Original Article
Prognostic value of cancer stem cell marker CD133 expression in pancreatic ductal adenocarcinoma (PDAC): a systematic review and meta-analysis

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Abstract: CD133 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. Although the expression of CD133 has been reported to correlate with poor prognosis of PDAC in most literatures, some controversies still exist. In this study, we aimed to investigate the correlation between CD133 expression and prognosis and clinicopathological features in PDAC. A search in the Medline, EMBASE and Chinese CNKI (China National Knowledge Infrastructure) database (up to 1 March 2015) was performed using the following keywords pancreatic cancer, CD133, AC133, prominin-1 etc. Data from eligible studies were extracted and included into meta-analysis using a random effects model. Outcomes included overall survival and various clinicopathological features. We performed a final analysis of 723 patients from 11 evaluable studies for prognostic value and 687 patients from 12 evaluable studies for clinicopathological features. Our study shows that the pooled hazard ratio (HR) of overexpression CD133 for overall survival in PDAC was 0.58 (95% confidence interval (CI): 0.49-0.67) by univariate analysis and 0.73 (95% CI: 0.52-1.03) by multivariate analysis. With respect to clinicopathological features, CD133 overexpression by immunohistochemistry (IHC) method was closely correlated with clinical TNM stage (TNM stage III+IV, OR=0.32, 95% CI: 0.19-0.54), tumor differentiation (poor differentiation, OR=0.56, 95% CI: 0.37-0.83), and lymph node metastasis (N1, 3.15, 95% CI: 1.56-6.36) in patients with PDAC. Our meta-analysis results suggest that CD133 is an efficient prognostic factor in PDAC. Overexpression of CD133 was significantly associated with clinical TNM stage, tumor differentiation and lymph node metastasis.

Keywords: Pancreatic neoplasms, CD133, meta analysis

Introduction

With a 5-year survival rate of only 5%, pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive human malignancies and the 4th leading cause of cancer related death [1]. Part of the reason for the fatal prognosis of patients with PDAC is the high rate of recurrence after tumor resection and a high resistance to chemotherapy. It is necessary to explore the biological index for the early diagnosis and prediction of PDAC which present as aggressive and recurrent malignancies.

Subpopulations of cells with malignant potential have recently been isolated from a variety of tumors by fluorescence-or magnetic-activated cell sorting (FACS, MACS) based on the expression of certain cell surface markers.

Evidence supports the cancer stem cell (CSC) hypothesis, according to which CSCs may be responsible for tumor initiation, metastasis, recurrence and resistance to treatment [2]. Among the various markers, CD133 is one of the most commonly used. Positivity for the surface proteins CD133 has been employed in many studies to isolate cells with stem cell-like and cancer-initiating properties from differrent solid tumors. Furthermore, its prognostic and clinicopathological values in differrent cancer types have been widely studied [3-5].

The effect of abnormalities expression of CD133 has been investigated for PDAC by using univariate or multivariate analysis, however, the relationship between CD133 and prognosis value was still controversial mainly because of the limited patient numbers of independent
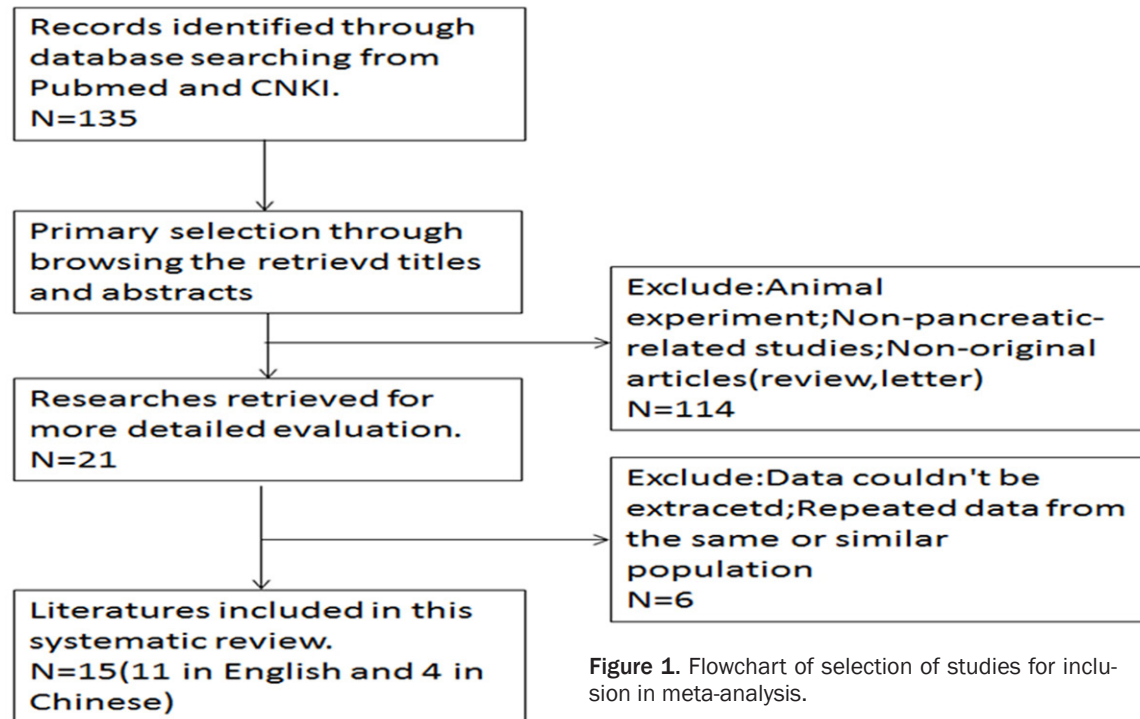
Figure 1. Flowchart of selection of studies for inclusion in meta-analysis.

