Clinicopathological significance of CD133 in lung cancer: A meta-analysis

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Abstract. CD133 is one of the most commonly used markers of lung cancer stem cells (CSCs), which are characterized by their ability for self-renewal and tumorigenicity. However, the clinical value and significance of CD133 in lung cancer remains controversial. Due to the limited size of the individual studies, the association between CD133 and the clinicopathological characteristics of lung cancer had not been fully elucidated. A meta-analysis based on published studies was performed with the aim of evaluating the effect of CD133 on the clinicopathological characteristics of lung cancer and to investigate the role of CSCs in the prognosis of lung cancer. A total of 15 eligible studies were included in this meta-analysis and our results indicated that a positive CD133 expression was significantly associated with poor differentiation and lymph node metastasis, although it was not associated with tumor stage or histological type. Therefore, CD133 may be considered as a prognostic maker of lung cancer. Further clinical studies, with larger patient samples, unified methods and cut-off levels to detect CD133 expression, classified by tumor stage, therapeutic schedule, follow-up time and survival events, are required to determine the role of CD133 in clinical application and the association between CD133 and the prognosis of lung cancer.

Introduction

Lung cancer remains the most common cause of cancer-related mortality worldwide (1). Patients affected by lung cancer exhibit a poor prognosis. Despite the significant advances in surgical techniques, chemotherapy and radiotherapy, the relapse rate is high and only a few patients achieve long-term survival, with an overall 5-year survival rate of ~15% (2). Evidence supports the cancer stem cell (CSC) hypothesis, according to which CSCs may be responsible for tumor initiation, metastasis, recurrence and resistance to treatment (3,4). Due to the characteristics of CSCs, the conventional therapies are unable to effectively eliminate these cells. The residual CSCs continue to proliferate, leading to the relapse of cancer. A variety of molecules have been investigated as putative markers of CSCs in malignancies, including lung cancer (5). Among the various markers, CD133 is one of the most commonly used. It is widely expressed in a number of malignancies, such as glioblastomas (6), hepatocellular (7), ovarian (8), colon (9) and lung (10) carcinomas.

A growing number of CD133-positive cancer cells have been identified in lung cancer (11,12). Eramo *et al* (10) observed that a rare population of CD133-positive cancer stem-like cells were able to self-renew and generate an unlimited progeny of non-tumorigenic cells, whereas the CD133-negative cancer cells lacked this potential. However, the association between CD133 expression and the clinicopathological characteristics of lung cancer remains unknown. Attempts to elucidate the association between CD133-positive cancer cells and clinicopathological characteristics in previous studies yielded controversial results (13,14). The limited sample availability resulted in discrepancies regarding the clinical significance determined by different CSCs studies. Therefore, we performed a systematic review of the literature with a meta-analysis to determine the association between CSCs marker CD133 and the clinicopathological characteristics of lung cancer and to investigate the role of CSCs in the prognosis of lung cancer.

Materials and methods

Literature search. Studies were identified via an electronic search through Medline, EMBASE and the China National Knowledge Infrastructure (CNKI) databases, using the key words 'lung cancer' and 'CD133', completed by the personal bibliography of two of the authors (Yaoxi Tan and Bo Chen). The bibliographies reported in all the identified studies were used to complete the study search. Review articles were scanned to identify additional eligible studies. The search was completed on November 15, 2012. To be eligible for inclusion in this systematic review, a study was required to meet the following criteria: i) it only included patients with primary lung cancer;

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Table I. Main characteristics of the 15 eligible studies.

First author	Year	Histological type	Method	Cut-off level	No. of patients	No. of case groups	Refs.
Shien	2012	NSCLC	IHC	>1% staining	30	9	(23)
Bertolini	2009	NSCLC	IHC	Strong staining	58	32	(13)
Salnikov	2010	NSCLC	IHC	Any staining	88	56	(28)
Cortes-Dericks	2012	AD	RT-PCR	Median	64	63	(18)
Li	2011	NSCLC	IHC	>1% staining	145	46	(22)
Herpel	2011	NSCLC	Tissue microarray	Diffuse expression or distinct staining in at least 1 out of 4 tissue cores per sample	86	13	(19)
Xu	2011	NSCLC	IHC	Any staining	103	51	(14)
Wei	2008	NSCLC	IHC	>10% staining	77	40	(26)
Li	2011	N/E LC	IHC	>10% staining	90	44	(21)
Yao	2010	LC	IHC	>10% staining	42	31	(27)
Lin	2009	SQ	IHC	Any staining	54	27	(29)
Gu	2010	NSCLC	IHC	>10% staining	44	30	(30)
Cheng	2010	NSCLC	IHC	>10% staining	65	45	(31)
Sun	2012	NSCLC	IHC	Method described by Xu and Yang (33)	67	42	(32)
Tirino	2009	NSCLC	Flow cytometry	NA	89	64	(20)

