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Original Article

Prognostic value of ALDH1 expression in lung cancer: a meta-analysis

Wei Huo, Min Du, Xinyan Pan, Xiaomin Zhu, Zhimin Li

Department of Medical Oncology, Dalian Municipal Central Hospital, Dalian, Liaoning, China

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Abstract: Objective: ALDH1 has recently been reported as a marker of cancer stem-like cells in lung cancer. However, the predictive value of ALDH1 in lung cancer remains controversial. In this study, we aimed to evaluate the association of ALDH1 expression with the clinicopathological features and outcomes of lung cancer patients through a meta-analysis. Methods: Publications that assessed the clinical or prognostic significance of ALDH1 in lung cancer up to October 2014 were identified. A meta-analysis was performed to clarify the association between ALDH1 expression and clinical outcomes. Results: Ten eligible publications with 1836 patients were included. The analysis of these data showed that ALDH1 expression was highly correlated with lymph node metastasis (pooled OR = 1.45, 95% CI: 1.04-2.02, P = 0.027), decreased overall survival (pooled RR: 2.25, 95% CI: 1.15-4.41, P = 0.019), and decreased disease-free survival (pooled RR: 1.63, 95% CI: 1.01-2.64, P = 0.047). Conclusion: Patients with ALDH1-positive lung cancer had poor prognosis, which was associated with common clinicopathological poor prognostic factors.

Keywords: Lung cancer, cancer stem cells, aldh1, outcome

Introduction

Primary lung cancer is one of the most common malignancies in the United States, with an estimated 215,020 new cases, comprising approximately 15% of new cancer diagnoses, and 161,840 deaths, accounting for nearly 29% of all cancer-related deaths in 2008 [1]. Despite the advances in diagnosis and treatment in the last few decades, lung cancer prognosis remains very poor, with five-year survival rate of 15% [2]. One of the most important reasons for such poor prognosis is the lack of an early and putative diagnostic biomarker to detect lung cancer. An increasing number of studies have shown that tumor progression is related to a small population of cancer cells, known as cancer stem cells (CSCs), which have the capabilities of multi-differentiation and self-renewal [3]. This hypothesis led to the investigation of CSCs, which might be associated with the clinical outcomes of cancer.

The ALDH1 superfamily represents a diverse group of enzymes that metabolize and detoxify various endogenous and exogenous aldehydes and oxidize retinol to synthesize retinoic acid, which is an important modulator of cell differentiation [4]. ALDH1 activity and/or antigen expression have been demonstrated to be strong in stem cell fractions in various cancers, which suggests that ALDH1 participates in maintaining CSCs. Over the past decade, several studies have evaluated the prognostic value of ALDH1 expression in lung cancer with conflicting results. Some concluded that ALDH1 expression exerts a favorable influence on survival [5], whereas others reported that ALDH1 expression is predictive of decreased survival outcome for lung cancer [6, 7]. We conducted a systematic review and meta-analysis to evaluate the association of ALDH1 expression with the common clinicopathological features and lung cancer patient outcomes.

Methods

Publication search

The studies were identified by searching the PubMed, Embase, and Web of Science databases. The studies eligible for this analysis were those that were updated on October 2014 with the use of the search terms “aldehyde

