

Diagnostic performance of SHOX2 promoter methylation as biomarker for lung cancer identification: A meta-analysis update

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Abstract

Objective: To evaluate the diagnostic performance of short state homeobox 2 (SHOX2) promoter methylation as biomarker for lung cancer identification through aggregating the open published data.

Methods: We did an electronic search in Pubmed, EMBASE, Ovid, Web of Science, Google Scholar, and the China National Knowledge Infrastructure (CNKI) to identify the publications related to SHOX2 promoter methylation and lung cancer. The diagnostic performance of sensitivity (SEN), specificity (SPE), odds ratio (DOR), and summary receiver operating characteristic (SROC) curve were aggregated by fixed or random effect model. Fagan's nomogram was used to investigate the post-test diagnostic probability. Deek's funnel plot and line regression test was applied to evaluate the publication bias.

Results: In total, 18 clinical studies about SHOX2 promoter methylation and lung cancer were included in the meta-analysis. The combined diagnostic SEN, SPE, DOR were 0.63 (95% CI = 0.54–0.70), 0.91 (95% CI = 0.87–0.94), and 16.84 (95% CI = 11.18–25.36) in random effect model respectively. The pooled area under the curve (AUC) of SROC was 0.88 (95% CI = 0.84–0.90). The post-test probability of lung cancer was 88% and 29% when SHOX2 methylated and unmethylated in humoral components given a pre-test probability of 50%. Deek's funnel plot and regression test indicated no publication bias ($p = 0.62$).

Conclusion: SHOX2 promoter methylation in humoral components may be a potential biomarker for lung cancer diagnosis with relative high diagnostic specificity.

KEYWORDS

diagnosis, lung carcinoma, meta-analysis, methylation

INTRODUCTION

Genetic abnormality and changes play an important role in carcinogenesis.¹ Epigenetic change is one of the molecular mechanisms that reflects the early stage of tumorigenesis.^{2,3} As an important way of epigenetics, DNA methylation plays an important role in controlling gene expression, maintaining genomic stability, cell differentiation, and embryonic development.^{4,5} In addition, DNA methylation is considered as an early event in the process of malignant transformation of cells.^{6–8} Therefore, detection of DNA abnormal methylation in tumor cells

becomes an important method for early diagnosis of carcinoma.

Lung cancer is the most common carcinoma of human beings and the leading cause of cancer related death globally.⁹ The general prognosis of lung cancer is poor because of the lack of effective tools for lung cancer identification in early stages. Recently, studies have shown DNA methylation detection in body fluid such as plasma and bronchoalveolar lavage fluid (BALF) as a promising method for lung cancer early identification.^{10,11}

Short state homeobox 2 (SHOX2), also known as homeobox protein OG12X, is encoded by SHOX2 gene,

which encodes 60 amino acid residues representing DNA binding domain. Homeobox proteins are considered as transcription regulators widely expressed in organisms and have complex biological functions. In recent years, it has been reported that the abnormal methylation of SHOX2 gene promoter region was related to the occurrence and development of lung cancer.^{12,13} The abnormal methylation of this gene may occur in the early stage of lung cancer and is related to the inactivation of this gene after methylation. Studies have also reviewed the occurrence rate of methylation in SHOX2 gene promoter region in lung cancer patients and found that SHOX2 methylation rate was higher in cancer cases than normal controls. The finding indicated that SHOX2 methylation detection may provide promising diagnostic information for lung cancer.^{14,15}

MATERIAL AND METHODS

Electronic publication search

Two authors (X.Z. and X.L.) did an electronic search of Pubmed, EMBASE, Ovid, Web of Science, Google Scholar, and the China National Knowledge Infrastructure (CNKI) to identify the publications related to SHOX2 promoter methylation and lung cancer. The following key words were applied for publication searching: “SHOX2”, “Short stature homeobox 2”, “OG12”, “SHOT”, “OG12X”, “non-small cell lung cancer”, “lung cancer”, “lung neoplasm”, “carcinoma, non-small cell lung”, “methylation”, and “hypermethylation”. The references of the included publications were also reviewed to find potential suitable studies.

