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RESEARCH ARTICLE

The prognostic value of CD133 expression in non-small cell lung cancer: a meta-analysis

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Abstract CD133 has been identified as a potential cancer stem cell (CSC) marker in non-small cell lung cancer (NSCLC). However, the clinical and prognostic significance of CD133 in NSCLC remains controversial. In this study, a meta-analysis with a total number of 13 studies was performed to clarify the association between CD133 expression and clinical outcomes in publications up to June 2013. Odds ratios (ORs) and their 95 % confidence intervals (CIs) were used to assess the association between CD133 expression and the clinicopathological characteristics of NSCLC. Hazard ratios (HRs) and their 95 % CI were used to quantify the predictive ability of CD133 on NSCLC prognosis. Analysis of these data showed that CD133 expression was not associated with any clinicopathological parameters except for histology (pooled OR=1.35, 95%CI=1.04–1.76, P=0.024) and tumor differentiation (pooled OR=3.19, 95%CI=1.10–9.21, P=0.032). Simultaneously, we also found that positive CD133 expression was not associated with disease-free survival (DFS) (pooled HR=1.76, 95 % CI=0.87–3.57, P=0.114, random-effect) but was associated with overall survival (OS) (pooled HR=2.06, 95 % CI=1.08–3.91, P=0.027, random-effect). Overall, it is appropriate to regard CD133 expression as a potential prognostic factor for the OS of NSCLC patients.

Keywords CD133 · NSCLC · Clinicopathological characteristics · Prognosis

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Introduction

Lung cancer (LC) remains the most frequent and the most lethal human malignancy worldwide [1], and among all LC cases, NSCLC accounts for approximately 85 % [2]. Significant diagnostic and therapeutic improvements have been made for NSCLC; however, the prognosis is still suboptimal, with an overall five-year survival rate of less than 15 % [3]. CSCs are defined as a distinct subpopulation of cancer-initiating cells, possessing the properties of self-renewal, asymmetric cell division [4]. In the last decade, there has been increasing evidence that CSCs play a pivotal role in drug resistance, tumor regeneration, and metastasis [5, 6]. A correlation has identified that in terms of chemoresistance and bad prognosis of NSCLC patient, tumor samples enriched with CSCs [7]. Recently, specific biomarkers of CSCs have been identified by major efforts, such as CD44, aldehyde dehydrogenase, CD133 [8].

CD133, also known as prominin-1, is a cell-surface glycoprotein comprising five transmembrane domains and two large glycosylated extracellular loops [9]. CD133 has been used as a potential CSC marker to identify subpopulations of cells in brain [10], colon [11], and some lung tumors [12]. Some studies showed that CD133 positive is associated with poor prognosis of some cancers such as gastric cancer [13], colon cancer [14], and also NSCLC patients [15–17]. However, conflicting results on the detection, tumorigenicity, clinical pathologic features, and progression of CD133 positive tumor cells [9, 18] indicate limited availability of samples which has resulted in variations in the clinical significance of the results. The present meta-analysis was conducted to determine the association between CD133 and common clinical

and pathologic features of NSCLC, considering the putative role of stem cells in the prediction of outcome in NSCLC.

Materials and methods

Search strategy, inclusion criteria, and data collection

The electronic database of PubMed was searched for studies that investigated the association of clinicopathological parameters and prognosis with CD133 expression in NSCLC to be included in the present meta-analysis. Studies were examined on papers imposed with an updating search on June 31, 2013. Search terms were (AC133 [all fields] OR AC-133 [all fields] OR (AC [all fields] AND 133 [all fields]) OR CD-133 [all fields] OR CD133 [all fields]) OR (CD [all fields] AND 133 [all fields]) OR "AC133 antigen" [supplementary concept] OR "AC133 antigen" [all fields] OR "prominin 1" [all fields] OR PROM1 [all fields] OR PROM-1 [all fields]) AND ("lung neoplasms" [MeSH terms] OR ("lung" [all fields] AND "neoplasms" [all fields]) OR "lung neoplasms" [all fields] OR ("lung" [all fields] AND "cancer" [all fields]) OR "lung cancer" [all fields]). The published studies that were eligible for inclusion in this meta-analysis should meet the following criteria: (1) the histological type of the tumors was NSCLC, and (2) it assesses the relationship between CD133 expression and clinical parameters or/and survival, and has been published as an English full paper. Studies that did not meet all inclusion criteria were excluded from our analysis.