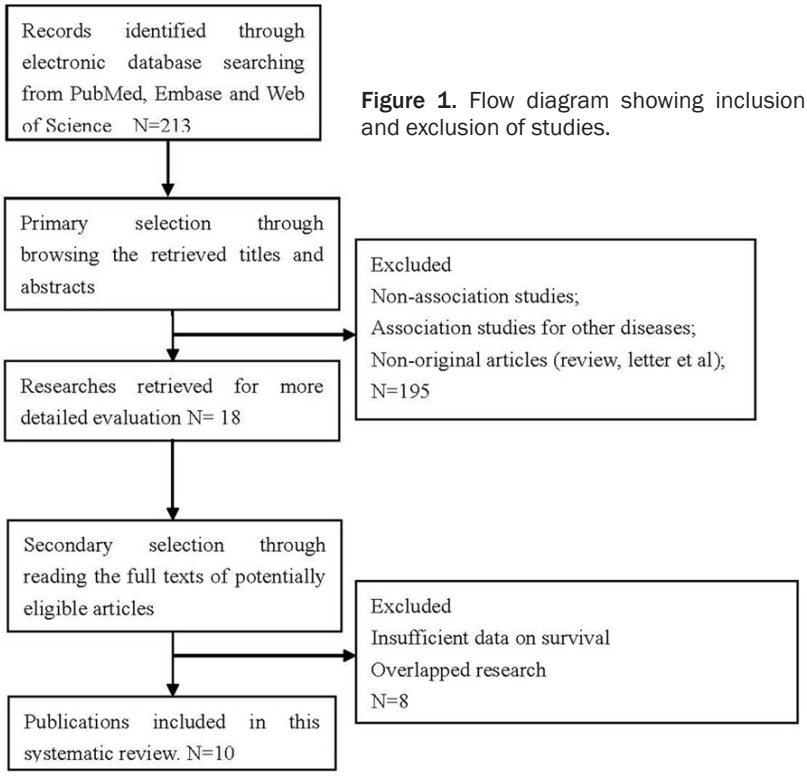

Figure 1. Flow diagram showing inclusion and exclusion of studies.

dehydrogenase 1" or "ALDH1" and "lung cancer" or "NSCLC" or "SCLC."All eligible studies were retrieved, and their bibliographies were checked for other relevant publications. Additional papers and book chapters were identified by a manual search of the references from the key articles. The search results were then screened according to the following inclusion criteria: (a) evaluation of the association between ALDH1 expression and either overall survival (OS) or prognostic factors of lung cancer, (b) inclusion of sufficient data to enable the estimation of an odds ratio (OR) with a 95% confidence interval (95% CI) or a relative risk (RR) of OS, and (c) English language publications. Letters to the editor, reviews, and articles published in a book or papers were excluded. The following information was extracted from each publication and used as a supplement, if available: author, publication year, country of the patient, tumor stage, number of patients, research technique used, and cutoff value of ALDH1. A lower limit of number of patients included in each study was not set for inclusion in the meta-analysis. Two of the authors of the present study carefully extracted the information from all eligible publications independently. Differences in the extraction of data were checked by a third investigator.

Statistical analysis

ORs with 95% CI were used to estimate the association between the expression of ALDH1 and the general prognostic markers for lung cancer, including smoking status, degree of differentiation, tumor TNM stage, and lymph node status. RR was used to assess the association of ALDH1 expression and survival outcome combined over studies. For RRs that were not provided directly in the published articles, the published data and figures from original papers were used to assess the RR according to the methods described by Parmar et al. [8]. The heterogeneity assumption was calculated by using a Q-test, and *P*-values greater than 0.05 indicated a lack of heterogeneity among studies. Thus, OR and RR were calculated by a fixed-effect model (Mantel-Haenszel method and chi-squared tests). Otherwise, a random-effect model (DerSimonian-Laird method) was used. The influence of individual studies on the summary effect estimate was determined through a sensitivity analysis. In addition, funnel plots and Egger's test were used to estimate the possible publication bias [9]. Kaplan-Meier curves were read by GetData Graph Digitizer 2.24. All statistical analyses were performed using Stata 12.0 for Windows (Stata Corporation, College Station, TX, USA).

Results

Study characteristics

Ten publications met the criteria for this analysis [5, 8, 10-17] (**Figure 1**). In the study of Dimou et al. [5], the ORs were presented separately according to a US study and a Greek study. Therefore, each study in the publication