reports. Here, we performed a systematic review and meta-analysis in the published literatures to clarify whether the expression of CD133 was associated with the clinicopathological features and prognosis of pancreatic cancer patients.

Materials and methods

Literature search strategy

A literature search via PubMed, EMBASE and CNKI (China National Knowledge Infrastructure) databases was conducted up to 1 Mar 2015. Search strings of PubMed was ("cd133" [Title/Abstract] OR "AC133 antigen" [Supplementary Concept] OR prominin-1 OR PROML1 OR AC141 antigen OR fudenine [Title/Abstract]) AND and AND (("carcinoma" [MeSH Terms] OR carcinoma [Text Word]) AND and AND ("Pancreatic Neoplasms" [Mesh]) OR (pancreatic cancer)). Titles and abstracts were reviewed to identify reports which examined the association of CD133 level with clinical outcomes, such as overall survival (OS) and clinicopathological features including sex, histology, differentiation and lymph node metastasis in PDAC patients.

Selection criteria

To be eligible for inclusion in this systematic review, a study was required to meet the follow-

ing criteria: (1) studies dealing with pancreatic cancer patients, diagnosis of PDAC was proven by histopathological methods; (2) articles investigated the association between CD133 and clinicopathological characteristics; (3) articles was published as a full-text article in English or Chinese; (4) expression of the proteins were evaluated in tumor tissues by IHC; (5) articles providing sufficient data to allow the estimation of hazard ration (HR) of overall survival (OS) and prognostic factors; (6) if the same group of patients were used to analyze more than once, the most complete research was selected for our study. The major exclusion criteria were (1) letters to the editor, reviews, and articles published in a book; (2) non-CD133 or PDAC; (3) duplication of a previous publication (**Figure 1**).

Data extraction

All data were independently reviewed by two reviewers with standardized data abstraction tool. The following information was extracted from each study: year of publication and first author's name; sample size, test method and cut-off level; tumor data including stage, grade and lymph node metastasis. Any disagreement within or between pairs was resolved by discussion. Staging of pancreatic cancer was based on the UICC classification revised in 2012 [6]. The software GetData Graph Digitizer 2.24

Table 1. Characteristics and results of eligible prognostic studies evaluating survival

