RESEARCH ARTICLE

Association between Cigarette Smoking and RASSF1A Gene Promoter Hypermethylation in Lung Cancer Patients: a Meta-analysis

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Abstract

Objectives: Epidemiological studies have shown that molecular mechanisms underlying the development of lung cancers differ between smokers and unsmokers. Aberrant promoter methylation in some tumor suppressor genes is frequent in lung tumors from smokers but rare in those from non-smokers. Recently, many studies have investigated the association between cigarette smoking and RASSF1A gene promoter hypermethylation in lung cancer patients, but a unanimous conclusion could not be reached. We therefore performed this meta-analysis to derive a more precise estimation of any association. Study Design: An electronic search of PubMed and Chinese Biomedicine databases was conducted to select studies. A total of 19 case-control studies were chosen, and odds ratios (ORs) with confidence intervals (CIs) were used to assess the strength of associations. Results: The case-control studies covered 2, 287 lung cancer patients: 63.4% (1449) of the patients were smokers, 36.6% (838) were unsmokers. The overall results suggested that smokers with lung cancer had a 1.297-fold (95% CI: 1.066~1.580, *p*=0.010, *p*=0.087) higher risk for RASSF1A gene hypermethylation than the non-smokers. In the stratified analysis, an increased risk of RASSF1A gene hypermethylation in smokers than in non-smokers was found in Asian (OR=1.481, 95%CI: 1.179~1.861, *p*=0.001, *p*=0.186). Conclusions: This meta-analysis supports the idea that RASSF1A gene hypermethylation is associated with cigarette smoking-induced lung cancer.

Keywords: Cigarette smoking - RASSF1A hypermethylation - meta-analysis - lung cancer

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Introduction

Lung cancer is one of the most frequent cause of cancer-related mortality in the world (Jemal et al., 2011). Primary lung cancer can be divided into two categories according to histopathology, non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). NSCLC, characterized by a long asymptomatic latency and poor prognosis, accounts for about 80% of primary lung cancers . NSCLC includes denocarcinoma (AC), squamous cell carcinoma (SCC), large cell carcinoma and others. Despite advances in both detection and treatment of lung cancer, most lung cancers are detected at a late stage, the overall 5-year survival rate is less than 15% (Jemal et al., 2011). Lung cancer was expected to accout for 26% of all female cancer deaths in 2013 (Siegel et al., 2013). Thus, it is necessary to identify biomarkers for effective early diagnosis of lung cancer.

Cigarette smoking has been definitely established as the major cause of lung cancer. Smoking is thought to be responsible for 17.2% of NSCLC cases in men and 11.6% of cases in women, which is much higher than nonsmokers with 1.3% in men and 1.4% in women (Villeneuve and Mao, 1994). However, the molecular mechanism responsible for the increased risk of lung cancer in young smokers remains unclear.

The genomic organization at 3p21.3, RASSF1A (Ras-association domain family 1 isoform A), a major isoform of RASSF1 (the Ras-association domain family 1), has a RAS association (RA) domain and a cysteine-rich diacylglycerol-binding domain (C1 domain) in the amino terminal region (Dammann et al., 2000; Burbee et al., 2001). RASSF1A is a cell cycle-related tumor suppressor protein that that decreases colony formation, suppresses anchorage-independent growth, and dramatically reduces tumorgenicity *in vivo*. Previous studies have reported the involvement of RASSF1A promoter methylation in several cancers, including prostate (Ge et al., 2014), Ovarian (Vo et al., 2013), lung cancer (Liu et al., 2013). It has been reported that the RASSF1A gene is frequently inactivated in primary lung cancers by the de novo methylation of CpG islands in the promoter region (Zochbauer-Muller et al., 2001; Tomizawa et al., 2002). RASSF1A methylation has been identified in 30-60% of NSCLC and has been associated with poorer patient prognosis and earlier recurrence of disease (Dammann et al., 2000; Burbee