Table 1. Characteristics of the included studies

Study	Patient's country	Year	Tumor stage	Histological type	Technique	Number of patients	cut-off for ALDH1 positive	Survival analysis
Jiang	USA	2009	I-IV	NSCLC	IHC	96	> 10% staining	OS
Sullivan	USA	2010	I-IV	NSCLC	IHC	282	ND	OS
Li X	China	2012	I-IV	LC	IHC	50	> 10% staining	OS
Cortes-Dericks	Italy	2012	I-III	AD	qRT-PCR	64	Median	DFS
Dimou1	USA	2012	I-IV	NSCLC	Immunofluorescence	134	an AQUA score of 1200	DFS
Dimou2	Greece	2012	I-IV	NSCLC	Immunofluorescence	296	an AQUA score of 1200	DFS
Okudela	Japan	2012	I	AD	IHC	177	> 85% staining	DFS
Shien	Japan	2012	III	NSCLC	IHC	150	> 10% staining	OS; DFS
Alamgeer	Australia	2013	I	NSCLC	IHC	267	> 10% staining	OS; DFS
Okudela	Japan	2013	I-IV	NSCLC	IHC	268	Scores of ≥ 10	DFS
Zenke	Japan	2013	I-IV	NSCLC	IHC	52	> 10% staining	DFS

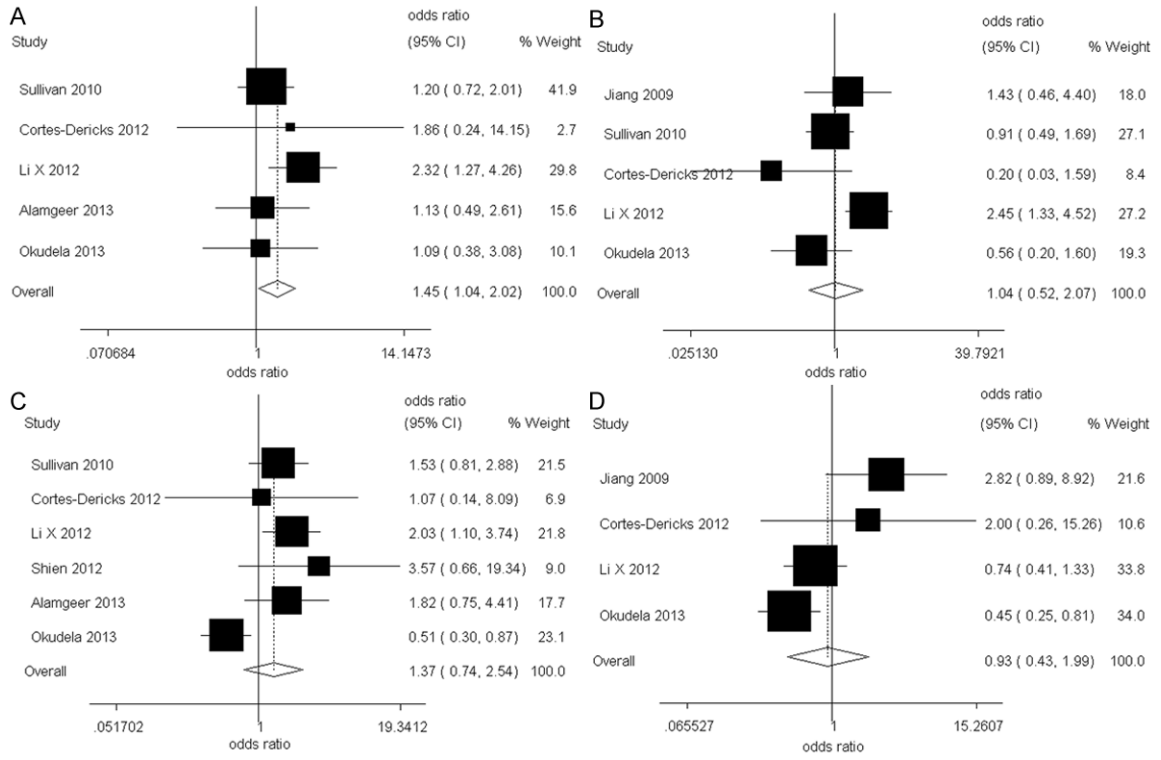

Figure 2. Forrest plot of ORs for the association of ALDH1 expression with the (A) lymph node metastasis. (B) Tumor TMN classification. (C) Smoking status. (D) Tumor grade.

was considered separately for analysis. Thus, 11 studies were involved in the meta-analysis. The main characteristics of the eligible studies are summarized in **Table 1**. Our analysis involved 1836 patients, ranging from 50 to 296 per study. Seven articles dealt with clinicopathological factors. All 11studies determined OSordisease-freesurvival(DFS).Immunohistochemistry (IHC) was the main method used to investigate ALDH1 expression in lung cancer specimens.

