Does adenomatous polyposis coli gene promoter 1A methylation increase non-small cell lung cancer risk? A meta-analysis

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Abstract
Background: The promoter region of the adenomatous polyposis coli (APC) gene is hypermethylated in several types of cancers, including non-small cell lung cancer (NSCLC). The prevalence of methylation in the promoter region of this gene in tumor tissues and autologous controls has not been consistent in previous studies. We evaluated the frequency of APC gene promoter 1A methylation between tumor tissues and autologous controls in NSCLC patients by meta-analysis.

Methods: Open published studies of APC gene promoter 1A methylation between tumor tissues and autologous samples in NSCLC patients were identified using a systematic search. Odds ratios (OR) and 95% confidence intervals (CI) of APC gene promoter 1A methylation in lung cancer tissues versus autologous controls were calculated. Fourteen studies, involving a total of 1345 patients and 2182 samples, were finally included.

Results: The pooled proportion of APC promoter 1A methylation was 0.62 (95% CI 0.52–0.72) and 0.34 (95% CI 0.21–0.50) in cancer tissues and autologous controls, respectively. The APC gene promoter 1A methylation rate in cancer tissues was much higher than in autologous controls, with a pooled OR of 3.66 (95% CI 2.12–6.33). A strong and significant correlation of APC gene promoter 1A methylation between tumor tissues and autologous controls was detected (correlation coefficient rpearson = 0.77; P = 0.0013).

Conclusion: The proportion of APC promoter 1A methylation in lung cancer tissues was higher than in autologous controls, indicating that promoter 1A methylation of the APC gene may play an important role in NSCLC carcinogenesis.

Introduction
Lung cancer, which accounted for 1.4 million deaths worldwide in 2008, is the leading cause of cancer related mortality in men and the second for women. An estimated 158 080 deaths, 72 160 in women and 85 920 in men, occurred in the United States in 2016. Lung cancer is divided into non-small cell lung cancer (NSCLC) and small-cell lung cancer (SCLC), according to biological behavior. Tobacco smoking is a confirmed risk factor for developing NSCLC, accounting for 85% of lung cancer-related death worldwide. However, other carcinogenesis may also play an important role in the development of NSCLC, such as epigenetic processes. DNA methylation, a commonly detected change in cancer, is deemed an important epigenetic mechanism for the silencing of tumor suppressor genes. The association between tumor suppressor gene promoter hypermethylation and NSCLC risk has previously been studied. Adenomatous polyposis coli (APC), a well-characterized tumor suppressor gene, is located on the long arm of chromosome 5 between positions 21 and 22. The promoter region of this gene is hypermethylated in various kinds of cancers, including NSCLC. However, the prevalence of methylation in the promoter region of this gene in tumor tissues and autologous controls has not been consistent in previous small sample studies. Therefore, we conducted a meta-analysis of the prevalence of
methylation in the promoter region of APC in tumor tissues and autologous controls using open published studies.

**Methods**

**Search strategy**

Medline, Embase, and CNKI databases were searched for open published studies of APC gene promoter 1A methylation in NSCLC published before December 2016. The following search terms were used as free text: “Non-small cell lung cancer/non-small cell lung carcinoma,” “NSCLC,” “Adenomatous polyposis coli,” “APC,” “methylation,” and “hypermethylation.” The search was limited to studies of humans and restricted to English and Chinese language. All potential relevant studies were assessed in detail and all citations in the included articles were further evaluated in order to identify additional suitable publications. The selection procedure for suitable articles was conducted using a Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement flow chart (Fig 1).

**Selection criteria**

The inclusion criteria were: (i) NSCLC without restriction of stage and pathology type, (ii) APC gene promoter 1A methylation frequency was clearly reported or could be calculated, (iii) only methylation-specific polymerase chain reaction (MSP) and quantitative methylation-specific polymerase chain reaction (qMSP) methods were used, (iv) methylation detection in lung cancer tissues and autologous controls included corresponding non-tumor lung tissues (CNTLT) or blood/serum/plasma or sputum/bronchoalveolar lavage fluid (BALF), and (v) studies in English and Chinese language only. The exclusion criteria were: (i) APC gene promoter 1A methylation detected in animals or cell lines, (ii) inadequate data to pool the prevalence of APC gene promoter 1A methylation, and (iii) case reports and review papers.

**Data extraction**

Two independent reviewers extracted the data. Any disagreement was solved in consultation with the third investigator. Data including publication year, patient eligibility, age, gender, pathology, methylation detection method, and autologous control types of the relevant studies were extracted by two reviewers and then verified by the third reviewer, as described in the Cochrane Handbook for systematic reviews. The number of patients, samples detected for APC gene promoter 1A methylation, and methylation frequency in both tumor tissues and autologous controls were extracted with caution.