Study selection criteria

The following study inclusion criteria were applied: (i) the SHOX2 promoter methylation rate was examined in plasma, BALF, or pleural effusion by MSP or other confirmed methods; (ii) studies were published in English or Chinese; (iii) SHOX2 methylation rate in lung cancer cases and controls can be extracted; and (iv) lung cancer diagnosis was confirmed by cytology or pathology. The exclusion criteria included: (i) duplicated publications or data; (ii) SHOX2 methylation was detected in cancer and normal lung tissue; and (iii) autologous controls were applied.

Data extraction

Two reviewers (J.L. and H.H.) made data extraction independently. The extracted data was listed as follows: (i) study ID showed as first author; (ii) time period of the data published; (iii) area the study performed; (iv) methylation rate in lung cancer and controls; (v) tissue type such as plasma, BALF, or pleural effusion; (vi) lung cancer stages of the included cases; and (vii) methylation detection methods.

Statistical methods

All data was managed by STATA12.0 statistical software (Stata Corp LP). The diagnostic sensitivity = true positive / (true positive + false negative), specificity = true negative / (true negative + false positive). The summary receiver operating characteristic (SROC) curve was applied to evaluate the feasibility of SHOX2 promoter methylation as biomarker for lung diagnosis. Fagan's nomogram was used to investigate the post-test diagnostic probability. Deek's funnel plot and line regression test were used to evaluate the publication bias. Two-tailed $p < 0.05$ was considered statistically significant.

RESULTS

Publication searching and main features of the included studies

In total, 766 articles were initially obtained from the relevant electronic databases. Forty-five duplicate articles or data were removed, and 721 publications were reviewed. After reading the titles and abstracts, 689 studies were eliminated because of obvious unqualification. Finally, 18 clinical studies relevant to SHOX2 promoter methylation as biomarker for lung cancer were included for meta-analysis (Figure 1). The main features of the 18 studies were demonstrated in Table 1.

Statistical heterogeneity among the included studies

The statistical heterogeneity among studies was evaluated by Q-test and expressed by I^2 . There were significant statistical heterogeneity among studies in sensitivity (SEN) ($Q = 182.75$, $I^2 = 89.06\%$, $p < 0.01$), specificity (SPE) ($Q = 89.32$, $I^2 = 77.61\%$, $p < 0.01$), and odds ratio (DOR) ($I^2 = 75.50\%$, $p < 0.01$). Therefore, the data of SEN, SPE, and DOR were aggregated in random effect model.

Meta-analysis

The combined diagnostic SEN, SPE, and DOR were 0.63 (95% CI = 0.54–0.70), 0.91 (95% CI = 0.87–0.94) (Figures 2), and 16.84 (95% CI = 11.18–25.36) (Figure 3) in random effect model. The pooled area under the curve (AUC) of SROC was 0.88 (95% CI = 0.84–0.90) (Figure 4). Fagan's nomogram indicated that if SHOX2 methylated in humoral components, the positive post-test probability of lung cancer was 88%, whereas the negative post-test probability was 29% when SHOX2 unmethylated in humoral components given a pre-test probability of 50% (Figure 5).

Publication bias evaluation

The Deek's funnel plot was general symmetrical and the line regress test did not indicated publication bias ($p = 0.62$) (Figure 6).

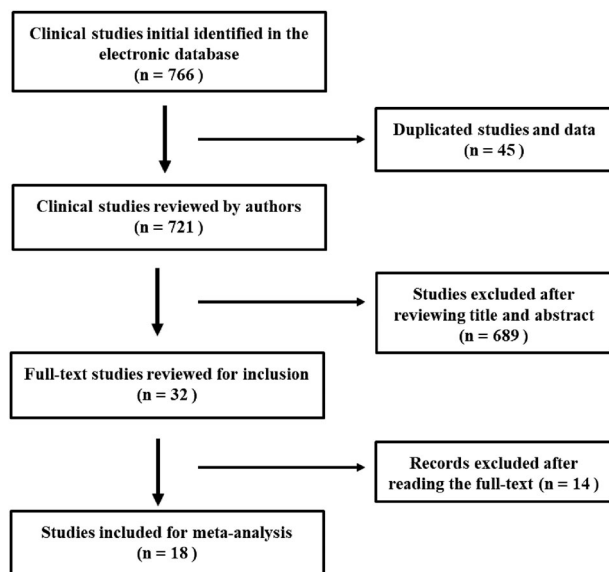


FIGURE 1 Flow chart of literature retrieval according to inclusion and exclusion criteria