Study	Country	N (M/F)	Samples	Staning pattern	Cut off value	Positve/Negative	Univariate HR (95%CI)/P value	Multivariate HR (95%CI)/P value
Hou YC 2014	China	96 (63/33)	TMA	Cytoplasm	> 0%	42/54	0.83/0.55-1.24/0.365	1.346 high/1.0 low/0.441-4.112/0.602
Kim HS 2012	Korea	42 (28/14)	Whole tissue sections	Cytoplasm and Membrane	> 3	14/28	0.42/0.19-0.92/0.014	1.445/1.012-2.214/0.022
Maeda S 2008	Japan	80 (52/28)	Whole tissue sections	Cytoplasm	> 0%	48/32	0.52/0.32-0.85/0.0002	2.151/1.211-3.869/0.009
Li XW 2011	China	35 (23/12)	Whole tissue sections	Membrane	≥ 10%	26/9	0.68/0.36-1.27/0.032	NA
Cen G 2010	China	71 (50/21)	Whole tissue sections	Cytoplasm and Membrane	> 5%	46/25	0.42/0.25-0.71/0.000	NA
Mizukami T 2014	Japan	17	Whole tissue sections	Membrane	> 1%	5/12	3.59/1.01-12.72/0.0406	NA
Chen K 2014	China	109 (71/38)	Whole tissue sections	Membrane	> 5%	65/44	0.62/0.42-0.901/0.013	1.008/0.227-4.486/0.992
Wang YC 2014	China	52 (31/21)	Whole tissue sections	Cytoplasm	> 3	39/13	0.35/0.19-0.65/0.001	NA
Kure S 2012	Japan	105 (61/44)	Whole tissue sections	Membrane	> 0%	45/60	0.75/0.51-1.11/0.1536	NA
Chen L 2014	China	65 (44/21)	Whole tissue sections	Membrane	> 0%	38/27	0.54/0.33-0.88/0.013	NA
Immervoll H 2008	Norway	51	TMA	Membrane	> 0%	41/10	0.69/0.33-1.46/0.123	NA

Abbreviations: N (M/F): number (male/female); TMA: tissue microarrays; NA: unknown.

Table 2. Main clinicopathological features and results of eligible studies

Study	Country	N (M/F)	Samples	P/N	Staning patterns	Cut off value	Lymph node metastasis		TNM stage		Differentiation	
							CD133+	CD133-	CD133+	CD133-	CD133+	CD133-
							Negative/Positive	Negative/Positive	I+II/III+IV	I+II/III+IV	Well/poor	Well/poor
Hou YC 2014	China	96 (63/33)	TMA	42/54	Cytoplasm	> 0%	20/22	27/27	39/3	51/3	36/6	42/12
Kim HS 2012	Korea	42 (28/14)	Whole tissue sections	14/28	Cytoplasm and Membrane	> 3	NA	NA	NA	NA	10/4	21/7
Maeda S 2008	Japan	80 (52/28)	Whole tissue sections	48/32	Cytoplasm	> 0%	11/37	18/14	42/6	28/4	48/2	31/1
Li XW 2011	China	35 (23/12)	Whole tissue sections	26/9	Membrane	≥ 10%	2/18	7/8	9/17	8/1	21/5	8/1
Wang X 2009	China	58 (36/22)	Whole tissue sections	37/21	Cytoplasm	> 0%	14/23	17/4	18/19	16/5	22/15	17/4
Cen G 2010	China	71 (50/21)	Whole tissue sections	46/25	Cytoplasm and Membrane	> 5%	10/20	33/8	23/7	36/3	10/20	33/8
Mizukami T 2014	Japan	17	Whole tissue sections	5/12	Membrane	> 1%	3/2	8/4	3/1	12/1	4/1	7/5
Chen K 2014	China	109 (71/38)	Whole tissue sections	65/44	Membrane	> 5%	31/34	34/10	51/14	41/3	45/20	37/7
Wang YC 2014	China	52 (31/21)	Whole tissue sections	39/13	Cytoplasm	> 3	9/30	9/4	19/20	11/2	19/20	11/2
Shimizu K 2009	Japan	10 (4/6)	Whole tissue sections	10/0	Cytoplasm and Membrane	> 0%	NA	NA	5/5	0/0	8/2	0/0
Vizio B 2012	Italy	37 (15/22)	TMA	11/26	Membrane	> 1%	NA	NA	NA	NA	NA	NA
Tsukamoto N 2013	Japan	80	TMA	29/51	Cytoplasm	> 5%	12/17	14/37	0/29	1/50	28/1	42/9

Abbreviations: N (M/F): number (male/female); TMA: tissue microarrays; P/N: positive/negative; NA unknown.

