Association of RASSF1A Promoter Methylation with Lung Cancer Risk: a Meta-analysis

Ying-Ze Huang, Wei Wu, Kun Wu, Xiao-Ning Xu, Wen-Ru Tang*

Abstract

RASSF1A, regarded as a candidate tumor suppressor, is frequently silenced and inactivated by methylation of its promoter region in many human tumors. However, the association between RASSF1A promoter methylation and lung cancer risk remains unclear. To provide a more reliable estimate we conducted a meta-analysis of cohort studies to evaluate the potential role of RASSF1A promoter methylation in lung carcinogenesis. Relevant studies were identified by searches of PubMed, Web of Science, ProQest and Medline databases using the following key words: ‘lung cancer or lung neoplasm or lung carcinoma’, ‘RASSF1A methylation’ or ‘RASSF1A hypermethylation’. According to the selection standard, 15 articles were identified and analysed by STATA 12.0 software. Combined odds ratio (OR) and 95% confidence interval (CI) were used to assess the strength of the association between RASSF1A promoter methylation and lung cancer risk. A chi-square-based Q test and sensitivity analyses were performed to test between-study heterogeneity and the contributions of single studies to the final results, respectively. Funnel plots were carried out to evaluate publication bias. Overall, a significant relationship between RASSF1A promoter methylation and lung cancer risk (OR, 16.12; 95%CI, 11.40-22.81; p<0.001) with no between-study heterogeneity. In subgroup analyses, increased risk of RASSF1A methylation in cases than controls was found for the NSCLC group (OR, 13.66, 95%CI, 9.529-19.57) and in the SCLC group (OR, 314.85, 95%CI, 48.93-2026.2).

Keywords: RASSF1A - lung cancer - methylation - NSCLC and SCLC

Introduction

Lung cancer is the most frequent cancer worldwide still. There were more than 1.8 million new cases (13% of total cancer incidence) and almost 1.6 million deaths (20% of total cancer mortality), as estimated in 2012 (WIL, D2014). Moreover, Lung cancer is the leading cause of cancer death in men in 87 countries and in women in 26 countries. Despite the advent of new diagnostic techniques, most lung cancers are detected at a late stage, and the 5-year survival rate of lung cancer is less than 15% in the US (Jemal et al., 2011). Once tumor cells have spread, the long-term prognosis is poor since no curative treatments are available. Thus, the development of biomarkers for effective early diagnosis of lung cancer is clearly necessary and he molecular biomarker is a new diagnostic technique for tumors (Hirsch et al., 2002).

The Ras association domain family 1 A (RASSF1A) gene, located on chromosome 3 at band p21.3 (3p21.3) which is common heterozygous and homoezygous deletions in different types of human tumors (Agathanggelou et al., 2003). It may serve as the effector that mediates the apoptotic effects of Ras by binding Ras in a guanosine triphosphate dependent manner (Hesson et al., 2007). What is more, RASSF1A is a frequent aberrant methylation gene in lung cancer, and high methylation of the RASSF1A promoter gene was reported in up to 60% of non-small cell lung cancer and 100% of small-cell lung cancer cases (Honorio et al., 2003; Grote et al., 2006). These findings suggest that RASSF1A is likely to be involved in the genesis of lung cancer, and plays an important role in the progression of tumorigenesis.

The effect of RASSF1A methylation abnormalities has been investigated in lung carcinoma. Many studies using univariate or multivariate analysis have been evaluated the association between promoter methylation of RASSF1A gene and the risk of lung cancer (Agathanggelou et al., 2001; Burbee et al., 2001; Wang et al., 2004). However, the results from those studies remain conflicting rather than conclusive. Thus, we carried out a meta-analysis based on all eligible case-control literature to assess the association of RASSF1A promoter methylation with the risk of lung carcinoma.