TABLE 1 General characteristics of the included studies

Author	Time	Sample size	Cancer		Control		TNM	Tissue	Country	Methods
			(M+/M-)	(M+/M-)	(M+/M-)	(M+/M-)				
Schmidt ¹²	2010	523	190/91	12/230	I-IV	BALF	German	MSP		
Christoph ¹³	2011	343	112/76	16/139	I-IV	Plasma	German	MSP		
Konecny ¹⁴	2016	63	31/6	4/22	NA	BALF	German	MSP		
Konecny ¹⁴	2016	59	20/11	6/22	NA	Plasma	German	MSP		
Peter ¹⁵	2014	118	48/27	1/42	NA	BALF	German	MSP		
Dietrich ¹⁶	2012	204	78/22	4/100	I-IV	BALF	German	MSP		
Dietrich ^{V16}	2012	114	7/51	0/56	NA	Pleural effusion	German	MSP		
Peter ¹⁷	2013	719	138/138	70/373	NA	Pleural effusion	German	MSP		
Li ¹⁸	2014	47	10/18	0/9	NA	Pleural effusion	China	MSP		
Zhang ¹⁹	2016	277	98/32	34/113	NA	BALF	China	MSP		
Wang ²⁰	2016	243	79/44	8/112	NA	BALF	China	MSP		
Ren ²¹	2017	253	79/44	10/120	I-IV	BALF	China	MSP		
Rong ²²	2018	48	18/20	2/8	III/IV	Plasma	China	MSP		
Wang ²³	2018	120	57/23	1/39	I-IV	Plasma	China	MSP		
Peng ²⁴	2018	48	18/20	2/8	NA	Plasma	China	MSP-DHPL		
Lin ²⁵	2019	202	29/4	18/151	I-IV	BALF	China	MSP		
Chen ²⁶	2019	276	101/30	22/123	I-IV	BALF	China	MSP		
Sun ²⁷	2020	180	53/67	3/57	I-IV	Plasma	China	MSP		
Sun ²⁷	2020	180	74/67	4/56	I-IV	BALF	China	MSP		
Tian ²⁸	2020	134	54/13	12/55	Early stage	BALF	China	MSP		
Yang ²⁹	2021	104	32/16	14/42	NA	Plasma	China	MSP		

Abbreviations: BALF, bronchoalveolar lavage fluid; MSP, methylation specific PCR; M+, methylation positive; M-, methylation negative; NA, not available.

DISCUSSION

DNA methylation of gene promoter region, the well-known mechanism in epigenetic modification, commonly occurs in tumor cells.^{30,31} DNA methylation of the promoter region is an important way of gene silencing especially for tumor genes. DNA methylation alteration appears to precede apparent malignancy in many cases or at least an early event in the procedure of carcinogenesis.³² Therefore, methylation can be applied as a promising method for carcinoma diagnosis or recurrence monitoring after effective treatment.

Methylation of SHOX2 in promoter region was widely observed in lung carcinomas. Schmidt et al.¹² performed a diagnostic study to investigate whether SHOX2 DNA methylation can be applied as a biomarker for the diagnosis of lung cancer based on bronchial aspirates. The authors found that the lung cancer identification SEN and SPE were 68% (95% CI = 62%–73%) and 95% (95% CI = 91%–97%), respectively. However, Dietrich et al.¹⁶ believed that when using bronchial lavage specimens of SHOX2 methylation as a diagnostic marker, the diagnostic SEN and SPE were 78% and 96%, respectively. Therefore, the diagnostic performance was quite different between publications because of study heterogeneity.