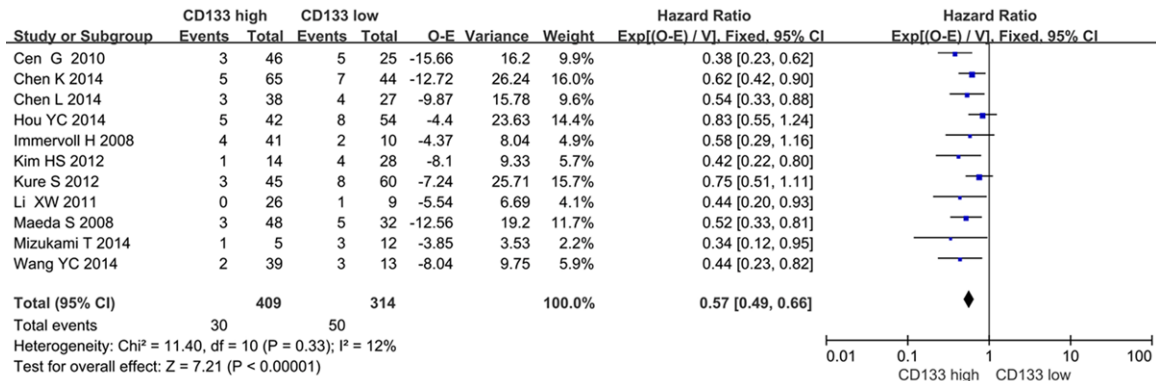**Figure 2.** CD133 and OS rate by univariate analysis.

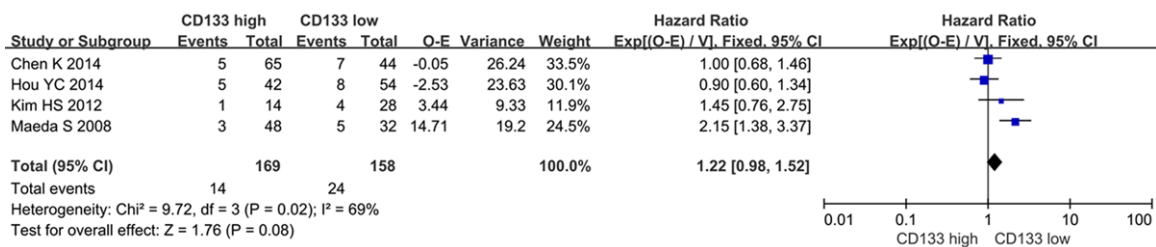**Figure 3.** CD133 and OS rate by multivariate analysis.

(http://getdata-graph-digitizer.com/) was applied to digitize and extract the data from the Kaplan-Meier curve in some articles.

Statistical analysis

The intensity of relationship between the expression levels of CD133 and overall survival were described as HRs. Software RevMan 5.3 (the Cochrane Collaboration, Copenhagen) was employed for data analysis. The data was analyzed by means of SPSS version 13.0 (SPSS Inc; Chicago, USA). *P* < 0.05 was considered as statistical significance. Comparisons of dichotomous measures were performed by pooled estimates of odds ratios (OR), as well as their 95% confidence intervals (CI). The pooled HR corresponding to the 95% CI was used to assess the prognostic value of CD133 in PDAC patients. Statistical heterogeneity was tested by Cochrane's Q test (Chi-squared test; Chi^2) and inconsistency. Fixed or Random model was used depending on heterogeneity analysis. If there was no obvious heterogeneity, the fixed-effects model (Mantel-Haenszel method) was used to estimate the pooled HR; otherwise, the random-effects model (DerSimonian and Laid method) was used [7].

Results

Literatures information

The entire literature search yielded a total of 15 studies (11 in English and 4 in Chinese) comprising 908 patients for the final analysis (**Figure 1**) [8-22]. The sample size of the studies included ranged from 10 to 109. The patients from all the studies were divided into a CD133 high group and CD133 low group. Eleven studies including 723 cases were available for our meta-analysis for the expression of CD133 and prognosis. Among all the included studies, twelve studies including 687 cases were available for our meta-analysis for the expression of CD133 and clinicopathological features. The individual characteristics and results of eligible prognostic studies evaluating surviving are summarized in **Table 1**. Main clinicopathological features and results of eligible studies are summarized in **Table 2**.