Materials and Methods

Search strategy and selection criteria

We conducted a comprehensive search strategy towards electronic databases, including PubMed, Web of Science, ProQest and Medline, using the key words: ‘lung
cancer or lung neoplasm or lung carcinoma’, ‘RASSF1A methylation’ or ‘hypermethylation’. The included articles meet the following criteria: 1. original study; 2. the diagnosis of lung cancer was based on histopathology; 3. the sample for the analysis was obtained from biopsy or surgical tumour tissue specimens; 4. studies were included if they had a case-control design and available frequency of the RASSF1A promoter methylation. 5. When the same or overlapping data appeared in multiple publications, we used the most recent or largest population; 6. the publication language was confined to English.

**Data extraction**

Data were extracted from each study by two reviewers independently using pre-specified selection standards. Decisions were made and disagreements about study selection were resolved by consensus or by involving a third reviewer. The following information was extracted from the studies: the first author’s last name, publication year, original country of patients in the subjects, and numbers of cases and controls, the number of RASSF1A methylation individuals in NSCLC and SCLC groups, etc.

**Statistical analysis**

The strength of the association between the RASSF1A promoter methylation and lung cancer risk was measured by pooled odds ratio (OR) with its 95% confidence interval (CI). The significance of the pooled OR was determined by the Z test and \( p < 0.05 \) was considered as statistically significant. Subgroup analysis was performed stratified by the study character of NSCLC and SCLC. The heterogeneity assumption was checked by chi-test based on Q-test (significance level of \( p < 0.10 \)) (Dickersin and Berlin, 1992). With a lack of heterogeneity among included studies, the pooled odds ratio estimates were calculated using the fixed-effects model (Mantel-Haenszel) (Mantel and Haenszel, 1959). Otherwise, the random-effects model (DerSimonian and Laird method) was used (DerSimonian and Laird, 1986). Sensitivity analyses were performed to assess the contributions of single studies to the final results. Begg’s funnel plots were used to examine whether the results of a meta-analysis may have been affected by publication bias. Egger’s test was implemented to testing for funnel plot asymmetry (Egger, 1997). All statistical analyses were performed using Stata statistical software (Stata/SE version 12.0 for Windows; Stata Corp, College Station, TX).

**Results**

**Eligible studies**

After being selected in accordance with the inclusive criteria, our final eligible studies included 15 studies, as shown in Figure 1 (Burbee et al., 2001; Wang et al., 2004; Safar et al., 2005; Chen et al., 2006; Wang et al., 2007; Yanagawa et al., 2007; Brock MV, 2008; Helmbold et al., 2009; Lin et al., 2009; Zhang et al., 2010; Lee et al., 2012; Li et al., 2012; Li et al., 2014; Zhai and Li, 2014).

**Study characteristics**

The characteristics of retained 15 studies are listed in Table 1. The subjects were conducted in 6 countries (Korea, China, Japan, USA, Sweden and Germany) and published between 2001 and 2014, twelve of the 15 studies were focus on non-small cell lung cancer, 2 of the 15 article were on small cell lung cancer investigation, one of the 15 paper contain NSCLC and SCLC. There

![Figure 1. Flow Chart of Study Identification](image1)