In the present meta-analysis, we combined 18 studies and pooled the open published data. Combined results showed the diagnostic SEN, SPE, and DOR were 0.63 (95% CI = 0.54–0.70), 0.91 (95% CI = 0.87–0.94), and 16.84 (95%

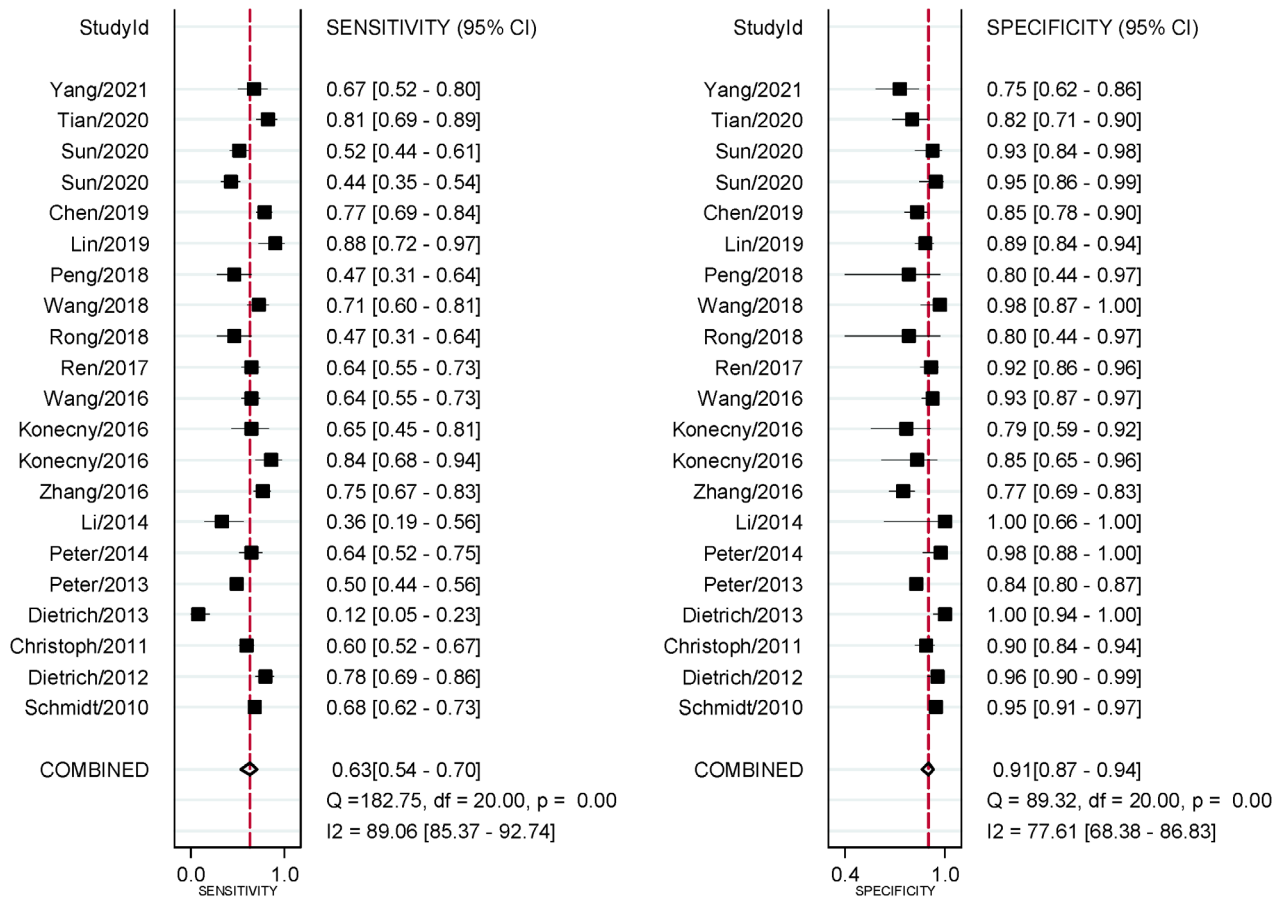


FIGURE 2 Forest plot for sensitivity and specificity of SHOX2 gene methylation as biomarker for lung cancer diagnosis

