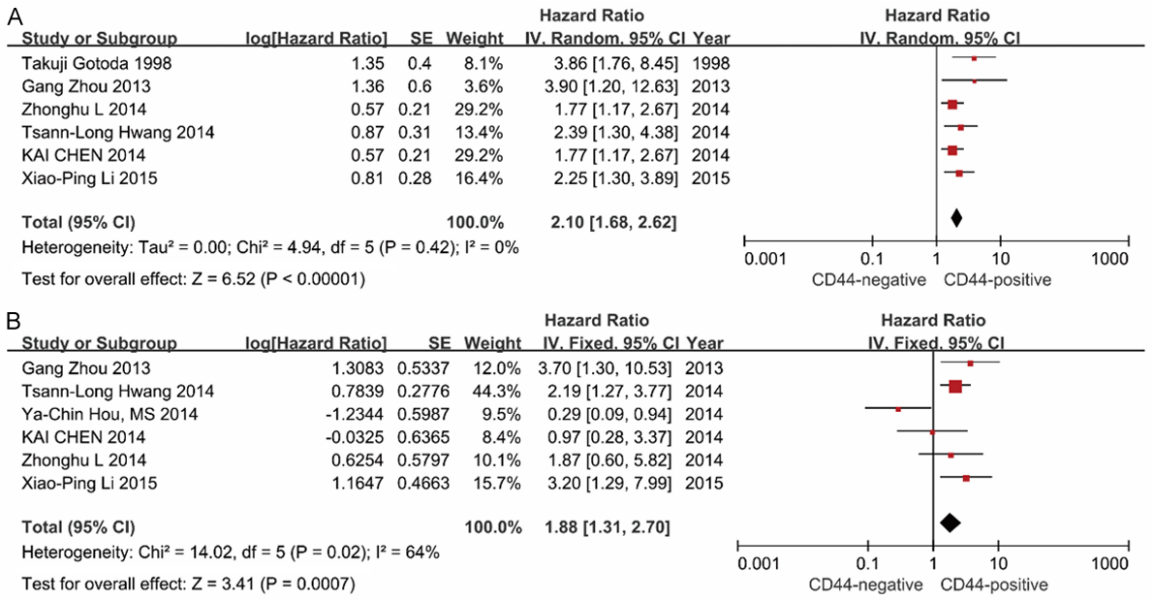

Figure 2. A. CD44 and OS rate by multivariate analysis. B. CD44 and OS rate by univariate analysis.

event cases and control cases. The heterogeneity among studies was evaluated using the Chi-square test and I^2 test. A fixed-effects model was performed in the arguments without significant heterogeneity, otherwise, a random-effect model was used. All *P*-values were for two tailed analysis and $P<0.05$ was considered statistically significant.

Results

We initially identified 675 references using keywords (**Figure 1**). After screening, we excluded 206 studies that were duplicate. Another 364 articles were excluded after a review of title, abstract and full texts, leaving seven studies that met the inclusion criteria and were used for analysis [10-16]. In total, there were 557 pancreatic cancer patients involved in this meta-analysis, including 303 patients with high CD44 expression and 254 patients with low CD44 expression. All included studies were reported by authors in Asia (six from China, one from Japan). The definition of "high" CD44 expression differed between studies. **Tables 1** and **2** summarized the characteristics and methodological quality of the included studies in our meta-analysis.

Prognostic value of CD44 expression

We extracted the HRs of each included study using available information or the methods described above. Each study correlated "high" CD44 with survival data. The study could be included in meta-analysis by univariate and multivariate analysis effect of CD44 on overall survival via sufficient data to estimate the HR and 95% CI. According to univariate analysis, increased expression of CD44 was significantly associated with poor over survival (OS) rate in a fixed-effects model (HR = 2.10, 95% CI = 1.68-2.62, P<0.001; **Figure 2A**). Furthermore, according to multivariate analysis, there was also a significant difference between CD44-positive and CD44-negative patients in a random-effects model (HR = 1.88, 95% CI = 1.31-2.70, P = 0.007; **Figure 2B**).

