P16\textsuperscript{INK4a} gene promoter methylation as a biomarker for the diagnosis of non-small cell lung cancer: An updated meta-analysis

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Keywords
Aberrant methylation; bronchoalveolar fluid; meta-analysis; P16\textsuperscript{INK4a} gene; serum.

Abstract

Background: This meta-analysis was conducted to investigate the diagnostic performance of P16\textsuperscript{INK4a} gene promoter methylation as a biomarker of non-small cell lung cancer (NSCLC).

Methods: Two reviewers independently searched the Web of Science, PubMed, Cochrane, Embase, China National Knowledge Infrastructure, and Chinese Biomedical Literature databases. Publications relevant to P16\textsuperscript{INK4a} gene promoter methylation in serum or bronchoalveolar fluid/sputum were screened and included in this meta-analysis. Pooled diagnostic sensitivity, specificity, positive and negative likelihood ratios, and diagnostic odds ratio were calculated.

Results: Twenty-six publications with 1768 lung cancer cases and 1323 controls were included. The pooled sensitivity, specificity, positive and negative likelihood ratios, and diagnostic odds ratio were 0.46 (95% confidence interval [CI] 0.43–0.48), 0.90 (95% CI 0.88–0.91), 6.33 (95% CI 3.89–10.30), 0.57 (95% CI 0.50–0.65) and 10.72 (95% CI 6.94–16.56), respectively, for P16\textsuperscript{INK4a} gene promoter methylation as a biomarker for the diagnosis of NSCLC. The area under the symmetric receiver operating characteristic curve was 0.75 with a standard error of 0.004. No publication bias was detected via line regression test (t = 0.95; P = 0.35) and Begg’s funnel plot.

Conclusion: P16\textsuperscript{INK4a} gene promoter methylation detection in serum or bronchoalveolar fluid/sputum may be a potential biomarker for NSCLC diagnosis; however, the sensitivity was relatively low, which is not suitable for NSCLC screening.

Introduction

Non-small cell lung cancer (NSCLC) is one of the most common clinically diagnosed malignant carcinomas. It is estimated that 234,030 new cases and 154,050 deaths from NSCLC will occur in the United States in 2018.\textsuperscript{1} The general prognosis of NSCLC is poor, particularly in advanced-stage patients, with an extremely low five-year survival rate. One of the major reasons for this poor prognosis is the lack of effective lung cancer screening or early diagnostic methods.\textsuperscript{2} Several studies have evaluated lung cancer screening methods such as X-ray,\textsuperscript{3,4} sputum cytology, and chest computed tomography (CT);\textsuperscript{5} however, such methods yield low sensitivity or specificity and thus are not adequate to diagnose NSCLC at an early stage.

Promoter methylation of tumor suppressor genes is frequently detected in cancer tissue and body fluid in malignant carcinomas such as lung,\textsuperscript{6,7} colorectal,\textsuperscript{8} and esophageal cancers. Previous studies have reported that methylation of the P16\textsuperscript{INK4a} gene promoter is common in lung cancer. The methylation frequency of P16\textsuperscript{INK4a} in serum or bronchoalveolar fluid (BAF)/sputum in lung cancer patients has been widely discussed; however, the exact diagnostic performance of P16\textsuperscript{INK4a} as a biomarker for...
NSCLC remains inconclusive. Therefore, we conducted this updated meta-analysis to further evaluate the diagnostic performance of P16INK4a as a biomarker for NSCLC.

**Methods**

**Electronic database search strategy**

Two reviewers independently searched the Web of Science, PubMed, Cochrane, Embase, China National Knowledge Infrastructure, and Chinese Biomedical Literature databases for studies relevant to P16INK4a gene promoter methylation in serum or BAF/sputum. The following keywords were used: non-small cell lung cancer; non-small cell carcinoma, NSCLC, P16, P16INK4a, cyclin-dependent kinase inhibitor 2A, CDKN2A; CDK4 inhibitor; multiple tumor suppressor 1; TP16; methylation; and hypermethylation. Relevant studies were identified and duplicated publications were excluded. The title and abstract were then reviewed to locate relevant studies. All potentially suitable studies were reviewed in full-text and all references of included publications were further screened to identify additional relevant publications. The publication search process is demonstrated in Figure 1.