Study characteristics

All 15 eligible studies were listed in **Tables 1** and **2**. Five reports originated from Japan, seven from China, one from Norway, one from South Korea, and one from Italy. One studies
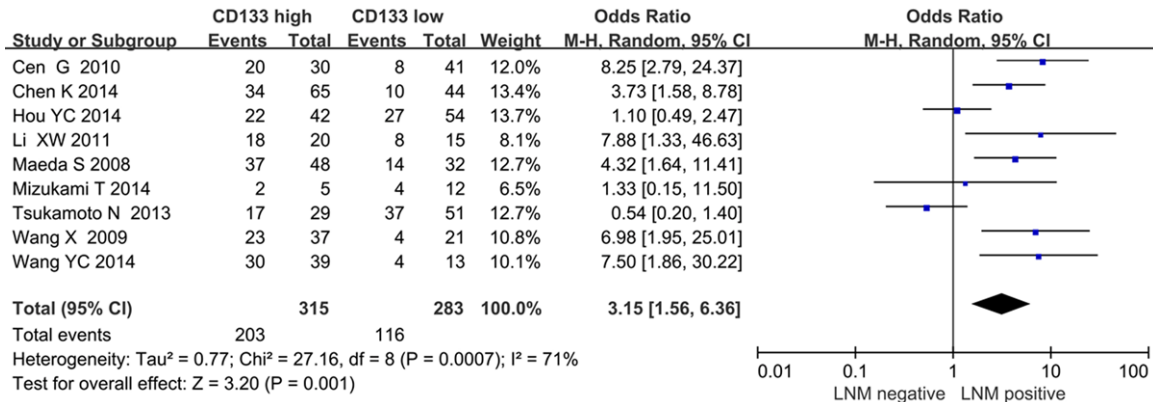
Study or Subgroup	CD133 high Events	Total	CD133 low Events	Total	Weight	Odds Ratio M-H, Random, 95% CI
Cen G 2010	20	30	8	41	12.0%	8.25 [2.79, 24.37]
Chen K 2014	34	65	10	44	13.4%	3.73 [1.58, 8.78]
Hou YC 2014	22	42	27	54	13.7%	1.10 [0.49, 2.47]
Li XW 2011	18	20	8	15	8.1%	7.88 [1.33, 46.63]
Maeda S 2008	37	48	14	32	12.7%	4.32 [1.64, 11.41]
Mizukami T 2014	2	5	4	12	6.5%	1.33 [0.15, 11.50]
Tsukamoto N 2013	17	29	37	51	12.7%	0.54 [0.20, 1.40]
Wang X 2009	23	37	4	21	10.8%	6.98 [1.95, 25.01]
Wang YC 2014	30	39	4	13	10.1%	7.50 [1.86, 30.22]
Total (95% CI)		315		283	100.0%	3.15 [1.56, 6.36]
Total events	203		116			

Heterogeneity: Tau² = 0.77; Chi² = 27.16, df = 8 (P = 0.0007); I² = 71%
Test for overall effect: Z = 3.20 (P = 0.001)

Figure 4. Correlation of CD133 with lymph node metastasis.

Study or Subgroup	CD133 high Events	Total	CD133 low Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Cen G 2010	23	30	36	39	13.4%	0.27 [0.06, 1.17]
Chen K 2014	51	65	41	44	19.3%	0.27 [0.07, 0.99]
Hou YC 2014	39	42	51	54	5.9%	0.76 [0.15, 4.00]
Li XW 2011	9	26	8	9	14.3%	0.07 [0.01, 0.62]
Maeda S 2008	42	48	28	32	7.7%	1.00 [0.26, 3.87]
Mizukami T 2014	3	4	12	13	2.6%	0.25 [0.01, 5.26]
Tsukamoto N 2013	0	29	1	51	2.0%	0.57 [0.02, 14.46]
Wang X 2009	18	37	16	21	19.3%	0.30 [0.09, 0.98]
Wang YC 2014	19	39	11	13	15.5%	0.17 [0.03, 0.88]
Total (95% CI)		320		276	100.0%	0.32 [0.19, 0.54]
Total events	204		204			

Heterogeneity: Chi² = 6.54, df = 8 (P = 0.59); I² = 0%
Test for overall effect: Z = 4.27 (P < 0.0001)

Figure 5. Correlation of CD133 with clinical TMN stage.

Study or Subgroup	CD133 high Events	Total	CD133 low Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Cen G 2010	10	30	33	41	28.9%	0.12 [0.04, 0.36]
Chen K 2014	45	65	37	44	21.1%	0.43 [0.16, 1.12]
Hou YC 2014	36	42	42	54	8.2%	1.71 [0.58, 5.03]
Kim HS 2012	10	14	21	28	6.2%	0.83 [0.20, 3.52]
Li XW 2011	21	26	8	9	3.6%	0.53 [0.05, 5.22]
Maeda S 2008	48	50	31	32	2.4%	0.77 [0.07, 8.91]
Mizukami T 2014	4	5	7	12	1.3%	2.86 [0.24, 33.90]
Tsukamoto N 2013	28	29	42	51	1.6%	6.00 [0.72, 50.02]
Wang X 2009	22	37	17	21	13.7%	0.35 [0.10, 1.23]
Wang YC 2014	19	39	11	13	13.2%	0.17 [0.03, 0.88]
Total (95% CI)		337		305	100.0%	0.56 [0.37, 0.83]
Total events	243		249			