**Table 1 Main Characteristics of the Studies Included in the Meta-analysis**

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Country</th>
<th>Lung cancer histology</th>
<th>Conrol</th>
<th>Case</th>
<th>Total cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zhai et al.</td>
<td>2014</td>
<td>China</td>
<td>NSCLC</td>
<td>0</td>
<td>40</td>
<td>52.38%</td>
</tr>
<tr>
<td>Li et al.</td>
<td>2014</td>
<td>China</td>
<td>NSCLC</td>
<td>0</td>
<td>56</td>
<td>85.71%</td>
</tr>
<tr>
<td>Li et al.</td>
<td>2012</td>
<td>China</td>
<td>SCLC</td>
<td>0</td>
<td>52</td>
<td>80.36%</td>
</tr>
<tr>
<td>Lee et al.</td>
<td>2012</td>
<td>Korea</td>
<td>NSCLC</td>
<td>1</td>
<td>40</td>
<td>44.66%</td>
</tr>
<tr>
<td>Zhang et al.</td>
<td>2010</td>
<td>China</td>
<td>NSCLC</td>
<td>0</td>
<td>20</td>
<td>38.67%</td>
</tr>
<tr>
<td>Helmbold et al.</td>
<td>2009</td>
<td>Germany</td>
<td>SCLC</td>
<td>0</td>
<td>18</td>
<td>94.44%</td>
</tr>
<tr>
<td>Lin et al.</td>
<td>2009</td>
<td>China</td>
<td>NSCLC</td>
<td>2</td>
<td>26</td>
<td>42.74%</td>
</tr>
<tr>
<td>Brock et al.</td>
<td>2008</td>
<td>USA</td>
<td>NSCLC</td>
<td>37</td>
<td>104</td>
<td>50.00%</td>
</tr>
<tr>
<td>Yanagawa et al.</td>
<td>2007</td>
<td>Japan</td>
<td>NSCLC</td>
<td>0</td>
<td>3</td>
<td>42.57%</td>
</tr>
<tr>
<td>Wang et al.</td>
<td>2007</td>
<td>China</td>
<td>SCLC</td>
<td>0</td>
<td>15</td>
<td>80.00%</td>
</tr>
<tr>
<td>Wang et al.</td>
<td>2007</td>
<td>China</td>
<td>NSCLC</td>
<td>0</td>
<td>15</td>
<td>30.67%</td>
</tr>
<tr>
<td>Chen et al.</td>
<td>2006</td>
<td>China</td>
<td>NSCLC</td>
<td>4</td>
<td>57</td>
<td>38.60%</td>
</tr>
<tr>
<td>Ito et al.</td>
<td>2005</td>
<td>Japan</td>
<td>NSCLC</td>
<td>0</td>
<td>138</td>
<td>31.88%</td>
</tr>
<tr>
<td>Safar et al.</td>
<td>2005</td>
<td>USA</td>
<td>NSCLC</td>
<td>2</td>
<td>32</td>
<td>18.10%</td>
</tr>
<tr>
<td>Wang et al.</td>
<td>2004</td>
<td>China</td>
<td>SCLC</td>
<td>4</td>
<td>119</td>
<td>38.66%</td>
</tr>
<tr>
<td>Burbee et al.</td>
<td>2001</td>
<td>Sweden</td>
<td>NSCLC</td>
<td>0</td>
<td>104</td>
<td>51.30%</td>
</tr>
</tbody>
</table>
Quantitative analysis

The main results of our meta-analysis and the heterogeneity test are shown in Figure 2. No statistically significant heterogeneity was observed in overall and stratified analyses; all the pooled odds ratios for risk were calculated by a fixed-effects model.

The combined results based on all studies showed the relationship between the RASSF1A promoter methylation and lung cancer significantly associated with increased risk of lung cancer (OR, 16.123; 95% CI, 11.395-22.813; p < 0.001; Figure 2). In other words, compared with healthy persons, lung cancer patients had a 16.123-fold higher risk for RASSF1A methylation. When stratifying for histological type of lung cancer, the increased risk of RASSF1A methylation in case and controls was found NSCLC group (OR = 13.655; 95% CI, 9.529-19.567) and in the SCLC group (OR = 314.853; 95% CI, 48.926-2026.156).

Sensitive analysis

Sensitive analyses were conducted to determine whether modification of the inclusive criteria of the meta-analysis affected the final results. When we excluded one study (Zhai and Li, 2014) to assess the publication bias of the studies. For overall and subgroup analyses, the shapes of Egger’s funnel plot revealed obvious symmetry (not shown). Then the results were confirmed by Beggs’s test (for total studies: p = 0.363; for the NSCLC group: p = 0.272; for the SCLC group: p = 0.117). Therefore, neither Beggs’s funnel plot nor Egger’s test detected any publication bias of the studies.