![Publication search process](image)

**Inclusion and exclusion criteria**

The identified studies were further reviewed to assess whether the inclusion criteria were fulfilled: (i) diagnostic studies relevant to P16INK4a promoter methylation and NSCLC; (ii) NSCLC diagnosis confirmed by pathology or cytology; (iii) P16INK4a gene promoter methylation was detected by methylation-specific PCR (MSP), real-time MSP (RT-MSP), or quantitative MSP (q-MSP); (iv) P16INK4a gene methylation status in serum or BAF/sputum in NSCLC and control subjects was available for each included study. The exclusion criteria were: (i) case reports or literature reviews; (ii) P16INK4a gene methylation status detected in other specimens, not in serum or BAF/sputum; (iii) studies published in languages other than English or Chinese; and (iv) insufficient data to calculate sensitivity and specificity.

**Statistical analysis**

The diagnostic sensitivity, specificity, and symmetric receiver operating characteristic (SROC) curve were pooled by fixed or random effects method according to the statistical heterogeneity across the included studies. Diagnostic sensitivity and specificity were calculated using the following equations: sensitivity = true positive/(true positive + false negative), specificity = true negative/(false positive + true negative).
false negative); specificity = true negative/(true negative + false positive). Publication bias was evaluated by Egger’s line regression test and Begger’s funnel plot. \( P < 0.05 \) was considered to indicate significant statistical difference.

## Results

### Study characteristics

Initially, 488 relevant publications were identified. After reviewing the title, abstract, and full text, 26 studies relevant to \( P16^{INK4a} \) gene promoter methylation as a biomarker for the diagnosis of NSCLC were included for quantitative analysis.\(^9\)–\(^34\) Sixteen publications evaluated \( P16^{INK4a} \) gene promoter methylation in serum and 10 in BAF/sputum. The general characteristics of the 26 studies are shown in Table 1.

### Pooled sensitivity and specificity

Because of significant statistical heterogeneity, the diagnostic sensitivity and specificity were pooled using the random effects method. The pooled sensitivity and specificity were 0.46 (95% confidence interval [CI] 0.43–0.48) (Fig 2) and 0.90 (95% CI 0.88–0.91) (Fig 3), respectively, for \( P16^{INK4a} \) gene promoter methylation as a biomarker for the diagnosis of NSCLC.

### Pooled positive and negative likelihood ratios

The diagnostic positive likelihood ratio (+LR) and negative likelihood ratio (−LR) were also pooled by random effect method because of significant heterogeneity. The pooled +LR and −LR were 6.33 (95% CI 3.89–10.30) (Fig 4) and 0.57 (95% CI 0.50–0.65) (Fig 5), respectively, for \( P16^{INK4a} \) gene promoter methylation as a biomarker for the diagnosis of NSCLC.

### Pooled diagnostic odds ratio

The pooled diagnostic odds ratio (DOR) was 10.72 (95% CI 6.94–16.56) for \( P16^{INK4a} \) gene promoter methylation as a biomarker for the diagnosis of NSCLC (Fig 6).