Heterogeneity: Chi² = 21.51, df = 9 (P = 0.01); I² = 58%
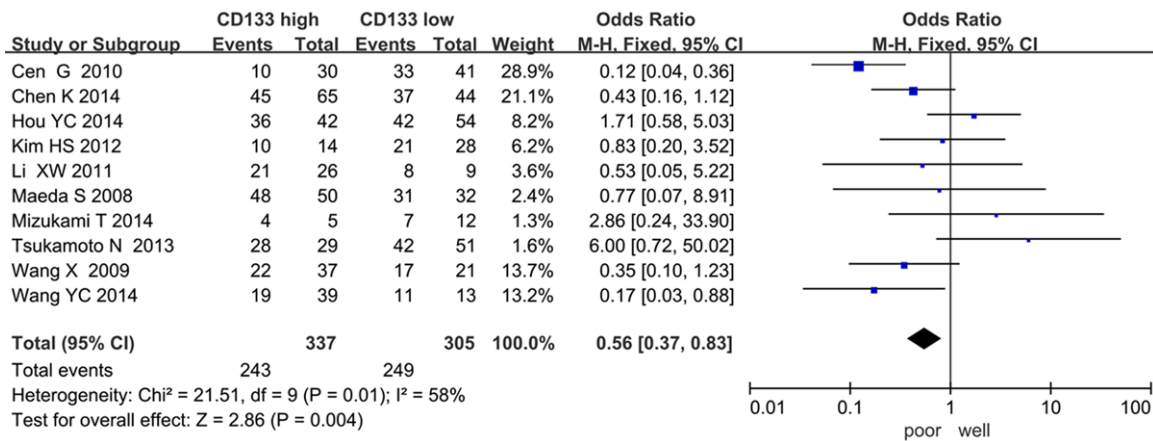Test for overall effect: Z = 2.86 (P = 0.004)

Figure 6. Correlation of CD133 with tumor differentiation.

reported that pre-operative therapy was performed on patients while the others had no relevant reports at all.

A total of 908 patients were included, most of them were male patients (> 478). In regarding to TNM stage, a median of 68.2% (1.25%-84.4%) patients were stage I or II, while the other 31.8% (15.6%-99.9%) were stage III or IV. Differentiated grading of tumor was reported in 11 studies and among those, roughly 23% were poorly differentiated. Around 53.3% (39.4%-74.3%) of reported patients were identified as metastatic lymph node status. Eleven reports

Table 3. Results of sensitivity analysis

Categories	Studies (n)	Effect model	HR (95% CI)	P value
Samples				
Whole tissue sections	9	random	0.54 (0.46-0.64)	< 0.00001
Tissue microarray	2	random	0.75 (0.53-1.07)	0.12
Staining patterns				
Membrane	6	random	0.60 (0.48-0.74)	< 0.00001
Cytoplasm	3	random	0.60 (0.41-0.87)	0.008
Membrane and Cytoplasm	2	random	0.42 (0.28-0.63)	< 0.00001
Cut off scores				
> 0%	5	random	0.66 (0.54-0.81)	< 0.00001
> 1%	1	fixed	3.59 (1.01-12.72)	0.0406
> 5%	2	random	0.52 (0.36-0.74)	0.0003
> 10%	1	fixed	0.68 (0.36-1.27)	0.032
Scores > 3	2	random	0.43 (0.26-0.70)	0.0008
Antibody				
Abcam	2	fixed	0.67 (0.47-0.95)	0.03
Santa Cruz	2	fixed	0.50 (0.34-0.73)	0.0004
Dako	2	fixed	0.51 (0.30-0.85)	0.01

pooled analysis, CD133 expression was significantly associated with lymph node metastasis (OR=3.15, 95% CI: 1.56-6.36, P=0.001 and I^2=71, random-effect), clinical TNM stage (OR=0.32, 95% CI: 0.19-0.54, P < 0.0001 and I^2=0, fixed-effect) and tumor differentiation (poor differentiation, OR=0.56, 95% CI: 0.37-0.83, P=0.004 and I^2=58, random-effect) in patients with PDAC.