Correlation of ALDH1 expression with clinicopathological parameters

The association between ALDH1 and several clinicopathological parameters is illustrated in **Figure 2**. ALDH1 expression was highly correlated with lymph node metastasis (pooled OR = 1.45, 95% CI: 1.04-2.02, P = 0.027 fixed effect) (**Figure 2A**). However, ALDH1 expression was not associated with tumor TMN classification (pooled OR = 1.04, 95% CI: 0.52-2.07, P =

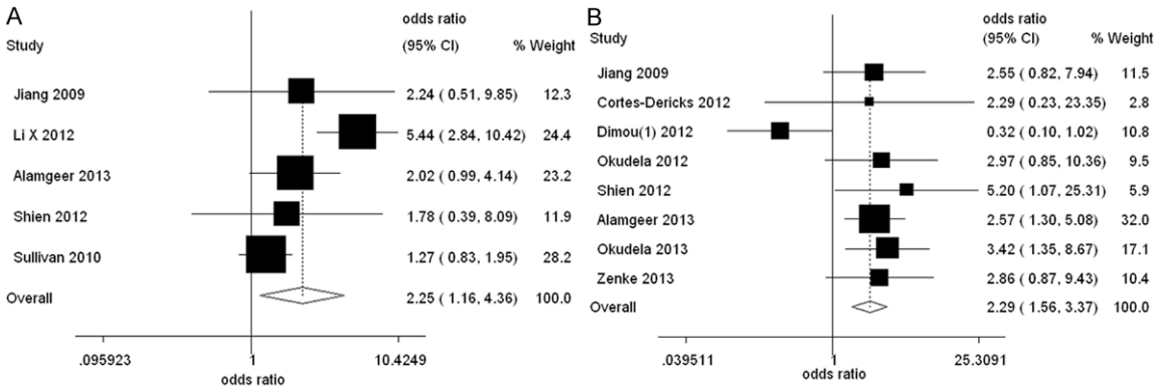

Figure 3. Analysis of ALDH1 expression and survival of lung cancer patients. Forest plot of RR for OS (A) and DFS (B) among included studies. Combined RR was calculated by a random mode.