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

First Author	Publication year	Country	Ethnicity	histology	Methods	Sample size	RASSF1A methylation in smoker		RASSF1A methylation in nonsmoker	
							Methylation	Total	Methylation	Total
Lee(Lee, Lee et al. 2012)	2012	Korea	Asian	NSCLC	MSP	206	61	146	16	60
Kang(kang 2011)	2011	China	Asian	NSCLC	MSP	53	6	40	3	13
Yanagawa(Yanagawa, Tamura et al. 2011)	2011	Japan	Asian	AC	MSP	62	18	36	9	26
Ye(Ye 2010)	2010	China	Asian	NSCLC	MSP	30	4	23	1	7
Zhang(Zhang, Zhang et al. 2010)	2010	China	Asian	NSCLC	nMSP	150	34	88	24	62
Peng(Peng, Shan et al. 2010)	2010	China	Asian	NSCLC	MSP	82	30	45	22	37
Liu(Liu 2010)	2010	China	Asian	NSCLC	MSP	96	28	48	19	48
Yang(Yang 2007)	2007	China	Asian	NSCLC	MSP	49	14	14	13	35
Yanagawa(Yanagawa, Tamura et al. 2007)	2007	Japan	Asian	NSCLC	MSP	101	32	73	10	28
Kim(Kim, Cha et al. 2007)	2007	Korea	Asian	NSCLC	MSP	99	34	79	6	20
Liu(Liu, Gao et al. 2007)	2007	Pennsylvania	Caucasian	NSCLC	MSP	122	39	81	18	41
Chen(Chen, Suzuki et al. 2006)	2006	Japan	Asian	NSCLC	MSP	114	26	72	18	42
Toyooka(Toyooka, Tokumo et al. 2006)	2006	Japanese	Asian	AC	MSP	164	34	86	19	78
Divine(Divine, Pulling et al. 2005)	2005	USA	Mix	AC	MSP	268	79	225	19	43
Marsit(Marsit, Kim et al. 2005)	2005	USA	Mix	NSCLC	MSP	178	3	13	80	165
Wang(Wang, Lee et al. 2004)	2004	USA	Mix	NSCLC	MSP	119	33	81	13	38
Tomizawa(Tomizawa, Iijima et al. 2004)	2004	Japan	Asian	NSCLC	MSP	90	16	61	10	29
Endoh(Endoh, Yatabe et al. 2003)	2003	Japan	Asian	NSCLC	MSP	100	22	53	20	47
Kim(Kim, Kim et al. 2003)	2003	Korea	Asian	NSCLC	MSP	204	62	185	3	19

Table 2. ORs and 95% CI for Cigarette Smoking and RASSF1A Methylation in Lung Cancer Patients

Population	Pooled OR [95% CI] *p*	Heterogeneity *p*-value	Publication Bias *p*-value Begg	*p*-value Egger
Asian	1.481(1.179-1.861)0.001	0.186	0.622	0.751
Caucasian	1.187(0.558-2.525)0.657	-	-	-
Mix	0.769(0.483-1.224)0.268	0.168	0.602	0.655
Overall	1.297(1.066-1.580)0.010	0.087	0.97	0.382

et al., 2001; Tomizawa et al., 2002; Endoh et al., 2003; Kim et al., 2003). Despite the significance of RASSF1A gene promoter methylation in predicting incidence or prognosis and in epigenetic therapy of lung cancer, the manner in which these epigenetic lesions accumulate during carcinogenesis is not completely understood.

Molecular epidemiological studies have begun to link specific environmental carcinogens, including those in tobacco smoke, with DNA methylation changes in lung cancer progression. However, the results are inconsistent and inconclusive. The present study mainly focused on RASSF1A gene , which is transcriptionally silenced predominantly through aberrant promoter hypermethylation. Here, we performed a meta-analysis to quantitatively analyze the correlation between cigarette smoking and RASSF1A gene promoter methylation in lung cancer patients.