Summary of pathological characteristics

The forest plot of OR (odds ratio) was assessed for association between CD44 expression and clinicopathological features such as lymph node metastasis (**Figure 3A**), Vascular invasion (**Figure 3B**), tumor differentiation (**Figure 3C**), tumor size (**Figure 3D**), and tumor location (**Figure 3E**). In pooled analysis, CD44 expression was significantly associated with lymph node metastasis (OR = 2.43, 95% CI = 1.64-3.61, P = 0.001 and I^2 = 38%, fixed-effect model), vascular invasion (OR = 1.75, 95% CI = 1.04-2.95, P = 0.04 and I^2 = 0, fixed-effect model), tumor differentiation (OR = 0.73, 95% CI = 0.45-1.18, P = 0.02 and I^2 = 44%, fixed-effect model), tumor location (OR = 0.73, 95%

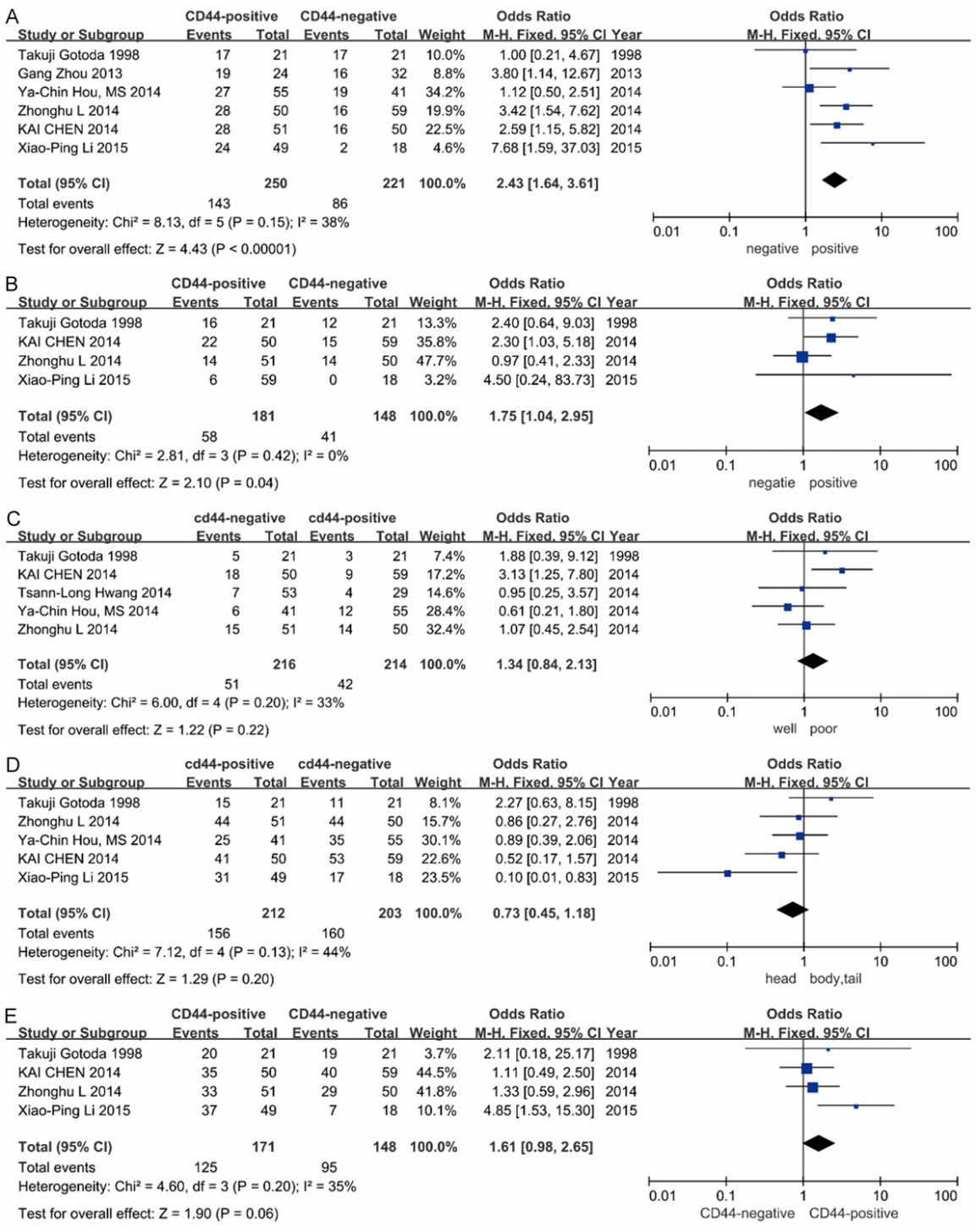

Figure 3. A. Correlation of CD44 with lymph node metastasis. B. Correlation of CD44 with vascular metastasis. C. Correlation of CD44 with tumor differentiation. D. Correlation of CD44 with tumor size. E. Correlation of CD44 with tumor location.

CI = 0.45-1.18, P = 0.20 and I^2 = 44%, fixed-effect model), tumor size (OR = 1.61, 95% CI = 0.98-2.65, P = 0.06 and I^2 = 35%, fixed-effect model) in patients with pancreatic cancer.

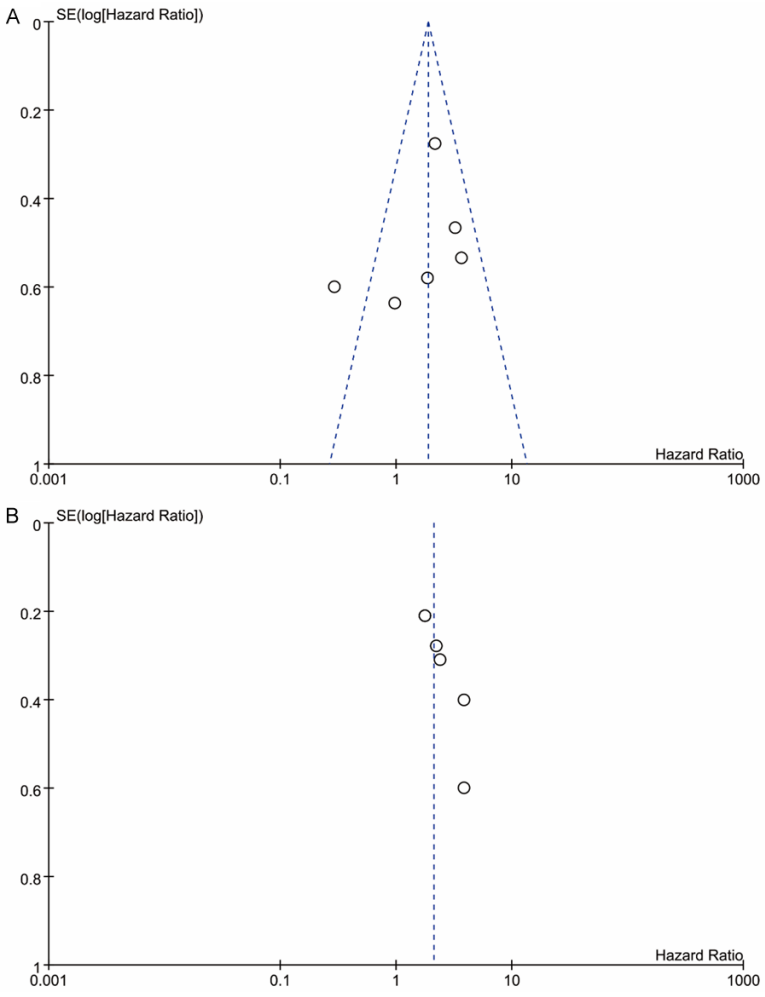

Figure 4. A. Funnel plots of publication bias: CD44 expression and OS rate by univariate analysis. B. Funnel plots of publication bias: CD44 expression and OS rate by multivariate analysis.