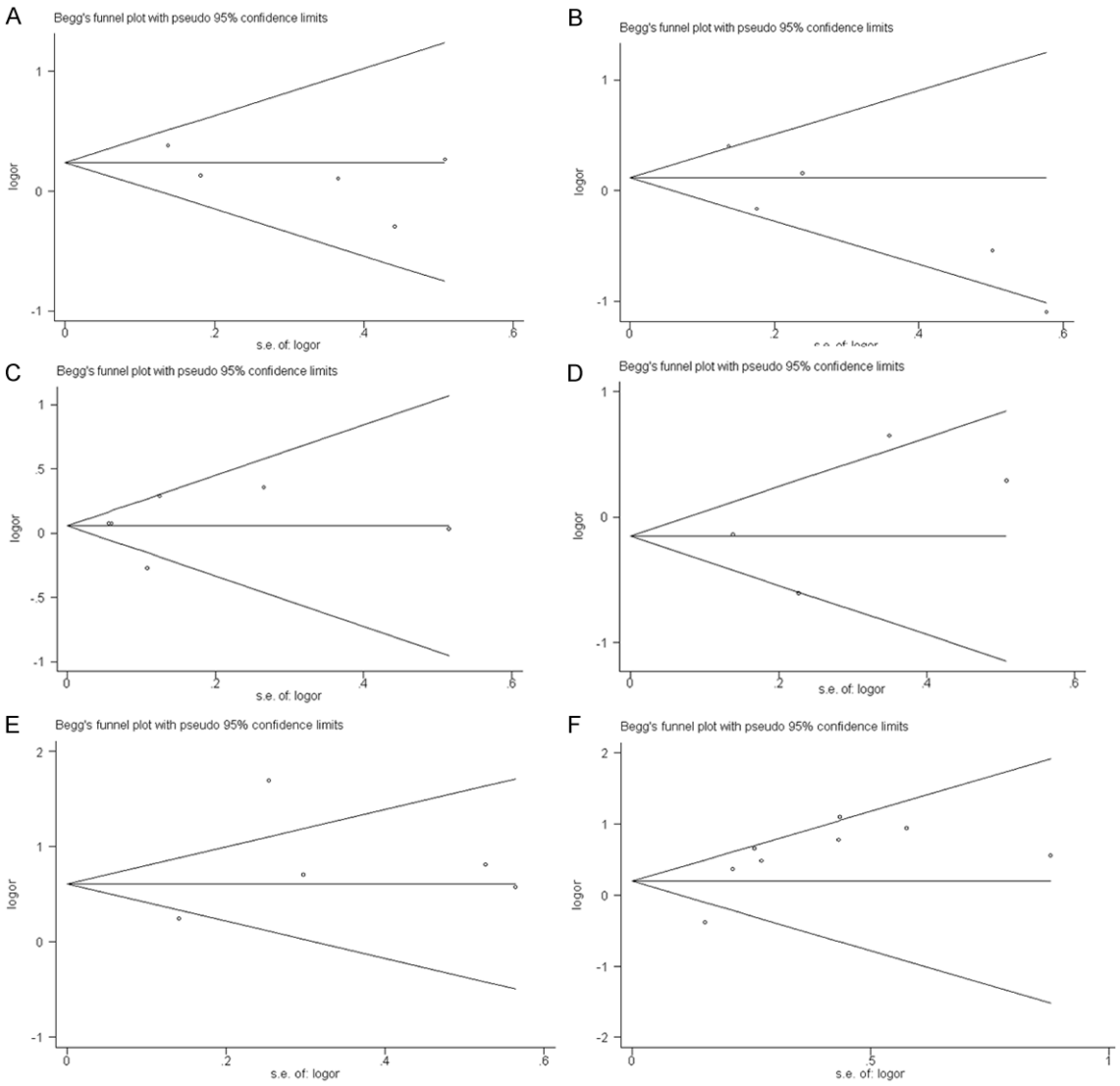

Figure 4. Funnel plots for publication bias. All the graphical funnel plots appeared to be symmetrical. A. Lymph node metastasis. B. Tumor TMN classification. C. Smoking status. D. Tumor grade. E. OS. F. DFS.