Materials and Methods

Publication Search

We searched the PubMed and Chinese biomedicine databases for all articles on the association between cigarette smoking and RASSF1A gene methylation in lung cancer (last search update, November 21, 2013). The following key words were used: 'smoking', ' Cigarette smoking', 'RASSF1A', 'methylation '. 'hypermethylation', Case-control studies containing frequencies of RASSF1A gene methylation in smoker and unsmoker patients were chosen. Of the studies with overlapping data published by the same author, only the most recent or complete study was included in this meta-analysis.

Statistical analysis

The strength of association was accessed by calculating crude odds ratios (ORs) and 95% confidence intervals (CIs). Heterogeneity assumption was evaluated by a chi-square based Q-test. A *P*-value of >0.05 for the Q-test indicated a lack of heterogeneity among the studies, the summary OR estimate of each study was calculated by the fixed effects model (Mantel and Haenszel, 1959; DerSimonian and Laird, 1986). The potential for publication bias was examined by a Begg's test (funnel plot method) and Egger's linear regression test ($P<0.05$ considered representative of statistical significance) (Egger et al., 1997). All analyses were performed using Stata software (version 8.2; Stata Corporation, College Station, TX).

Results

Eligible studies

In this meta-analysis, we identified 19 studies with 2287 lung cancer patients on the association between cigarette smoking and RASSF1A gene methylation, including 1449/838 smokers /unsmokers (Table 1). The following information was extracted from study: first author, publication year, country, ethnicity, histology of lung cancer, method, number of participants RASSF1A methylation frequencies in smokers and nonsmokers. In studies defining nonsmoker, there were three different definitions of nonsmoker: (1) less than 20 pack-years; (2) less than 100 cigarettes in entire lifetime; (3) daily cigarette consumption *years of smoking = 0. In our meta-analysis, we combined nonsmokers according to their original group in each individual study.

Meta-analysis

In the meta-analysis, 2287 lung cancer patients including 1449 smokers and 838 nonsmokers were included in pooling the overall correlation estimation.

The results of the association between the cigarette smoking and the frequency of RASSF1A methylation and the heterogeneity test are shown in Table 2. Under the fixed-effects model, compared to nonsmoker patients,

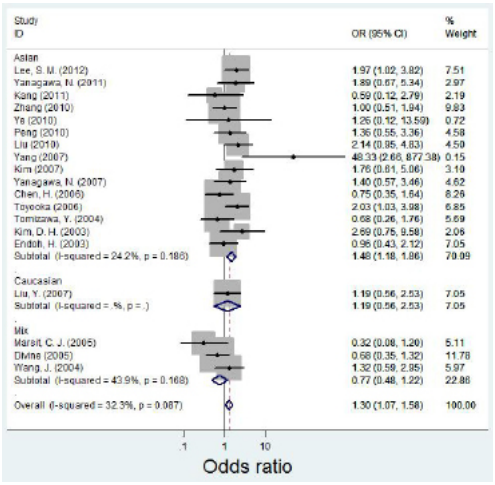

Figure 1. Forest Plot of ORs of RASSF1A Methylation in Smoker Compared to Nonsmoker Lung Cancer Patients. The squares and horizontal lines correspond to the study-specific OR and 95% CI. The area of the squares reflects the study-specific weight. The diamond represents the pooled OR and 95% CI

smoker with lung cancer had a

1.297-fold higher risk for methylation (Figure 1). Stratified analysis also revealed that the associations varied among the subjects from different regions. The association between cigarette smoking and RASSF1A methylation tended to be stronger in 15 Asian studies (OR =1.481, 95% CI: 1.179~1.861, p=0.001, p=0.186) compared to the other studies (Caucasian: OR=1.187, 95%CI =0.558-2.525, p=0.657; Mix: OR=0.769, 95%CI =0.483-1.224, p=0.268) (Figure 1).

Publication bias

Funnel plot and Egger's test were performed to quantitatively evaluate the publication bias of literatures on RASSF1A methylation. The results of Begg's test provided statistical evidence for funnel plot symmetry (p=0.970) in overall results, suggesting the absence of publication bias.