### Table 1 Study characteristics

| Study            | Year | Area | NSCLC | Control | Tp | Fp | Fn | Tn | Specimen |
|------------------|------|------|-------|---------|----|----|----|----|----------|
| Kersting et al. \(^9\) | 2000 | US   | 31    | 25      | 18 | 7  | 13 | 18 | Serum    |
| Bearzatto et al. \(^10\) | 2002 | Italy | 30    | 15      | 12 | 0  | 18 | 15 | Serum    |
| Wu et al. \(^12\) | 2002 | China | 14    | 26      | 4  | 0  | 10 | 26 | Serum    |
| Cai et al. \(^12\) | 2003 | China | 49    | 55      | 15 | 1  | 34 | 54 | Serum    |
| Kim et al. \(^13\) | 2004 | Korea | 85    | 127     | 14 | 8  | 71 | 119| Serum    |
| Fujisawa et al. \(^14\) | 2005 | US   | 111   | 80      | 14 | 3  | 97 | 77 | Serum    |
| Kong et al. \(^15\) | 2007 | China | 64    | 46      | 19 | 0  | 45 | 46 | Serum    |
| Hsu et al. \(^16\) | 2007 | China | 51    | 33      | 21 | 3  | 30 | 30 | Serum    |
| Zhang et al. \(^17\) | 2008 | China | 95    | 22      | 52 | 2  | 43 | 20 | Serum    |
| Ma et al. \(^18\) | 2009 | China | 62    | 19      | 32 | 2  | 30 | 19 | Serum    |
| Hu et al. \(^19\) | 2009 | China | 46    | 21      | 22 | 1  | 24 | 20 | Serum    |
| Chen et al. \(^20\) | 2010 | China | 159   | 81      | 39 | 0  | 120| 81 | Serum    |
| Wang et al. \(^21\) | 2016 | China | 50    | 50      | 22 | 0  | 28 | 50 | Serum    |
| Wan et al. \(^22\) | 2017 | China | 98    | 60      | 69 | 9  | 29 | 51 | Serum    |
| Liu et al. \(^23\) | 2017 | China | 120   | 46      | 45 | 3  | 75 | 43 | Serum    |
| Destro et al. \(^24\) | 2004 | Italy | 24    | 100     | 16 | 4  | 8  | 96| BAF/sputum |
| Konno et al. \(^25\) | 2004 | Japan | 78    | 94      | 44 | 20 | 34 | 74 | BAF/sputum |
| Wang et al. \(^26\) | 2004 | China | 34    | 21      | 11 | 0  | 23 | 21 | BAF/sputum |
| Georgiou et al. \(^27\) | 2007 | Greece | 80   | 40      | 55 | 9  | 25 | 31 | BAF/sputum |
| Liu et al. \(^28\) | 2008 | China | 58    | 107     | 41 | 55 | 17 | 52 | BAF/sputum |
| Zhang et al. \(^29\) | 2004 | China | 44    | 20      | 27 | 3  | 17 | 17 | BAF/sputum |
| Guo et al. \(^30\) | 2008 | China | 100   | 50      | 61 | 0  | 39 | 50 | BAF/sputum |
| Hu et al. \(^31\) | 2009 | China | 42    | 25      | 20 | 0  | 22 | 25 | BAF/sputum |
| Peng et al. \(^32\) | 2010 | China | 82    | 25      | 60 | 0  | 22 | 25 | BAF/sputum |
| Zhang et al. \(^33\) | 2012 | China | 41    | 15      | 21 | 2  | 20 | 13 | BAF/sputum |
| Sun et al. \(^34\) | 2012 | China | 120   | 120     | 56 | 6  | 64 | 114| BAF/sputum |

BAF, bronchoalveolar fluid; fn, false negative; fp, false positive; NSCLC, non-small cell lung cancer; tn, true negative; tp, true positive; US, United States.
Figure 2 Forest plot if the sensitivity of \(P16^{INK4a}\) gene promoter methylation as a biomarker for the diagnosis of non-small cell lung cancer. CI, confidence interval.

Sensitivity (95% CI)

| Author          | Sensitivity |
|-----------------|-------------|
| Kersting        | 0.58 (0.39–0.75) |
| Bearzatto       | 0.40 (0.23–0.59) |
| Wu J            | 0.29 (0.08–0.58) |
| Cai ZX          | 0.31 (0.18–0.45) |
| Kim H           | 0.16 (0.09–0.26) |
| Keichi Fujiwara | 0.13 (0.07–0.20) |
| Kong YM         | 0.30 (0.19–0.42) |
| Han-Shui Hsu    | 0.41 (0.28–0.56) |
| Zhang LP        | 0.55 (0.44–0.65) |
| Ma XP           | 0.52 (0.39–0.65) |
| Hu ZJ           | 0.48 (0.33–0.63) |
| Chen SH         | 0.25 (0.18–0.32) |
| Wang PP         | 0.44 (0.30–0.59) |
| Wan LL          | 0.70 (0.60–0.79) |
| Liu Y           | 0.38 (0.29–0.47) |
| Destro          | 0.67 (0.45–0.84) |
| Konno           | 0.56 (0.45–0.68) |
| Wang X          | 0.32 (0.17–0.51) |
| Georgiou        | 0.69 (0.57–0.79) |
| Liu Y           | 0.71 (0.57–0.82) |
| Zhang W         | 0.61 (0.45–0.76) |
| Guo XJ          | 0.61 (0.51–0.71) |
| Hu XJ           | 0.48 (0.32–0.64) |
| Peng ZM         | 0.73 (0.62–0.82) |
| Zhang W         | 0.51 (0.35–0.67) |
| Sun N           | 0.47 (0.38–0.56) |

Pooled Sensitivity = 0.46 (0.43–0.48)
Chi-square = 249.45; df = 25 (P = 0.0000)
Inconsistency (I-square) = 90.0%

Figure 3 Forest plot for specificity of \(P16^{INK4a}\) gene promoter methylation as a biomarker for the diagnosis of non-small cell lung cancer. CI, confidence interval.