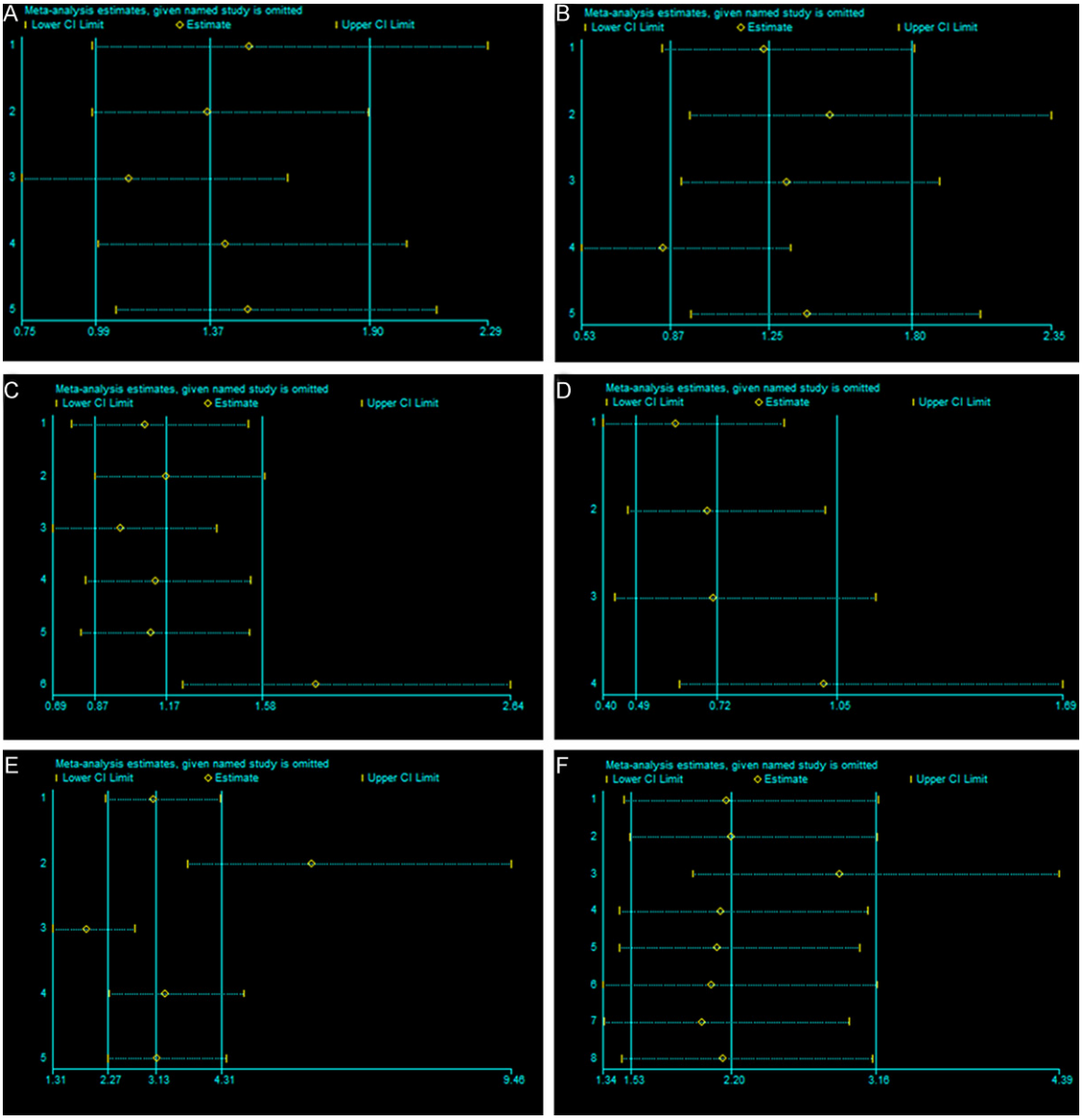

Figure 5. Sensitivity analyses of all the studies. A. Lymph node metastasis. B. Tumor TMN classification. C. Smoking status. D. Tumor grade. E. OS. F. DFS.

0.914 random effect) (**Figure 2B**), smoking status (pooled OR = 1.37, 95% CI: 0.74-2.54, P = 0.62 random-effect) (**Figure 2C**), or tumor grade (pooled OR = 0.93, 95% CI: 0.43-1.99, P = 0.846 random-effect) (**Figure 2D**).

ALDH1 expression and 5-year survival outcome

The OS and/or DFS of 1836 patients in 11 studies were analyzed by using the methods described earlier. The main results of this meta-analysis are shown in **Figure 3**. Five-year OS rate was extracted from five studies. The meta-analysis of the five studies for the prognostic value of ALDH1 expression showed that ALDH1 expression was associated with poor OS. This finding was obtained from DerSimonian-Laird random-effect model with a value of 2.25 (95% CI: 1.15-4.41, P = 0.019) (**Figure 3A**), although heterogeneity existed among studies (I^2 = 84.9%, Ph = 0.000).

ALDH1 expression and DFS in lung cancer

The meta-analysis of eight applicable studies showed that ALDH1 expression was associated with poor DFS (RR: 1.63, 95% CI: 1.01-2.64, P = 0.047; **Figure 3B**), despite the fact that the

studies displayed heterogeneity (I^2 = 79.9%, Ph = 0.000) (**Figure 3B**).