Data extraction

To minimize the bias and to improve the reliability, two reviewers checked all potentially relevant studies independently. The following characteristics were extracted from eligible studies: name of first author, name of journal, year of publication, sample size, test method, cutoff value, gender, smoking status, histological type, depth of invasion, lymph node metastasis, metastasis, tumor grade, stage, differentiation, and HR estimation and its 95 % CI. When HR and its 95 % CI were described in original articles, these values were obtained directly. When these statistical variables were not provided, they were extracted and calculated from Kaplan-Meier survival curve by the HR digitizer software Engauge 4.0.

Statistical methods

Odds ratios (ORs) with 95 % CI were used to estimate the association between CD133 expression and the general prognostic markers for NSCLC, including gender, smoking status, histological type, depth of invasion, lymph node metastasis, metastasis, tumor grade, stage, and differentiation. Pooled HR with 95 % CI was used to estimate the relationship of CD133 expression with survival of NSCLC. Based on the sample size of the DFS (762 cases) and OS (1065 cases), 1.76 and 2.06 difference HRs between CD133 low and high expression levels with both power of 1.0 could be determined, respectively. The statistical heterogeneity within studies was tested with the chi-squared-based Q-test ($P>0.10$) and I^2 ($I^2<50$ %, no heterogeneity); fixed-effects model was used with absence of heterogeneity across studies. Otherwise, the random-effects model was used. Evidence of publication bias was sought by Egger's and Begg's