Sensitivity analyses

We performed sensitivity analyses to investigate the potential impact of each study on pooled HR values. Three factors were included: whole tissue sections versus tissue microarray, membrane staining versus cytoplasm staining, and different cutoff scores. We observed that CD133 expression was associated with worse 5-year OS rate (HR=0.54, 95% CI=0.46-0.64, P < 0.00001) when IHC carried on the whole tissue sections. No significant difference was observed when IHC was carried out using tissue microarray (HR=0.75, 95% CI=0.53-1.07, P=0.12). When the staining patterns were considered, no evident publication bias existed. Five articles (cut off scores > 0%) and the other six articles (1 > 1%, 2 > 5%, 1 > 10% and 2 scores > 3, respectively) demonstrate significant difference between the CD133-high and CD133-low patients. An analysis was performed on the different antibodies used in the studies, which showed that no evident publication bias (**Table 3**).

Publication bias

We performed an analysis to evaluate the influence of individual studies on the summary of 5-year OS rate. In all included studies, no funnel plot asymmetry was found. Sensitive analysis was performed to investigate the effect of every study on the overall meta-analysis by omitting one study each time, and the omission of any study made no significant difference, demonstrating that our results were statistically reliable (**Figures 7, 8**).

used whole tissue sections for immunohistochemical analyses and four utilized tissue microarray.

CD133 overexpression and 5-year overall survival

We evaluated whether CD133 expression levels were associated with the overall survival in patients with PDAC. Of the 11 trials evaluable for systematic review, 11 and 4 could be included in meta-analysis by univariate and multivariate analysis effect of CD133 on overall survival due to sufficient data to estimate the HR and 95% CI. According to univariate analysis, CD133 overexpression was significantly associated with poor 5-year OS rate in a random-effects model (HR=0.58, 95% CI 0.49-0.67, P < 0.00001) (**Figure 2**). Furthermore, according to multivariate analysis, there was no significant difference between CD133-high and CD133-low groups in a random-effects model (HR= 0.73, 95% CI=0.52-1.03, P=0.07) (**Figure 3**).

CD133 overexpression and clinicopothological features

The forest plot of OR (odds ratio) was assessed for association between CD133 and clinicopathological features such as lymph node metastasis (**Figure 4**), clinical TNM stage (**Figure 5**), tumor differentiation (**Figure 6**). In
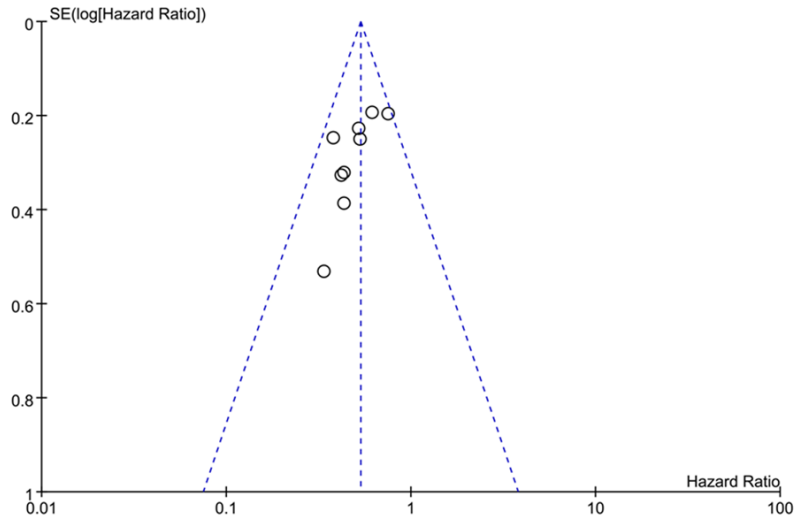
Figure 7. Funnel plots of publication bias: CD133 expression and OS rate by univariate analysis.
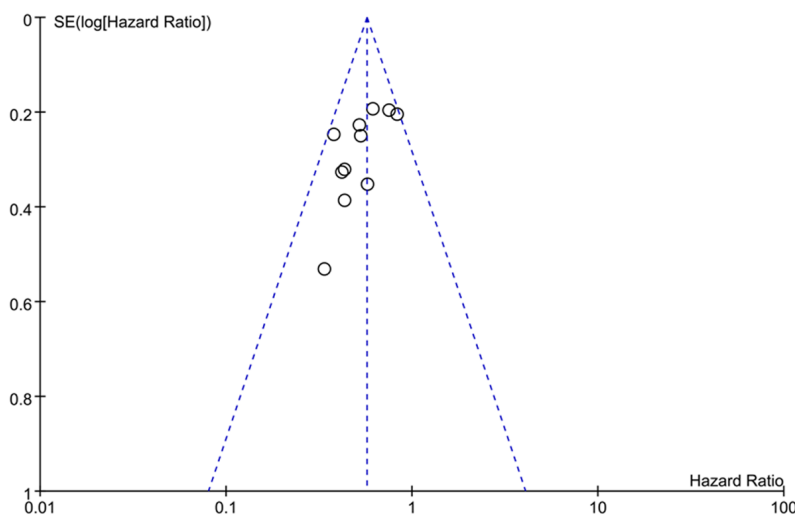

Figure 8. Funnel plots of publication bias: CD133 expression and OS rate by multivariate analysis.