Publication bias and sensitivity analysis

No significant publication bias existed in any of the clinicopathological parameters because the value of P > 0.05 in Egger's test (**Figure 4A-E**). Moreover, no evidence of obvious publication bias existed in OS (Egger's test, P = 0.052) (**Figure 4F**). This finding was strong evidence verifying that ALDH1 was an independent prognostic factor for patients with lung cancer.

To gauge result stability, a sensitivity analysis was performed. One study was deleted. All sensitivity analyses assessing the clinicopathological parameters were not obviously changed. Moreover, the result showed that pooled RRs of OS and DFS were not significantly changed, suggesting the robustness of our results (**Figure 5A-F**).

Discussion

The present meta-analysis is the first study to estimate the association between stem cell marker ALDH1 and lung cancer survival systematically. The presence of both significant and nonsignificant studies addresses the importance of stem cells in lung cancer. Thus, performing a quantitative aggregation of the survival results is necessary. The present results indicate that stem cell marker ALDH1 is significantly associated with lymph node metastasis, as well as with OS and DFS. The results suggest that this marker could be developed for clinical applications.

ALDH1 belongs to the aldehyde dehydrogenase superfamily, which is responsible for the oxidation of aldehydes to their corresponding carboxylic acids [18]. Previous studies have demonstrated that ALDH1 positive tumor cells possess the CSC phenotype, which contributes to self-renewal and tumorigenic capabilities [19]. ALDH1 overexpression results in increased cell proliferation and, interestingly, increased resistance to chemotherapeutic agents [20]. However, controversies remain as to whether a correlation exists between ALDH1 expression and either poor prognosis or the clinicopathological parameters in patients with lung cancer. Dimou et al. [5] demonstrated that patients with non-small cell lung cancer (NSCLC) with high expressions of ALDH1 survive longer and have lower recurrence rates. Jiang et al. [6] showed that high ALDH1 expression is associated with poor prognosis in patients with early-stage NSCLC. Similarly, Sullivan et al. [18] showed that ALDH1expression has a negative effect on survival in their cohort, although this effect was not independent in the proportional hazards model.

The possible reason for the discrepancy is that no unique scoring standard was used to evaluate the immunostaining results. In the articles by Dimou et al., clone 44 was used forALDH1 detection, as validated by Western blot analysis for specificity [21]. In another two studies [6, 19], subjective determinations of expression were used, and cases were classified as positive or negative on the basis of a semiquantitative rule that uses the product of the percentage of cell positive and the intensity of staining after pathology review.

Although our study revealed the positive correlation of CSC marker ALDH1 and lymph node metastasis with the survival of patients with lung cancer, ALDH1 itself as a biomarker has its limitations in predicting prognosis and clinicopathological parameters in patients. First, OS and DFS were determined from unadjusted RRs in the published papers, and RRs from the survival curves might be less reliable than those from direct analysis of variance. Ideally, measurements should be directly obtained from the statistical data in published papers and then adjusted by using other prognostic factors. Second, using a standard threshold to assess biomarkers is of great importance. Although IHC was the most commonly applied method, differences in cutoff values for positive ALDH1 expression may have contributed to the observed heterogeneity. Third, the OR of each study is generally small, and the conclusion might be affected by one or two reports with large ORs. All of these factors might partly influence the significance of ALDH1 expression in the survival and clinicopathological analysis.

In summary, this meta-analysis indicated that ALDH1 expression was associated with lymph node metastasis in lung cancer. Moreover, ALDH1-positiveexpression was associated with a worse outcome than that stemming from ALDH1-negative expression, and ALDH1 was an independent factor associated with reduced survival. The relative simplicity of the methodology for the use of ALDH1 expression to identify CSCs suggests that this marker should be fur-

ther evaluated for its potential use in identifying lung cancer stem cells in clinical practice.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Zhimin Li, Department of Medical Oncology, Dalian Municipal Central Hospital, 42 Xuegong Street, Shahekou-District, Dalian 116033, China. E-mail: zhiminli72@126.com

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