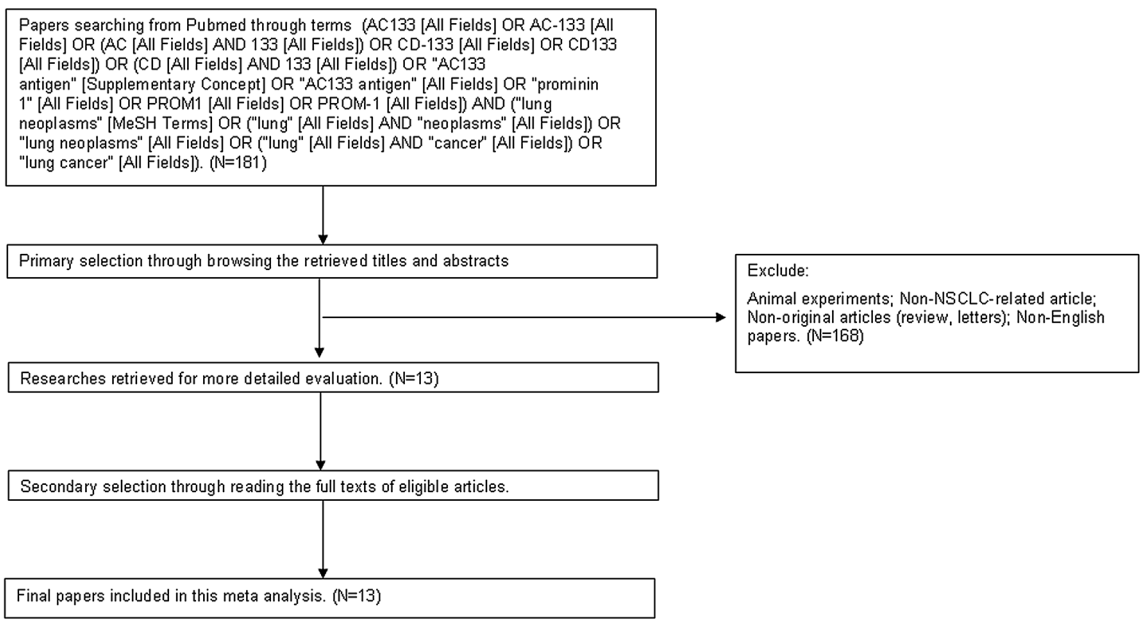

Fig. 1 Flow chart for selection of studies for inclusion in this meta-analysis

Table 1 Main characteristics and results of the eligible studies

No. of paper	First author	Journal	Year	Country	methods	Cutoff	Number of patients	HR estimation
(1)	A. V. Salnikov	Int J Cancer	2009	German	IHC	<20 %	88	Survival curve
(2)	Y. H. Xu	Saudi Med J	2010	China	IHC	<10 %	102	HR+95%CI
(3)	M. Janikova	Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub	2010	Czech	IHC	0	116	Survival curve
(4)	J. P. Sullivan	Cancer Res	2010	USA	IHC	0	207	HR+95%CI
(5)	T. Woo	Int J Clin Exp Pathol	2011	Japan	IHC	<17.5 %	177	HR+95%CI
(6)	E. Herpel	Anticancer Res	2011	German	IHC	0	86	HR+95%CI
(7)	F. Li	Med oncol	2011	Japan	IHC	<=1 %	145	HR+95%CI
(8)	K. Okudela	Pathol Int	2012	Japan	IHC	<=17.5 %	177	HR+95%CI
(9)	S. W. Wu	BMC Cancer	2012	China	IHC	<=1	305	HR+95%CI
(10)	L. C. Dericks	Eur J Cardiothorac Surg	2012	Italy	qPCR	<1	61	HR+95%CI
(11)	K. Shien	Lung Cancer	2012	Japan	IHC	<=1 %	29	No available date
(12)	H. Mizugaki	Int J Clin Oncol	2013	Japan	IHC	0	161	HR+95%CI
(13)	L. D. Li	J Exp Clin Cancer Res	2013	China	qPCR	–	73	No available date

IHC immunohistochemistry, *qPCR* quantitative real-time polymerase chain reaction

test. The significance of the intercept was determined by the *t* test suggested by Egger and Begg ($P<0.05$ was considered representative of statistically significant publication bias). All the statistical analyses were performed using Stata/SE 10.0 for Windows (Stata Corporation, College Station, TX, USA).

Results

Description of studies

Combined search in PubMed on the terms indicated in *Materials and methods* retrieved 181 hits, and when excluding animal experiments, non-NSCLC-related studies, non-original English articles, or lack of data on the association of CD133 expression with clinicopathological features and/or overall survival, only 13 publications met the inclusion criteria for the present analysis (Fig. 1). Ten studies reported the association between CD133 expression and the different clinicopathological parameters. When assessing the pooled HR and its 95%CI, two studies were excluded because of insufficient information to calculate the HR. Of these studies, the sample sizes ranged from 29 to 305 patients. Expression of CD133 was evaluated by Immunohistochemistry (IHC) in 11 studies, by quantitative real-time polymerase chain reaction (qPCR) in 2 studies. In 9 of the 11 studies, the useful data for calculation of overall HR were obtained directly from the original articles, and in 2 studies, HRs and 95%CIs were extrapolated from Kaplan-Meier survival curves. Table 1 showed all the studies included in the meta-analysis in detail.

Correlation of CD133 expression with clinicopathological characteristics

The main results of the meta-analysis were summarized in Table 2. The results showed that CD133 was not associated

Table 2 Main results for meta-analysis between CD133 and clinicopathological parameters

Association between CD133 and clinical parameters	No. of studies	Pooled OR (95%CI)	*P*	Heterogeneity test (Q, I^2,)
Sex (female vs. male)	(1)(2)(4)(6)(7)(9)(10)(11)(12)	0.93 (0.70–1.23)	0.597	5.90, 0.0 %, 0.597(fixed-effect)
Smoking status (no vs. yes)	(2)(4)(7)(10)(11)(12)	0.72 (0.49–1.05)	0.085	7.97, 37.3 %, (fixed-effect)
Histology (SCC vs. ADC)	(1)(2)(4)(6)(7)(9)(11)(12)(13)	1.35 (1.04–1.76)	0.024	8.70, 8.1 %, (fixed-effect)
Depth of invasion (T1/2 vs. T3/4)	(1)(2)(6)	1.14 (0.62–2.09)	0.675	0.61, 0.0 %, (fixed-effect)
Lymph node metastasis (N0 vs. N1)	(2)(4)(6)(9)(10)(12)	1.57 (0.78–3.14)	0.205	17.44, 71.3 %, (random-effect)
Metastasis (no vs. yes)	(1)(2)	0.57 (0.25–1.28)	0.174	0.15, 0.0 %, (fixed-effect)
Stage (I/II vs. III/IV)	(1)(4)(7)(9)	1.30 (0.24–7.14)	0.765	39.5, 92.4 %, (random-effect)
Grade (G1/2 vs. G3)	(6)(7)(10)	0.79 (0.43–1.48)	0.466	3.36, 40.5 %, (fixed-effect)
Differentiation (well vs. moderate and poor)	(2)(9)(12)	3.19 (1.10–9.21)	0.032	7.98, 74.9 %, (random-effect)