Specificity (95% CI)

| Author          | Specificity |
|-----------------|-------------|
| Kersting        | 0.72 (0.51–0.88) |
| Bearzatto       | 1.00 (0.78–1.00) |
| Wu J            | 1.00 (0.87–1.00) |
| Cai ZX          | 0.98 (0.90–1.00) |
| Kim H           | 0.94 (0.88–0.97) |
| Keichi Fujiwara | 0.96 (0.89–0.99) |
| Kong YM         | 1.00 (0.92–1.00) |
| Han-Shui Hsu    | 0.91 (0.76–0.98) |
| Zhang LP        | 0.91 (0.71–0.99) |
| Ma XP           | 1.00 (0.82–1.00) |
| Hu ZJ           | 0.95 (0.76–1.00) |
| Chen SH         | 1.00 (0.96–1.00) |
| Wang PP         | 1.00 (0.93–1.00) |
| Wan LL          | 0.85 (0.73–0.93) |
| Liu Y           | 0.93 (0.82–0.99) |
| Destro          | 0.96 (0.90–0.99) |
| Konno           | 0.79 (0.69–0.86) |
| Wang X          | 1.00 (0.84–1.00) |
| Georgiou        | 0.78 (0.62–0.89) |
| Liu Y           | 0.49 (0.39–0.58) |
| Zhang W         | 0.85 (0.62–0.97) |
| Guo XJ          | 1.00 (0.93–1.00) |
| Hu XJ           | 1.00 (0.86–1.00) |
| Peng ZM         | 1.00 (0.86–1.00) |
| Zhang W         | 0.87 (0.60–0.98) |
| Sun N           | 0.95 (0.89–0.98) |

Pooled Specificity = 0.80 (0.88–0.91)
Chi-square = 238.78; df = 25 (P = 0.0000)
Inconsistency (I-square) = 89.5%
Figure 4 Forest plot of the negative likelihood ratio (LR). CI, confidence interval.

Random Effects Model
Pooled Positive LR = 6.33 (3.89–10.30)
Cochran-Q = 140.09; df = 25 (P = 0.0000)
Inconsistency (I-square) = 82.2%
Tau-squared = 0.9658

Positive LR (95% CI)
Kersting: 2.07 (1.03–4.16)
Bearzatto: 12.90 (0.82–204.19)
Wu J: 16.20 (0.93–280.84)
Cai ZX: 16.84 (2.31–122.84)
Kim H: 2.61 (1.15–5.96)
Keiichi Fujiwara: 3.36 (1.00–11.32)
Kong YM: 29.20 (1.75–455.43)
Han-Shui Hsu: 4.53 (1.47–13.99)
Zhang LP: 6.02 (1.59–22.86)
Ma XP: 20.63 (1.32–321.94)
Hu ZJ: 10.04 (1.45–69.64)
Chen SH: 40.49 (2.52–650.39)
Wang PP: 45.00 (2.80–722.05)
Wan LL: 4.69 (2.54–8.69)
Liu Y: 5.75 (1.88–17.59)
Destro: 16.67 (6.13–45.35)
Konno: 2.65 (1.72–4.10)
Wang X: 14.46 (0.90–233.21)
Georgiou: 3.06 (1.69–5.53)
Liu Y: 1.38 (1.07–1.76)
Zhang W: 4.09 (1.40–11.92)
Guo XJ: 62.11 (3.92–983.89)
Hu XJ: 24.79 (1.56–392.80)
Peng ZM: 37.90 (2.43–591.83)
Zhang W: 3.84 (1.02–14.44)
Sun N: 9.33 (4.18–20.83)

Figure 5 Forest plot of the positive likelihood ratio (LR). CI, confidence interval.