**Statistical analysis**

Meta-Analyst 3.13 (http://www.biomedcentral.com) and Stata/SE 11.0 (StataCorp LP, http://www.stata.com) were
used for statistical analysis. The methylation status in tumor tissues and controls was calculated as a methylation proportion. The methylation proportion was then used to pool the overall proportion by DerSimonian–Laird random effects method and was demonstrated as odds ratios (OR) and 95% confidence intervals (CI). Statistical heterogeneity among the studies was evaluated by $I^2$. If significant heterogeneity was found ($I^2 > 50\%$), the data was pooled using the DerSimonian–Laird random effect method. Inversely, a fixed-effect method was applied. Publication bias was detected by Egger’s test. The association of $APC$ gene promoter 1A methylation between tumor tissues and autologous controls was evaluated by Spearman’s rank correlation test.

### Results

#### Study characteristics

Fourteen studies involving a total of 1345 patients and 2182 samples were finally included in this meta-analysis. The detailed selection procedure is demonstrated in Figure 1 and the patient’s characteristics are summarized in Table 1.

#### Pooled results from the meta-analysis

The methylation proportion of $APC$ gene promoter 1A ranged from 10% to 96% in cancer tissues and 0% to 88% in autologous controls, respectively. The pooled proportion of $APC$ promoter 1A methylation was 0.62 (95% CI 0.52–0.72) (Fig 2a) and 0.34 (95% CI 0.21–0.50) (Fig 2b) in cancer tissues and autologous controls, respectively. The pooled proportion was then used to evaluate their contribution to heterogeneity; however, none of these was a source of significant heterogeneity among the studies (Table 2). Only a small portion of heterogeneity can be explained by these factors. However, we still performed subgroup analysis according to the different factors. In subgroup analysis, the pooled odds of methylation for qMSP ($P = 0.076$), serum ($P = 0.286$), and sputum ($P = 0.385$) subgroups were not statistically different (Table 3).

### Sensitivity analysis

When omitting a single article in the random effect model, the OR ranged from 3.13 (95% CI 1.87–5.22) to 3.99 (95% CI 2.23–7.16) with only a slight change (Fig 4). Sensitivity analysis indicated that the pooled OR was not sensitive to

### Table 1 General characteristics of included studies

| Author            | Publication year | Race       | Age (year) | Gender | Sample size | Histology | Control type |
|-------------------|------------------|------------|------------|--------|-------------|-----------|--------------|
| Virmani et al.    | 2001             | White      | n/a        | n/a    | 48          | 18        | MSP          | CNTLT        |
| Brabender et al.  | 2001             | White      | 63.3 (34–82) | 69/22 | 91          | 91        | QMSP         | 43 33 5 CNTLT |
| Usadel et al.     | 2001             | White      | 64.2 ± 9.6  | 49/50  | 99          | 89        | QMSP         | 35 47 17 Serum |
| Yanagawa et al.   | 2003             | Asia-Pacific | 67.3 (35–86) | 54/21  | 75          | 75        | MSP          | 29 43 3 CNTLT |
| Toyooka et al.    | 2003             | Mixed      | 26–87      | 355/159| 514         | 84        | MSP          | 194 299 21 CNTLT |
| Pan et al.        | 2004             | Asia-Pacific | 53.0 (36–68) | n/a    | 17          | 17        | MSP          | n/a n/a n/a CNTLT |
| Yang et al.       | 2005             | Asia-Pacific | 56 ± 11    | n/a    | 49          | 49        | MSP          | n/a n/a n/a CNTLT |
| Vallbohmer et al. | 2006             | White      | 63 (34–83) | 69/22  | 91          | 91        | MSP          | 43 33 15 CNTLT |
| Kim et al.        | 2007             | Asia-Pacific | 63 ± 8.4  | 80/19  | 99          | 99        | MSP          | 61 38 0 CNTLT |
| Mi et al.         | 2008             | Asia-Pacific | 63.9      | 5/5    | 10          | 10        | MSP          | n/a n/a n/a CNTLT |
| Feng et al.       | 2008             | White      | 64.3       | 26/23  | 49          | 49        | MSP          | 14 20 15 CNTLT |
| Pan et al.        | 2009             | Asia-Pacific | 53 (35–71) | 51/27  | 78          | 40        | MSP          | n/a n/a n/a Serum |
| Zhang et al.      | 2011             | Asia-Pacific | 59 (35–80) | 58/20  | 78          | 78        | MSP          | 36 30 12 CNTLT |
| Kang Chunyan and Tang Shoupeng | 2011 | Asia-Pacific | 52.9 (31–78) | 40/7  | 47          | 47        | MSP          | 29 12 6 Sputum |

Ad, adenocarcinoma; C, control; CNTLT, corresponding non-tumor lung tissues; F, female; M, male; n/a, not available; qMSP, quantitative methylation specific PCR; Sq, squamous cell carcinoma; T, tumor tissue.
a single study, demonstrating that the results were relatively stable.