SCC squamous cell carcinomas, *ADC* adenocarcinomas

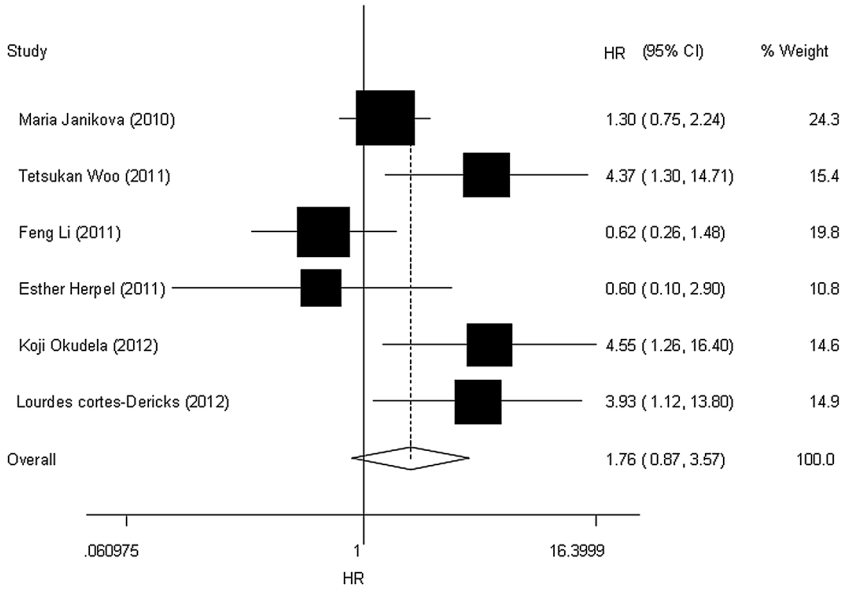

Fig. 2 Forest plot showed that the CD133 expression was not associated with DFS of NSCLC

with clinicopathological parameters such as sex, smoking status, depth of invasion, lymph node metastasis, metastasis, tumor stage, and grade, except for histology (pooled OR= 1.35, 95%CI=1.04–1.76, P=0.024) and tumor differentiation (pooled OR=3.19, 95%CI=1.10–9.21, P=0.032).

Impact of CD133 expression on DFS and OS of NSCLC

The different results obtained from previous studies on the impact of CD133 expression on DFS and OS. The pooled HR of the DFS was 1.76 (95 %CI=0.87–3.57, P=0.114, random-effect, Fig. 2), with an I^2 of 62.9 %. When limiting the terminal event to OS, pooled HR was 2.06 (95 %CI=1.08–3.91, P=0.027 random-effect, Fig. 3), with an I^2 of 87.6 %.

Test for heterogeneity

There was significant heterogeneity for survival evaluation (I^2= 62.9 % for DFS; I^2=87.6 % for OS). Then, we assessed the source of heterogeneity for additive model by population (Asian vs. the others) and CD133 cutoff value (IHC<=1 % vs. IHC <10 %). The results were shown in Table 3. For the DFS, cutoff value (χ^2=8.64,P=0.003), but not population (χ^2=0.11,P=0.736), was found to contribute to substantial heterogeneity. Moreover, high CD133 expression with <10 % cutoff level seemed to be a worse prognosis factor compared with low CD133 expression (pooled HR=4.27, 95%CI=2.08–8.79). Nevertheless, these significant difference was not found between the CD133 high or low when the cutoff value was IHC <=1 % (pooled HR=0.97, 95%CI=0.57–1.64).