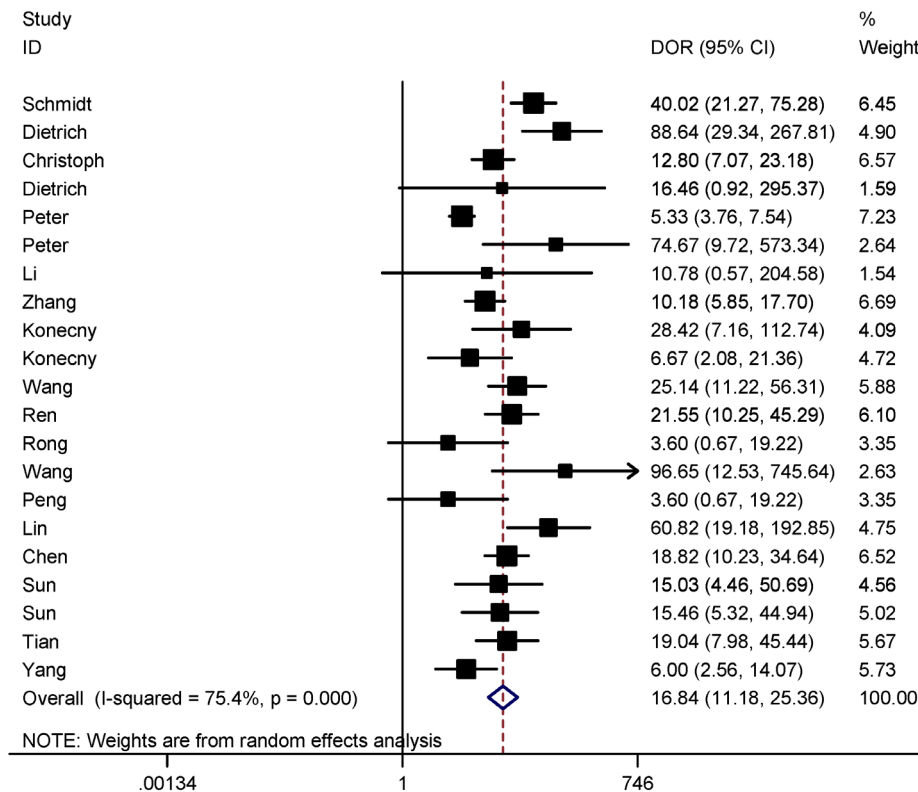


FIGURE 3 Forest plot for DOR of SHOX2 gene methylation as biomarker for lung cancer diagnosis

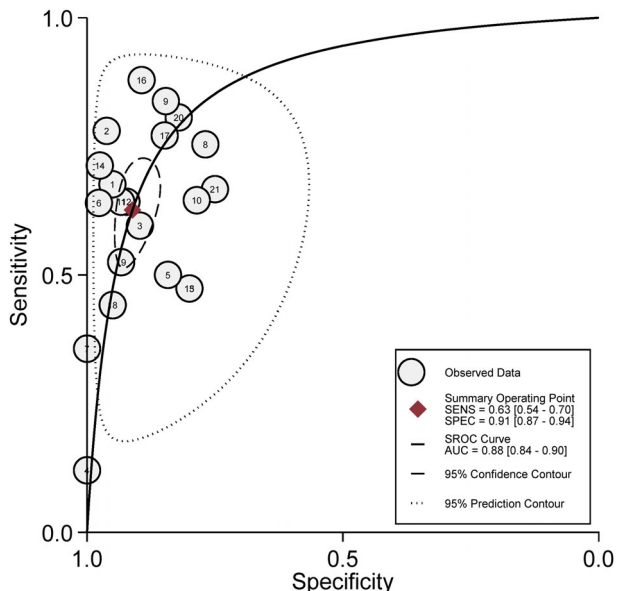


FIGURE 4 Summary receiver operating characteristic (SROC) curve of SHOX2 gene methylation for lung cancer diagnosis