**Correlation of APC gene promoter 1A methylation between tumor tissues and autologous controls**

The methylation proportion of APC gene promoter 1A ranged from 10% to 96% in cancer tissues and 0% to 88% in autologous controls, respectively. A strong and significant correlation of APC gene promoter 1A methylation between tumor tissues and autologous controls was detected with a correlation coefficient of $r_{\text{pearson}} = 0.77$ ($P = 0.0013$) (Fig 5).

**Publication bias**

Begger’s funnel plot and Egger’s line regression tests were applied to assess publication bias. The funnel plot demonstrated a little asymmetry (Fig 6). However, the
Egger’s line regression test, which can provide exact statistical analysis of the extent of asymmetry in a funnel plot, did not show any evidence of statistical publication bias ($t = 0.49; P = 0.64$).

**Discussion**

DNA methylation is one of the epigenetic modifications that affects cytosines in dinucleotide: cytosine-phosphate-guanine (CpG). The covalent addition of a methyl group on the C5 of the Cp transforms it into a methylated cytosine (Cm).\(^2^,\(^3^,\(^2^,\(^2^\)\) Tumor suppressor gene transcriptional silencing by CpG island hypermethylation of its promoter region is an important component for the initiation and progression of lung cancer.\(^2^\)\(^3^,\(^2^,\(^2^\)\) Accumulating evidence has shown that hypermethylation of CpG islands in promoter regions acts as an important mechanism for the inactivation of tumor-suppressor genes, including the cell cycle, DNA repair, and apoptosis.\(^2^\)\(^4^,\(^2^\) The APC gene, mapped on chromosome 5q21 (18), is a well known tumor suppressor gene.\(^2^\)\(^5^,\(^2^\) The protein product of the APC gene is an important component of the Wnt signaling pathway, which binds to and inactivates β-catenin.\(^2^\)\(^6^\) Impaired APC gene function usually leads to a lack of degradation β-catenin, which could cause the loss of cell growth control. Growing evidence shows that APC gene promoter 1A methylation can be detected not only in colorectal cancer but also in other types of cancers, including NSCLC.\(^8^,\(^9^,\(^1^,\(^5^\)\) However, there has been significant variation in the range of APC gene promoter 1A methylation in tumor tissues and autologous controls in previous studies using a small sample.\(^5^,\(^2^,\(^2^\)\) Therefore, we performed this meta-analysis using published studies on APC gene promoter 1A methylation in NSCLC patients in order to gain an objective consensus.

Fourteen articles with a total of 1345 patients (2182 samples) were included in this meta-analysis. Great variety in the range of APC gene promoter 1A methylation was found. The proportion of APC gene promoter 1A methylation ranged from 10% to 96%, with a pooled proportion of 0.62 (95% CI 0.52–0.72) in NSCLC tumor tissues. In autologous controls, the methylation rate ranged from 0% to 88%, with a pooled proportion of 0.34 (95% CI 0.21–0.50). The methylation frequency in tumor tissues was much higher than in autologous controls, with a pooled OR of 3.66 (95% CI 2.12–6.33), indicating that APC gene promoter 1A methylation may play an important role in NSCLC carcinogenesis. Because of the obvious statistical heterogeneity, we further performed meta-regression using the Knapp-Hartung modification, which restricted the maximum likelihood method to estimate the variance between studies. The obvious heterogeneity could not be explained by control type (CNTLT, sputum, serum), gender, age, ethnicity, histology type, smoking status, clinical stage, sample size, or methylation detection method. Only a small portion of heterogeneity can be explained by these factors. However, we still performed subgroup analysis according to the different characteristics. In subgroup analysis, the significant odds of APC gene promoter 1A methylation proportion in tumor tissues compared with autologous was changed in qMSP ($P = 0.076$), serum