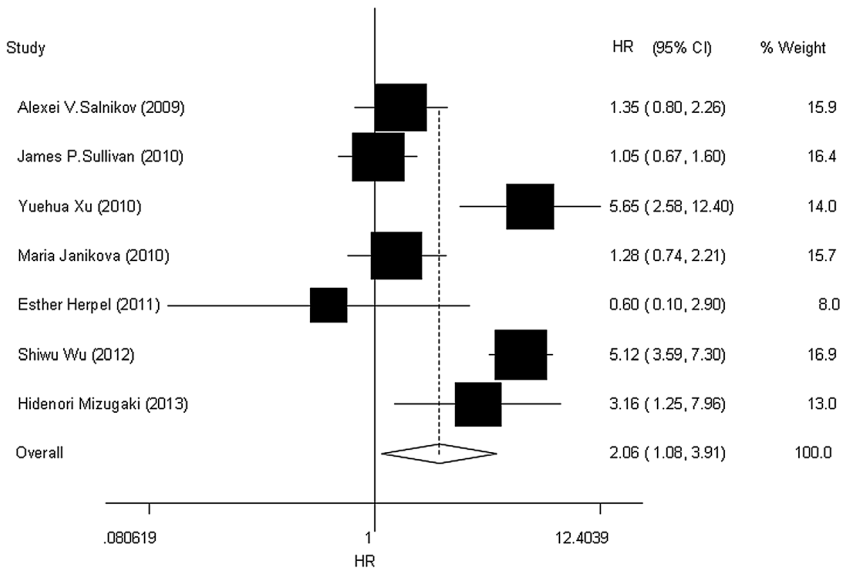

Fig. 3 Forest plot showed that the CD133 expression was associated with OS of NSCLC

Table 3 Heterogeneity analysis for survival

End points of survival	Subgroups	Pooled HR (95%CI)	I^2	Studies (n)	Heterogeneity test (χ^2, P)
DFS	Population				0.11, 0.736
	Asian	2.17 (0.53–8.99)	79.4 %	3	
	Non-Asian	1.53 (0.66–3.54)	44.9 %	3	
	Cutoff value				8.64, 0.003
	IHC<=1 %	1.01 (0.65–1.58)	16.5 %	3	
	IHC <10 %	4.45 (1.85–10.8)	0.0 %	3	
OS	Population				46.00, <0.001
	Asian	1.17 (0.86–1.55)	0.0 %	4	
	Non-Asian	4.93 (3.63–6.69)	0.0 %	3	
	Cutoff value				16.19, <0.001
	IHC<=1 %	1.59 (0.73–3.50)	79.8 %	4	
	IHC <10 %	2.81 (1.10–7.19)	88.4 %	3	

DFS disease free survival, OS overall survival

Simultaneously, population (χ^2=46.00,P<0.001) and cutoff value (χ^2=16.19,P<0.001) were found to associated with heterogeneity for OS. Moreover, high CD133 expression on the subgroups of non-Asian population (pooled HR=4.93, 95%CI=3.63–6.69) and IHC <10 % cutoff level (pooled HR=2.81, 95%CI=1.10–7.19) was more significantly prognostic parameter than low CD133 expression for OS. However, no difference had been found in the other corresponding subgroups.

Publication bias

Begg's funnel plot with pseudo 95 % confidence limits and Egger's publication bias plot were performed to estimate the publication bias of the included literature about DFS (Fig. 4a) and OS (Fig. 4b). Begg's and Egger's test did not reveal any evidence of obvious asymmetry in the overall meta-analysis of all studies.