Evaluation of heterogeneity and publication bias

We performed an analysis to evaluate the influence of individual studies on the summary of OS rate. In all included studies, one funnel plot asymmetry was found (**Figure 4A**) in multivariate HR analysis, while no funnel plot asymmetry was found (**Figure 4B**) in univariate HR analysis.

Discussion

To date, surgical resection is the only potentially effective therapy for pancreatic cancer [17]. Although there is consistency regarding the role of screening patients with pancreatic cancer, there is no consensus on the most effective method for applying in screenings [18]. Searching and developing biomarkers of prognosis to guide medical therapy in malignant cancers is vitally important [19]. Among them, CD44 is considered to be a promising one, which is the cancer stem cell biomarker. Recent studies have suggested that CD44 expression correlates with favorable prognosis in various cancers [20-22]. To our knowledge, this is the first meta-analysis focused on association of CD44 with overall survival and clinical features in patients with pancreatic cancer. The pooled multivariate HR and univariate HR derived from 7 studies evaluating the relationship between CD44 and overall survival of patients with pancreatic cancer was 1.88 (1.31-2.70) and 2.10 (1.68-2.62), respectively, demonstrating that CD44 upregulation is a poor prognostic marker in this population. Moreover, patients with pancreatic cancer had an even worse prognosis when CD44 expression was low or negative. Together, these results indicate that CD44 expression is significantly associated with OS, tumor differentiation, lymph node metastasis, vessel metastasis and tumor size. Metastasis is responsible for most cancer-associated deaths and worse prognosis [23]. Previous studies have proposed that CD44 is closely related to metastasis [24], which is consistent with our meta-analysis.

When pooling the HR of all studies, the association between high expression of CD44 and poor prognosis in patients with PDAC was accompanied by strong heterogeneity. However, as revealed by one-way sensitivity analysis, heterogeneity was not caused by any single study. Importantly, high quality studies that performed with multivariate analysis homogeneously confirmed our initial results that high expression of CD44 was associated with poor prognosis. Thus, the source of heterogeneity

appears to be related to studies of lower quality that presented only with univariate analysis by illustrating Kaplan-Meier survival curves. Although immunohistochemistry was the most commonly used method, differences in cut-off values for elevated expression of CD44 may have contributed to the observed heterogeneity.

Although the results revealed a significant correlation between the cancer stem cell (CSC) marker CD44 and pancreatic cancer patients' survival, some limitations in our meta-analysis are evident. The risks calculated in our meta-analysis may be overestimated due to publication and reporting bias. We did not include unpublished papers and abstracts into meta-analysis because the required data were available only in full publications. Positive results tend to be more acceptable by journals, whereas negative results are always rejected or even not submitted for review. Furthermore, another potential source of bias is related to the method used to extrapolate the HR, although we tried to identify all relevant data. HR was extracted from the data included in the article directly or calculated from the survival curves. The method of extrapolating HR from survival curves seems to be less reliable because this strategy did not completely eliminate inaccuracy in the extracted survival rates. Moreover, different objects included in these studies may have a different impact on overall survival, so this factor should be taken into consideration. Therefore, more meticulous research should be conducted. At last, most studies didn't provide supplemental chemo- or radio-therapy data which also were linked to the prognosis of PDAC patients. All of these factors may partly influence the significance of CD44 expression in the survival.

In conclusion, our meta-analysis results suggest that CD44 is an efficient prognostic factor for pancreatic cancer. Upregulation of CD44 is significantly associated with clinical significance and over survival.

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Disclosure of conflict of interest

None.

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