NSCLC, non-small-cell lung cancer; AD, adenocarcinoma; LC, lung cancer; SQ, squamous cell carcinoma; N/E LC, neuroendocrine lung cancer; IHC, immunohistochemistry; RT-PCR, reverse transcriptase-polymerase chain reaction; NA, not available.

ii) it investigated the association between CD133 and clinico-pathological characteristics; iii) it was published as a full-text article in English or Chinese and; iv) it reported the number of CD133-positive and -negative patients. When duplicate studies were published, only the most recent or most informative was included in the analysis, to avoid overlap between cohorts.

Data extraction. The following information was extracted from each study: i) year of publication and first author's name; ii) sample size, test method and cut-off level; iii) tumor data including stage, grade, histological type and lymph node metastasis. Information was carefully extracted from all the eligible studies independently by two of the authors of the present study (Yaoxi Tan and Bo Chen). Differences in the extraction of data were assessed by a third investigator (Jianqing Wu).

Statistical analysis. To assess the association between CD133 and the clinicopathological characteristics of lung cancer including stage, grade, histological type and lymph node metastasis, odds ratios (ORs) with 95% confidence intervals (CIs) were calculated. The heterogeneity of combined ORs was initially evaluated by graphical examination of the forest plots. Statistical assessment was then performed using a χ^2-based test of homogeneity and evaluation of the inconsistency index (I^2) statistic. The I^2 statistic was defined as the percentage of variability due to heterogeneity rather than chance, with values >50% representing the possibility of substantial heterogeneity (15). P<0.05 was considered to indicate a

statistically significant difference. If no obvious heterogeneity existed, the OR was calculated by the fixed-effects model (the Mantel-Haenszel method) and χ^2 tests. Otherwise, the random-effects model (the DerSimonian-Laird method) was used. In addition, evidence of publication bias was determined using the Egger's (16) and Begg's methods (17). All the calculations were performed using the Stata statistical software version 12.0 (StataCorp, College Station, TX, USA).

Results

Study characteristics. In total, 15 studies published between 2008 and 2012 were selected for this systematic review. The study sample size ranged from 30 to 145 subjects. All studies investigated the association between CD133 and the clinicopathological characteristics of lung cancer. Cortes-Dericks *et al* (18) used reverse transcriptase-poly-merase chain reaction (RT-PCR) to detect the expression of CD133, whereas Herpel *et al* (19) used tissue microarray, Tirino *et al* (20) used flow cytometry and the remaining studies used immunohistochemistry (IHC) with different cut-off levels. A total of 11 studies investigated non-small-cell lung cancer (NSCLC) alone, one study investigated adenocarcinoma and one squamous cell carcinoma. One study included adenocarcinoma, squamous cell carcinoma and small-cell lung cancer (SCLC). Li *et al* (21) conducted the study on patients with neuroendocrine lung cancer. The main characteristics of the 15 eligible publications are presented in Table I.
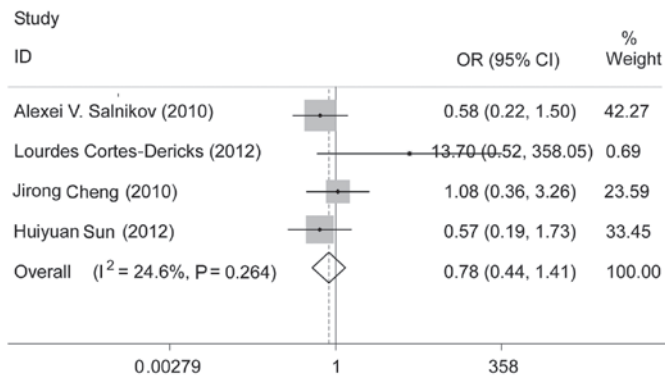
Figure 1. Correlation of CD133 with tumor stage.