Discussion

With a poor prognosis, pancreatic cancer poses a great burden in all countries [23]. Up to now, CEA and CA 19-9 are the most widely applied markers in gastrointestinal malignancies and elevated levels of both CEA and CA19-9 have also been suggested to be associated with poor prognosis in pancreatic cancer [24]. But approximately 5% of the population do not secrete CA19-9. Therefore, much effort has focused on enhancing the performance of CA19-9 by including it within larger panels of markers. Recently, CD133 is considered to be a potential prognostic marker in a number of cancers.

To get a better understanding of the relationship between CD133 expression and PDAC, this meta-analysis was carried out including 723 patients from 11 evaluable studies for prognostic value and 687 patients from 12 evaluable studies for clinicopathological features. In the present study, the pooled data revealed the CD133 expression in whole tissue sections, revealed a poor prognostic outcome in patients expressing high levels of CD133. With reference to clinicopathological features, CD133 expression was closely associated with tumor grades, tumor size, lymph node metastasis. Many articles have proved that CD133 was closely related with lymph node metastasis [25], which were well supported by our meta-analysis. It is believed that the correlation of lymph node metastasis were of high prognostic value. The results of our meta-analysis supported that the function of the lymph node metastasis might be dependent on CD133. Moreover, we found this marker to be inversely associated with tumor differentiation grade, in line with studies reporting both the presence of CD133 in different types of stem cells and in several solid tumors [26], and its down-regulated expression in differentiated cells. In accordance with some other types of malignances, CD133 overexpressed PDAC patients had lower 5-year overall survival in comparing to negative ones.

In our study, CD133 expression was associated with worse 5-year OS rate when IHC carried on the whole tissue sections, while the result of 2 reports detecting CD133 by tissue microarrays

showed no significant difference. A potential disadvantage of tissue microarrays sections compared with full tissue sections is that donor cores may not be representative of the whole tumor.

A large variance is also noted for the cytoplasmic or membrane CD133 positivity. Maeda et al. [9] observed cytoplasmic CD133 positivity in up to 15% of tumor cells, while Welsch et al. [27] reported that the prominent expression pattern of CD133 was an apical membrane staining. In the present study, 11 reports with cytoplasmic, membranous or combining with cytoplasmic CD133 staining, 5-year OS rate was poorer in CD133-high expression group. CD133 staining type had no significant effect on the prognosis of PDAC.

Although immunohistochemistry was the most commonly applied method, the cutoff value was defined differently in these studies. In our study, we found that cutoff score also had no significant effect on the prognosis of PDAC.

Although the data revealed the positive correlation between CSCs marker CD133 and PDAC patients' survival, certain limitations in our meta-analysis need to be pointed out. First, we did not include unpublished papers and abstracts into meta-analysis because the required data was available only in full publications. The OR of each study is generally small and the conclusion might be affected by one or two reports with large ORs. Secondly, all those studies are mostly based on Asian population. There is little study on this topic based on Western populations. Thirdly, although we tried to identify all relevant data, another potential source of bias is related to the method used to extrapolate the HR. HR was extracted from the data included in the article directly or calculated from the survival curves. Actually, 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. Fourthly, most studies didn't provide supplemental chemo- or radio-therapy data which also influenced the prognosis of PDAC patients. All of these factors might partly influence the significance of CD133 expression in the survival and the clinicopathological analysis.

Although the relationship between CD133 and pancreatic cancer stem cell remains obscure,

emerging evidence have disclosed the potential correlation. The present meta-analysis indicates that CD133 expression is associated with a poor OS in PDAC patients. Given the insights from the strong correlations between CD133 and prognosis/clinicopathological features, it could help in the development of strategies to pancreatic cancer. Higher quality studies with more volume of patients are needed to clarify the clinicopathological factors associated with CD133-positive pancreatic cancer and its impact on survival outcomes. More importantly, an improved knowledge in cancer biology and cancer stem cell can further potentiate the induction of new targeted therapeutics.

Disclosure of conflict of interest

None.

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