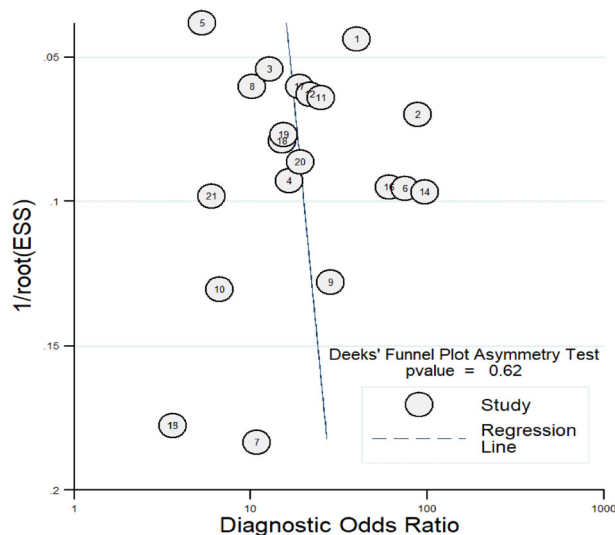


FIGURE 6 Deek's funnel plot in evaluation the publication bias

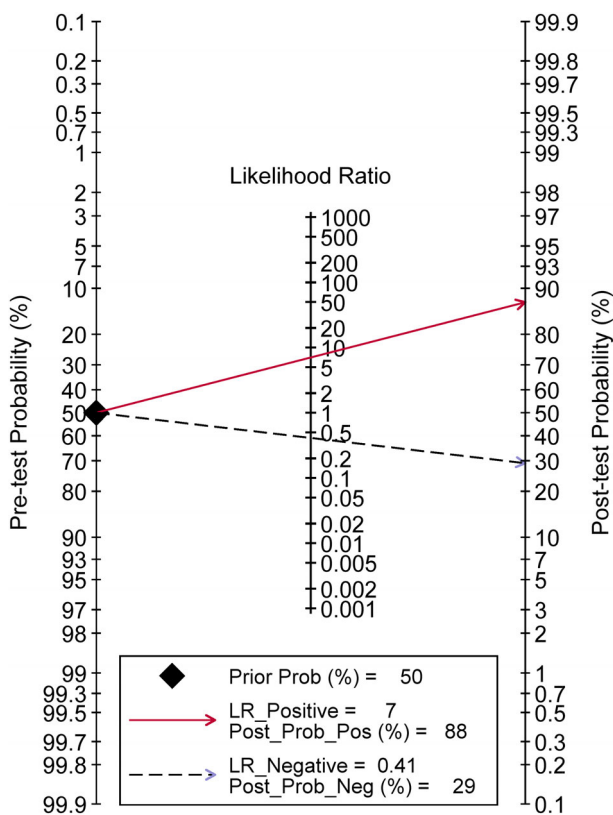


FIGURE 5 Fagan's nomogram of SHOX2 gene methylation as biomarker for lung cancer diagnosis

CI = 11.18–25.36), respectively. The lung cancer identification SPE was extremely high, but the SEN was relative low. The low SEN may hinder its clinical application as a lung cancer screening tool. According to Fagan's nomogram, the positive and negative post-probability of lung cancer was 88%

and 29% when SHOX2 methylated and unmethylated in humoral components given a pre-test probability of 50%. The post-probability of lung cancer diagnosis was obviously elevated after examination SHOX2 methylation status in body fluid.

Lung cancer is one of the most common malignant tumors in the world.³³ To date, there are few effective and clinically feasible methods for early diagnosis and screening of lung cancer.^{34–37} Although histology and cytology are the gold standard for the diagnosis of lung cancer, patients are often in advanced stages at the time of diagnosis by histology and cytology examination. Therefore, new diagnostic methods with non-invasive or mini-invasive with high sensitivity and specificity are urgently needed to improve the diagnosis efficacy and thereafter, decrease the mortality. SHOX2 gene methylation analysis is considered to be a diagnostic method with wide clinical application. SHOX2 gene methylation detection, combined with histology, cytology, and imaging, can improve the identification power of lung cancer and may become an effective tool for lung cancer early diagnosis.

In conclusion, based on the present open published data, SHOX2 promoter methylation in humoral components may be a potential biomarker for lung cancer diagnosis with relative diagnostic specificity. However, because of limitations of statistical heterogeneity and language restriction, high quality diagnostic works are required to further validation our findings. Furthermore, simply relying on detection of SHOX2 gene promoter methylation as the diagnostic reference of lung cancer has limited clinical application value. Therefore, comprehensive judgment should be based on combination of imaging and other diagnostic methods to improve the diagnostic accuracy.

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