Figure 2. Correlation of CD133 with tumor differentiation.

Main results of the meta-analysis

Correlation of CD133 with tumor stage. Among the selected studies, 8 analyzed the association between CD133 and tumor stage. Cortes-Dericks *et al* (18) and Li *et al* (21) observed that positive CD133 expression exhibited a significant association with tumor stage. Of the 8 studies, one study (22) was limited to stage I and one study (19) assessed patients with stage I-II disease. Two studies (13,21) were performed on stage I-IV lung cancer of different histological types. Excluding the four studies mentioned above, we performed a meta-analysis of the studies which investigated stage I-III cancer. A comparison of stage I-II to stage III revealed that there was no association between a positive CD133 expression and tumor stage (pooled OR=0.78, 95% CI: 0.44-1.41 and P=0.411) (Fig. 1). There was no evident publication bias (Egger's test, P=0.089), a finding also supported by the Begg's funnel plots (figure not shown).

Correlation of CD133 with tumor differentiation. Eleven studies investigated the association between CD133 and tumor differentiation. Three of those studies (13,14,18) concluded that positive CD133 expression was associated with poorly differentiated tumors. Significant heterogeneity (pooled OR=1.17, 95% CI: 0.68-2.00 and P=0.567; random effects, I^2=55.3% and P=0.013) existed when we analyzed all 11 studies. Excluding the studies using RT-PCR (18) and tissue microarray (19), we performed a subgroup analysis among the studies using IHC. However, significant heterogeneity (pooled OR=1.39, 95% CI: 0.81-2.37 and P=0.229; random effects, I^2=52.2% and P=0.033) was still evident. We excluded one study (13) which was conducted on European patients and analyzed the studies that investigated Asian patients using IHC. The comparison of poor to high tumor differentiation revealed that a positive CD133 expression was significantly correlated with poor differentiation (pooled OR=1.66, 95% CI: 1.15-2.40 and P=0.006) without significant heterogeneity (I^2=23.5% and P=0.242) (Fig. 2). No evident publication bias existed (Egger's test, P=0.684), a finding also supported by the Begg's funnel plots (figure not shown).

Correlation of CD133 with histological type. A total of 12 studies were found to be eligible for the analysis of the association between a positive CD133 expression and histological type. Of these, Bertolini *et al* (13) observed a correlation between a positive CD133 expression and adenocarcinoma. We excluded two more studies, one of which (18) investigated adenocarcinoma alone, while the other (21)
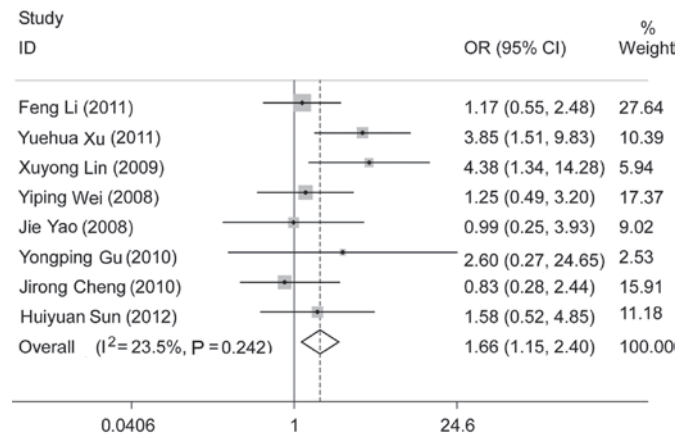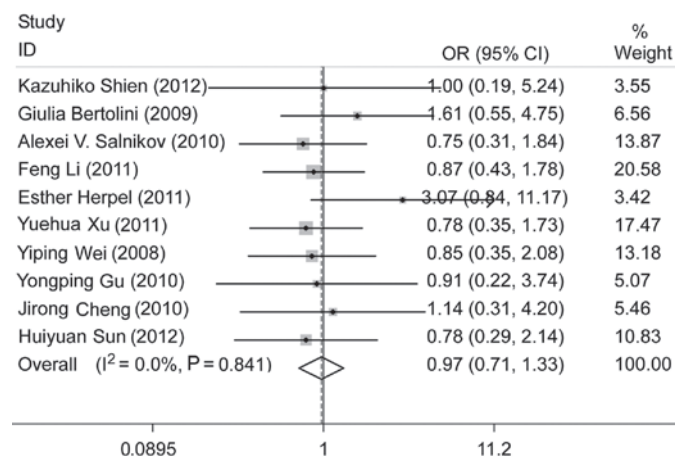