**Table 2** Meta-regression analysis

| Heterogeneity sources | Coefficient (95% CI) | t | P | $\tau^2$ | I$^2$ (%) | R$^2$ (%) adjusted |
|-----------------------|----------------------|---|---|---------|----------|------------------|
| Control type          | −0.24 (−1.79 to 1.30) | −0.35 | 0.73 | 0.93  | 78.85 | −11.28 |
| Proportion of men     | −2.30 (−8.64 to 4.03) | −0.81 | 0.44 | 0.93  | 81.35 | −5.55 |
| Mean age              | 0.01 (−0.17 to 0.19)  | 0.17 | 0.87 | 1.17  | 81.88 | −16.92 |
| Ethnicity             | −5.53 (−2.81 to 1.76) | −0.54 | 0.60 | 1.30  | 82.38 | −30.20 |
| Proportion of ad      | 3.20 (−4.02 to 10.43) | 1.00 | 0.34 | 0.87  | 79.85 | −0.59 |
| Proportion of smokers | −2.50 (−8.28 to 3.28) | −1.00 | 0.34 | 0.85  | 79.59 | 3.18 |
| Proportion of early stage | −10.71 (−32.60 to 11.16) | −1.20 | 0.28 | 0.97  | 81.87 | 7.40 |
| Sample size           | 0.001 (−0.003 to 0.006) | 0.64 | 0.54 | 0.87  | 75.62 | −4.39 |
| Detection methods     | 0.77 (−1.01 to 2.56)  | 0.94 | 0.36 | 0.83  | 77.17 | 1.52 |

CI, confidence interval; CNTLT, corresponding non-tumor lung tissues; MSP, methylation specific PCR; OR, odds ratio; qMSP, quantitative methylation specific PCR.

**Table 3** Subgroup meta-analysis

| Subgroup | Pooled effect size |
|----------|-------------------|
|          | OR  | 95% CI          | P   |
| Total    | 3.66 | 2.12–6.33       | 0.000 |
| Subgroup |      |                  |      |
| Ethnicity |      |                  |      |
| Asia-Pacific | 3.00 | 1.51–5.94       | 0.002 |
| White    | 6.35 | 1.64–24.51      | 0.007 |
| Method   |      |                  |      |
| MSP      | 3.23 | 1.85–5.62       | 0.000 |
| qMSP     | 7.10 | 0.81–62.00      | 0.076 |
| Control type |      |                  |      |
| CNTLT    | 3.82 | 2.15–6.80       | 0.000 |
| Serum    | 4.84 | 0.27–87.96      | 0.286 |
| Sputum   | 1.46 | 0.62–3.45       | 0.385 |

CI, confidence interval; CNTLT, corresponding non-tumor lung tissues; MSP, methylation specific PCR; OR, odds ratio; qMSP, quantitative methylation specific PCR.
(P = 0.286), and sputum (P = 0.385) subgroups. However, as only a small number of subjects were included in this subgroup analysis, the results should be interpreted with caution.

We also found a strong and significant correlation of APC gene promoter 1A methylation between tumor tissues and autologous controls (r_{pearson} = 0.77; P = 0.0013). The significant correlation indicates that the higher the proportion of APC gene promoter 1A hypermethylation in autologous controls, the higher the proportion of this gene promoter hypermethylation in tumor tissues. The detection of APC gene promoter 1A methylation in autologous samples, such as sputum and serum, may represent a method to diagnose NSCLC.24

However, several potential limitations should be considered when interpreting the results of this meta-analysis. Firstly, obvious heterogeneity across studies was detected (I^2 = 78.1%), which could decrease the statistical power of the results.27,28 The 95% CIs were enlarged using a random effect model with significant statistical heterogeneity. Accordingly, our conclusions should be interpreted with caution. Secondly, the pooled proportion and OR was calculated using a contingency table, which was not adjusted for contributing factors. However, the lack of adequate data in the original included studies made this limitation inevitable. Thirdly, hypermethylation in the promoter region of tumor-suppressor genes may link and interact with each other, indicating that an analysis of the status of single gene promoter methylation may not be sufficient.

In spite of these limitations, this meta-analysis also has merit: (i) all of the subjects included in this meta-analysis were NSCLC patients with confirmed pathology, which indicated that the clinical heterogeneity among patients was not significant; (ii) the controls were derived from autologous samples with concordance in clinical characteristics such as age, ethnicity, smoking history, and clinical stage and; (iii) no obvious publication bias was found using an Egger’s line regression test. However, completely ruling out the small number of studies and the possible existence of unpublished articles suitable for inclusion was impossible.

In conclusion, this meta-analysis showed that the prevalence of APC promoter 1A methylation in lung cancer tissues was higher than in autologous controls, indicating that promoter methylation of this gene may play an important role in NSCLC carcinogenesis.

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Disclosure

No authors report any conflict of interest.

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