Random Effects Model
Pooled Negative LR = 0.57 (0.50–0.65)
Cochran-Q = 232.86; df = 25 (P = 0.0000)
Inconsistency (I-square) = 89.3%
Tau-squared = 0.0862

Negative LR (95% CI)
Kersting: 0.58 (0.36–0.94)
Bearzatto: 0.62 (0.46–0.83)
Wu J: 0.71 (0.51–1.00)
Cai ZX: 0.71 (0.58–0.85)
Kim H: 0.89 (0.80–0.99)
Keiichi Fujiwara: 0.91 (0.84–0.99)
Kong YM: 0.71 (0.60–0.83)
Han-Shui Hsu: 0.65 (0.50–0.83)
Zhang LP: 0.50 (0.38–0.64)
Ma XP: 0.50 (0.38–0.65)
Hu ZJ: 0.55 (0.41–0.73)
Chen SH: 0.76 (0.69–0.83)
Wang PP: 0.56 (0.44–0.72)
Wan LL: 0.35 (0.25–0.48)
Liu Y: 0.67 (0.57–0.78)
Destro: 0.35 (0.20–0.61)
Konno: 0.55 (0.42–0.73)
Wang X: 0.69 (0.54–0.87)
Georgiou: 0.40 (0.28–0.58)
Liu Y: 0.60 (0.39–0.94)
Zhang W: 0.45 (0.30–0.69)
Guo XJ: 0.39 (0.31–0.50)
Hu XJ: 0.53 (0.40–0.71)
Peng ZM: 0.28 (0.19–0.39)
Zhang W: 0.56 (0.39–0.82)
Sun N: 0.56 (0.47–0.67)
Figure 6 Forest plot of the diagnostic odds ratio (OR).

Figure 7 The pooled symmetric receiver operating characteristic (SROC) curve for P16 gene promoter methylation for the diagnosis of non-small cell lung cancer. AUC, area under the curve; SE, standard error.
The area under the SROC curve was 0.75 with a standard error of 0.004 for P16INK4a gene promoter methylation as a biomarker for the diagnosis of lung cancer (Fig 7).

Subgroup analysis

We also conducted subgroup analysis, detecting P16INK4a gene promoter methylation in serum or BAF/sputum. The pooled diagnostic performances in serum and BAF/sputum are shown in Table 2.

Evaluation of publication bias

Publication bias was evaluated by Egger’s line regression test and Begg’s funnel plot. No publication bias was detected by line regression test (t = 0.95; P = 0.35) or Begg’s funnel plot (Fig 8).

Discussion

In China, lung cancer is the most commonly diagnosed malignant carcinoma and the leading cause of cancer mortality in both men and women, particularly in men aged ≥75 years. As most NSCLC patients are only diagnosed at locally advanced-stage or after remote metastasis has occurred, they are ineligible for surgery. Prognosis is poor, with an extremely low five-year survival rate, because of the lack of effective methods for lung cancer screening or early diagnosis.

Promoter methylation of tumor suppressor genes is common in body fluid and can be used as a lung cancer diagnosis method or biomarker. Several studies have evaluated its clinical application with acceptable diagnostic performance and high specificity.35–38

The P16INK4a gene, also known as the CDKN2A gene, is located on chromosome 9 (9p21.3) and plays an important role in regulating the cell cycle.39 The promoter region of P16INK4a is usually hypermethylated in cancer cells of NSCLC patients. Studies have found that P16INK4a methylation can be detected in body fluid, such as serum and sputum,23,24 indicating that detection of P16INK4a methylation status may be used as an important tool for lung cancer diagnosis, screening, or the monitoring of recurrence.

Two previous meta-analyses evaluated P16INK4a methylation in serum and sputum as a biomarker for lung cancer diagnosis and concluded that detection of P16INK4a promoter methylation via these methods was a useful tool for lung cancer diagnosis.40,41 However, several recently published relevant studies were not included in these meta-analyses. Therefore, we performed an updated meta-analysis, including recently published relevant publications and further evaluated the clinical value of P16INK4a methylation as a biomarker for NSCLC diagnosis. We confirmed that P16INK4a gene promoter methylation detection in serum or BAF/sputum may be a potential biomarker for NSCLC diagnosis; however, the sensitivity was relatively low and was thus not suitable for NSCLC screening.

Although our results indicate that P16INK4a gene promoter methylation represents a promising method for...
NSCLC diagnosis, there was significant statistical heterogeneity in the process of data merging, which inevitably affected our results. Furthermore, the sample sizes of the included studies were relatively small, which can reduce the statistical power of each included study. Large-scale prospective diagnostic tests should be conducted by multiple health centers to further evaluate the clinical application value of \( P16^{INK4a} \) gene promoter methylation as an NSCLC diagnostic method.

**Disclosure**

No authors report any conflict of interest.

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