Figure 3. Correlation of CD133 with histological type.

investigated neuroendocrine lung cancer. We analyzed the remaining 9 studies which were conducted on patients with NSCLC. There was no significant association between positive CD133 expression and histological type (adenocarcinoma vs. non-adenocarcinoma), with pooled OR=0.97, 95% CI: 0.71-1.33 and P=0.86 (Fig. 3). No evident publication bias existed (Egger's test, P=0.143), a finding also supported by the Begg's funnel plots (figure not shown).

Correlation of CD133 with lymph node metastasis. Eleven publications investigated the association between CD133 expression and lymph node metastasis. One study (23), which was limited to patients with N2 or N3 NSCLC undergoing induction chemoradiotherapy (CRT), was excluded. We observed that CD133 was associated with nodal status (pooled OR=2.30, 95% CI: 1.36-3.89 and P=0.002), with a positive CD133 expression in tumors with lymph node metastasis (N+ compared to N0) (Fig. 4). No evident publication bias existed (Egger's test, P=0.639), a finding also supported by the Begg's funnel plots (figure not shown). However, there was significant heterogeneity. We performed a subgroup analysis among the studies that used IHC and observed that positive CD133 expression was associated with nodal status (pooled OR=2.98, 95% CI, 2.04-4.35 and P<0.001), without significant heterogeneity (Fig. 5).
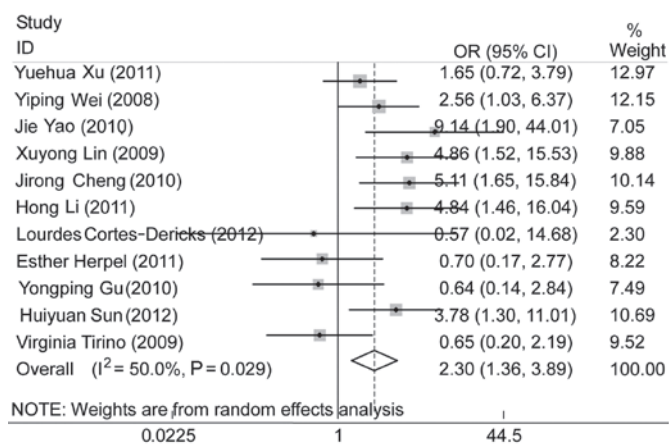
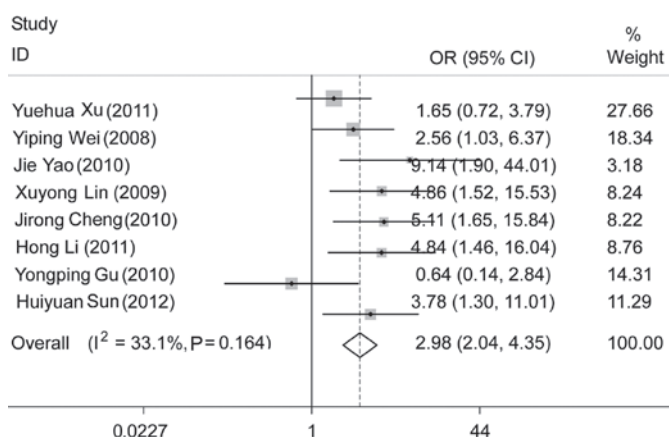Figure 4. Correlation of CD133 with lymph node metastasis.

Figure 5. Subgroup analysis of the correlation of CD133 with lymph node metastasis.

