RESEARCH ARTICLE

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

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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 databasesusing 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 analysised 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 homozygous 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

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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 (Mantele-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
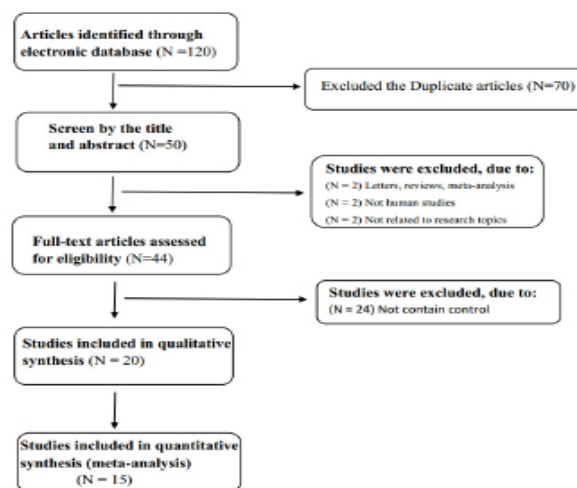

Figure 1. Flow Chart of Study Identification

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

| Author	Year	Country	Lung cancer histology	Concrol			Case			Total cases
				M	T	%	M	T	%	
Zhai et al.	2014	China	NSCLC	0	40	0.00%	22	42	52.38%	82
Li et al.	2014	China	NSCLC	0	56	0.00%	48	56	85.71%	112
Li et al.	2012	China	SCLC	0	52	0.00%	45	56	80.36%	108
Lee et al.	2012	Korea	NSCLC	1	40	2.50%	92	206	44.66%	246
Zhang et al.	2010	China	NSCLC	0	20	0.00%	58	150	38.67%	170
Helmbold et al.	2009	Germany	SCLC	0	18	0.00%	17	18	94.44%	36
Lin et al.	2009	China	NSCLC	2	26	7.69%	53	124	42.74%	150
Brock et al.	2008	USA	NSCLC	37	104	35.58%	25	50	50.00%	154
Yanagawa et al.	2007	Japan	NSCLC	0	3	0.00%	43	101	42.57%	104
Wang et al.	2007	China	SCLC	0	15	0.00%	4	5	80.00%	20
Wang et al.	2007	China	NSCLC	0	15	0.00%	23	75	30.67%	90
Chen et al.	2006	China	NSCLC	4	57	7.02%	44	114	38.60%	171
Ito er al.	2005	Japan	NSCLC	0	138	0.00%	44	138	31.88%	276
Safar et al.	2005	USA	NSCLC	2	32	6.25%	19	105	18.10%	137
Wang et al.	2004	China	NSCLC	4	119	3.36%	46	119	38.66%	238
Burbee et al.	2001	Sweden	NSCLC	0	104	0.00%	79	154	51.30%	258
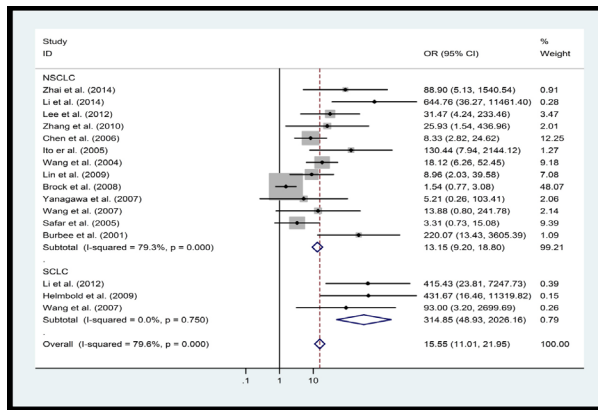
Figure 2. Forest Plot of Meta-analysis for Association between *RASSF1A* Methylation and Lung Cancer. The forest plot displays the effect size and 95% confidence intervals for each study, subgroup and overall. The pooled odds ratios for risk are from fixed-effects models using STATA12.0

was 43.75% of lung cancer patients had the methylated *RASSF1A* allele with a frequency ranging from 18.10% to 94.44% in individual trials.

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 was 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 heathy person, 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 studies is that sample size of the methylation patients were found in the NSCLC group (Brock MV, 2008). There was almost no difference between the remaining 14 studies and the previous 15 in terms of odds ratios and 95% confidence intervals (28.509, 17.82-45.62), indicating that our results were statistically robust.

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 Begg's test (for total studies: *p*=0.363; for the NSCLC group: *p*=0.272; for the SCLC group: *p*=0.117). Therefore, neither Begg'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 a 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 Begg'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,

RASSF1A 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.

Acknowledgements

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