Discussion

In this study, we investigated that cigarette smoking is positively related to RASSF1A hypermethylation in tumor tissues from lung cancer patients. The frequency of RASSF1A hypermethylation was 1.297 times higher in smoker patients than that in nonsmoker patients. The association appeared to be stronger in Asian patients. We speculated that RASSF1A hypermethylation might be an early marker for cancer diagnosis, particularly in cigarette smoking patients.

Epidemiological studies indicate that some characteristics of lung cancer among smokers significantly differ from those of unmokers. Kras mutations appear more often in smoker lung cancers, while EGFR is activated by gene mutations in unsmoker lung cancers. The biological consequences of Kras and EGFR mutations share similaries in regulation of cell proliferation. It is well known that RASSF1A could also lead to cell cycle arrest by participating in the Rb family cell cycle checkpoint (Hesson et al., 2007). Inhibition of cyclin D1 by RASSF1A occurs post-transcriptionally and may be at the level of translational control (Shivakumar et al., 2002). Moreover, RASSF1A serve as Ras effector, whose function analysis has revealed an involvement in apoptotic signaling (Khokhlatchev et al., 2002), microtubule stabilization (Liu et al., 2003) and mitotic progression (Tomizawa et al., 2002; Mathe, 2004). It has been reported that the RASSF1A gene is frequently inactivated in lung cancer by the de novo methylation of CpG islands in the promoter region (Zochbauer-Muller et al., 2001; Honorio et al., 2003; Grote et al., 2006). RASSF1A promoter methylation might play a pivotal role in the development of Lung cancer (Liu et al., 2013).

Links among cigarette smoking, RASSF1A hypermethylation, and lung cancer remain unclear. DNA methylation is mediated by the members of the DNA methyltransferase (DNMT) family, conventionally classified as de novo (DNMT3a and DNMT3b) and maintenance (DNMT1). It is estimated that DNMT1 overexpression was found in many types of cancers including lung cancers, particularly in patients who were smokers (Lin et al., 2007; Lin et al., 2010). DNMT1 accumulation and subsequent hypermethylation of the promoter of RASSF1A may lead to tumorigenesis and poor prognosis and provide an important link between tobacco smoking and lung cancer. Moreover, expression ofΔDNMT3B variants, a subfamily of DNMT3B, was detected in 80% tumors but in only 18% of the corresponding normal lungs (Wang et al., 2008). DNMT3B may play an important role in the development of promoter methylation. Demethylation by 5'-aza-2'-deoxycytidine treatment resulted in the restoration of RASSF1A gene expression and the transfection and expression of RASSF1A in lung cancer cells resulted in the suppression of colony formation, anchorage-independent soft agar growth and tumorigenesis in nude mice (Burbee et al., 2001). These findings indicated that tobaccoinduced DNMTs overexpression might be responsible for maintaining the hypermethylation status of RASSF1A gene.

There are some limitations that are present in this analysis, which may affect the objectivity of the conclusion of this meta-analysis. It is well known that the risk for developing lung cancer in smokers is 8 to 13 times higher than that in nonsmokers, while the risk of RASSF1A methylation in lung cancer patients with smoking habits was only 1.297 times. It is possible that differences in RASSF1A hypermethylation between smokers and unsmokers are influenced by other factors, such as gender, age, state of lung cancer, histological tumour type. Therefore, a meta-analysis including more high-quality designed epidemiology studie is necessary in the future in this field.

This meta-analysis with sufficient individual data to be stratify results by ethnicity. This analysis supports conclusions that the cigarette smoking had increased risk of RASSF1A methylation (OR =1.297, 95% CI: 1.066~1.580, p=0.010, p=0.087) in lung cancer patients, especially in the case of Asian (OR =1.481, 95% CI: 1.179~1.861, p=0.001, p=0.186) , which highlight the

potential importance of RASSF1A promoter methylation in early cancer diagnosis.

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