Discussion

Accumulating evidence indicates that specific subpopulations of cancer cells with stem cell characteristics within the majority of tumors may be crucial in the pathogenesis of malignant tumors, including lung cancer. Several methods were used to identify and enrich CSCs from lung cancer, e.g., side populations and stem cell markers of normal tissue (24). CD133 is one of the most extensively used markers in lung cancer, although its function in lung cancer has not been fully elucidated. It remains controversial whether CD133 is associated with clinicopathological characteristics and prognosis of lung cancer. The aim of this current meta-analysis was to investigate the correlation between CD133 expression and the clinicopathological characteristics in patients with lung cancer, since therapeutic decisions are directly associated with clinicopathological characteristics, such as tumor type and differentiation. Our results indicated that a positive CD133 expression was significantly associated with differentiation and lymph node metastasis, although it was not associated with tumor stage or histological type.

Several studies (14,18,21,23,25-27) reported that CD133 expression was correlated with poor prognosis in lung cancer, whereas other authors (13,19,22,28) observed no such

association. The patient populations varied among these studies. For example, Herpel *et al* (19) conducted the study on previously untreated stage I-II NSCLC patients, while Shien *et al* (23) investigated patients with locally advanced N2 or N3 NSCLC, who underwent induction CRT followed by surgery. The methods used to detect CD133 expression were also different, including IHC, RT-PCR and tissue microarray. Even among the studies using IHC, the cut-off level and the antibodies used varied widely. In addition, certain studies reported disease-free survival, whereas others reported overall survival with different follow-up times. Therefore, due to these considerable differences, we were not able to directly perform a systematic review to assess the correlation between CD133 expression and the prognosis of lung cancer.

The studies mentioned previously provided important information regarding the indirect prognostic value of CD133 and the therapy targeting CSCs in lung cancer. Eramo *et al* (10) observed that CD133-positive stem-like cancer cells were capable of self-renewal. The injection of immunocompromised mice with CD133-positive lung cancer cells readily generated tumor xenografts phenotypically identical to the original tumor, leading to the conclusion that CD133 was a reliable marker of lung cancer. We inferred that there was a larger percentage of CSCs in the majority of lung cancer tissue, with a higher number of CD133-positive lung cancer cells. One of the properties of CSCs was the ability to undergo asymmetrical division, leading to pluripotential differentiation and metastasis (28). This finding was consistent with the results of our meta-analysis, suggesting that a positive CD133 expression was clearly associated with poor tumor differentiation and lymph node metastasis. Poor differentiation and metastasis were significantly associated with poor survival of cancer. In brief, positive CD133 expression was most likely correlated with poor prognosis of lung cancer. It should also be noted that, since CD133 positivity was correlated with the expression of resistance-related proteins (28), the proportion of CSCs in patients who had received radiotherapy and/or chemotherapy was higher compared to that in patients who received surgery alone. As a result, it was not clear whether CD133 expression was of higher prognostic value in patients who received radiotherapy and/or chemotherapy. Further studies are required to elucidate the direct association between CD133 expression and the prognosis of lung cancer.

A meta-analysis is a quantitative approach in which individual study findings on the same topic are statistically integrated and analyzed. With more samples, the results of a meta-analysis are more reliable compared to those of a single study. However, the present meta-analysis had certain limitations. When analyzing whether CD133 expression was associated with tumor differentiation or lymph node metastasis, there was significant heterogeneity. The methods used to detect CD133 expression varied widely among the studies and heterogeneity was eliminated following exclusion of the studies that did not use IHC. Although IHC was the most commonly applied method, the cut-off level was defined differently among the studies. In addition, the IHC results were based on the primary antibody used and different antibodies were used by the eligible studies. The dilution of the antibody also varied, leading to differences in the sensitivity of the method, depending on the antibody concentration. Another factor was

the effect of ethnicity. The majority of the studies included were conducted on Asian patients. There is the possibility that different ethnic groups exhibit differences in CD133 expression, leading to heterogeneity and bias.

Although there was no evident publication bias in this meta-analysis, we were not able to completely exclude biases. For example, the study was restricted to studies published in English and Chinese, which may lead to bias.

In conclusion, our meta-analysis suggests that CD133 expression is significantly associated with poor differentiation and lymph node metastasis in lung cancer. CD133 expression is most likely correlated with poor prognosis of lung cancer. However, further studies are required, with larger patient samples, unified methods and cut-off levels to detect CD133 expression, classified by tumor stage, therapeutic schedule, follow-up time and survival events, to confirm the findings of the present meta-analysis.

Acknowledgements

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