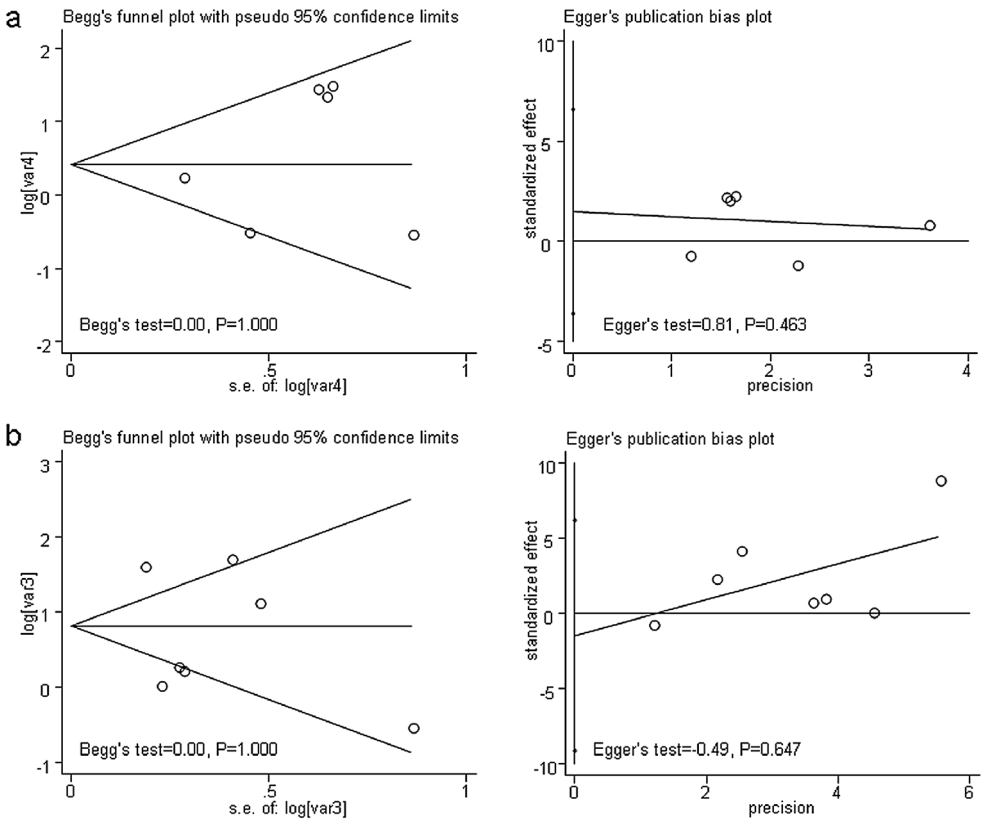

Fig. 4 Begg's and Egger's funnel plot estimated the publication bias of the included literature for DFS (**a**) and OS (**b**)

Discussion

Knowledge regarding altered expression of genes during carcinogenesis may result in the discovery of biological markers for the evaluation of cancer diagnosis and prognosis, which may aid the improvement of diagnosis, treatment, and prevention of malignancies [19]. Recent studies have showed that some functional molecules may modulate response to platinum-based chemotherapy and serve as promising biomarkers for individualized chemotherapy of NSCLC patients [20, 21]. There has been great interest in identifying prognostic and predictive markers for patients with NSCLC helping guide clinical decision-making regarding therapy and outcomes.

CD133 has been used as a potential CSC marker. Recently, it has been reported that CD133 expression may serve as a promising biomarker in prognosis of colorectal and gastric cancers [22, 23]. Liu et al found that CD133 positive cells of NSCLC displayed CSCs characteristics of greater sphere-forming ability, cisplatin resistance, and migration ability [24]. Recent study also shows that NSCLC contains CD133 expressing cells, which is essential for tumor cell propagation and metastasis [25]. Moreover, CD133 expression was positively associated with poor prognosis [15, 26]. However, the conflicting results were also reported [9, 18]. Therefore, we performed this meta-analysis to identify the association between CD133 and clinicopathological outcomes, which showed that CD133 high expression was not positively correlated with any clinicopathological parameters except for adenocarcinoma (ADC) and tumor poor differentiation, which was accordant with recent founding that CD133 expression is more frequently upregulated in ADC than squamous cell carcinoma (SCC) patients [18]. Simultaneously, we also found that CD133 high expression was not associated with DFS but significantly associated with a worse OS compared with low CD133 expression, which was supported by the notions of the previous studies [15, 27].

This systematic review has some limitations. Firstly, the number of included studies, as well as the included NSCLC patients in each study, was relatively small. Secondly, though no significant heterogeneity across studies was detected in our study, we could not fully neglect potential heterogeneity. The subgroup analysis was used to assess the sources of heterogeneity. We found cutoff value was more contributed to heterogeneity both in DFS and OS. Thirdly, the methods used for the assessment of the level of CD133 expression and the cutoff values of defining the specimens as positive CD133 expression in NSCLC patients differed among these studies. The results changed according to the cutoff value. Therefore, some new studies with same cutoff values must be recruited and combined for further evaluation. Fourthly, non-original English articles were excluded in our analysis, which may introduce bias. However, articles published in other languages can be more difficult to locate, and may require translation, which will delay the conclusion of a review. Therefore, additional studies with the larger sample sizes, high quality, and different ethnic background are needed to make a more definitive conclusion.

In summary, our meta-analysis showed that high CD133 expression was not correlated with clinicopathological parameters except for histology and tumor differentiation. Simultaneously, CD133 overexpression predicted a poor overall survival. Therefore, it is appropriate to regard CD133 expression as a potential prognostic factor for NSCLC patients.

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Conflicts of interest None

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