Bias diagnosis

Begg’s funnel plot and Egger’s test were carried out to assess the publication bias of the studies. For overall and subgroup analyses, the shapes of Egger’s funnel plot revealed obvious symmetry (not shown). Then the results were confirmed by Beggs’s test (for total studies: p = 0.363; for the NSCLC group: p = 0.272; for the SCLC group: p = 0.117). Therefore, neither Beggs’s funnel plot nor Egger’s test detected any publication bias of the studies.

Discussion

It is known that methylation is a major epigenetic modification in mammals, and changes in methylation patterns play an important role in tumorigenesis in humans. In particular, promoter CpG island hypermethylation is closely linked to inactivation and silencing, resulting in tumor suppressor loss of expression, and affects the development of carcinogenesis (Baylin, 2005; Hesson et al., 2007). Aberrant promoter region methylation of tumor-suppressor genes is association with the mechanism for carcinogenesis. Similarly, abnormal methylation of RASSF1A within the promoter region has been reported in various tumor types (Amin and Banerjee, 2012). To determine whether RASSF1A methylation can serve as a biomarker for risk of lung cancer, we undertook a systematic review and meta-analysis of the literature.

To our knowledge, this is the first meta-analysis of published studies to evaluate the relationship between RASSF1A promoter methylation and lung cancer risk. Our analyses, combining 15 independent studies, revealed that the methylation of RASSF1A promoter does increase the risk of lung cancer. In particular, the overall OR for methylation status in lung cancer versus normal lung tissue was 16.123 (95% CI, 11.395-22.813; p < 0.001; Figure 2), suggesting a strong association of the methylation of RASSF1A promoter with lung cancer. Next, we stratified the association between RASSF1A promoter methylation and lung cancer risk by NSCLC and SCLC, found subgroup analysis by lung cancer histology showed NSCLC group (OR = 13.655; 95% CI, 9.529-19.567) and in the SCLC group (OR = 314.853; 95% CI, 48.926-2026.156), indicating a parallel effect of RASSF1A promoter methylation on lung cancer risk among different lung cancer histology.

Assessment of the between-study heterogeneity is an essential requirement in meta-analyses (Joseph Lau, 1998). In our study, heterogeneity within the subjects was demonstrated by chi-square-based Q test. After systematically examined, we found that no significant heterogeneity between the enrolled 15 studies. In sensitivity analyses we found that there was no single sensitive study in our meta-analysis. In addition to between-study heterogeneity, publication bias has also been recognized as a major concern in robust meta-analyses. Thereby, we used Beggs’s funnel plot and Egger’s test to assess whether the studies included could be affected by publication bias. As a result, no evidence of publication bias was found. Taken together, these results indicated a credibly related of RASSF1A methylation with lung carcinoma.

Lung cancer remains the most frequent cancer worldwide, so development of efficient diagnostic methods to enable its early detection plays an essential role in increasing the survival rate of patients with lung cancer. Our result showed a strong positive association of RASSF1A methylation with risk of lung cancer, which is consistent with previous findings that RASSF1A methylation could be used as an independent clinical diagnosis factor for lung cancer (Niklinski et al., 2005; Fischer et al., 2007; Zhai and Li, 2014). Therefore,
methylation is a potential to be used as a molecular marker for early detection and monitoring in carcinoma of the lungs.

For NSCLC and SCLC, during our meta-analysis, we found a difference significant association with RASSF1A methylation. Overall, the small cell lung cancer had a high risk for non-small cell lung cancer. As to the cumulative meta-analysis, with the increasing number of included studies, the dynamic change trend was stable and the estimates gradually became consistent. The sensitive analysis confirmed that there was no change in the odds ratios and the 95% confidence interval when altering the included studies; both the Begg’s funnel plot and Egger’s test supported the robust conclusion. As Li et al. (Li et al., 2014) confirmed that there was a significant relationship between RASSF1A methylation in lung carcinoma.

In conclusion, there exists a strong association between methylation of the RASSF1A promoter and risk of lung cancer. Although further investigations with large number of samples are required to confirm the associations between RASSF1A promoter methylation and lung cancer, the findings in the present study highlight a promising potential for RASSF1A promoter methylation in lung cancer risk prediction.

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References


