Systematic Review and Meta-Analysis

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Associations of *RASSF1A*, *RARβ*, and *CDH1* promoter hypermethylation with oral cancer risk

A PRISMA-compliant meta-analysis

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Abstract

Background: Oral tumor is a heterogeneous group of tumors, in which it has several different histopathological and molecular features. Recently, genetic and epigenetic alterations are often detected in the development of oral cancer. Gene promoter hypermethylation leads to the silencing of cancer related genes without changes of genes sequence. To clarify the effect of RAS association domain family protein 1a (RASSF1A), retinoic acid receptor beta (RARβ), and E-cadherin (CDH1) promoter hypermethylation on the risk of oral cancer, we performed this meta-analysis.

Methods: PubMed, Web of Science, Embase, and Chinese National Knowledge Infrastructure (CNKI) databases were retrieved to identify eligible articles. Stata 12.0 software was used to analyze extracted data of the included articles. Odds ratios (ORs) with the corresponding 95% confidence interval (95% CI) were calculated to evaluate the associations of *RASSF1A*, *RARβ*, and *CDH1* promoter hypermethylation with oral cancer risk.

Results: Around 23 literatures with 29 studies were included in the final meta-analysis, in which 12 studies were about *RASSF1A* promoter methylation, 4 studies were about *RARβ* promoter methylation, and 13 studies were about *CDH1* promoter methylation. Overall, the results of this meta-analysis showed that there were significant associations between *RASSF1A*, *RARβ*, and *CDH1* promoter hypermethylation and oral cancer risk (*RASSF1A*, OR = 11.8, 95% CI = 6.14–22.66; *RARβ*, OR = 20.35, 95% CI = 5.64–73.39; *CDH1*, OR = 13.46, 95% CI = 5.31–34.17). In addition, we found that *RASSF1A* promoter hypermethylation exerted higher frequency in the tongue tumor than other site tumor in mouth (*RASSF1A*, tongue tumor vs other site tumor in mouth, unmethylation vs methylation, OR = 0.65, 95%CI = 0.44–0.98).

Conclusion: *RASSF1A*, *RARβ*, and *CDH1* promoter hypermethylation might significantly increase the risk of oral cancer.

Abbreviations: 95% CI = 95% confidence interval, CDH1 = E-cadherin, CNKI = Chinese National Knowledge Infrastructure, log OR = log odds ratio, MDM = murine double minute, MDM2 = murine double minute 2, NOS = Newcastle–Ottawa scale, ORs = odds ratios, OSCC = oral squamous cell carcinoma, RARβ = retinoic acid receptor beta, RASSF = RAS association family, RASSF1A = RAS association domain family protein 1a, s.e.of: log OR = standard error of log odds ratio, SGC = salivary gland carcinoma.

Keywords: *CDH1*, oral cancer risk, promoter hypermethylation, *RARβ*, *RASSF1A*

1. Introduction

Oral cancer is one of the most frequent tumor of head and neck tumor, in which oral squamous cell carcinoma (OSCC) accounts for approximately 90% of all oral malignancies.[1] As the sixth most common cancer worldwide, OSCC has a high mortality and low cure rate.[2] Although advancements have been made in the prevention and treatment of oral cancer, the 5-year survival rate of OSCC still remained approximately 50%.[3] In addition, invasion, lymph node metastasis, and distant metastasis of tumor cells were often found in the development of mouth cancer. Therefore, early detection was very important to the treatments of oral cancer, and therefore reduced the mortality rates of mouth cancer. It was commonly believed that environmental factors such as drinking, smoking, chewing betel nuts, and ultraviolet radiation modulated multistep process of oral carcinogenesis. Furthermore, some other intrinsic factors such as genetic and epigenetic alternations were also involved in the multistep carcinogenesis of oral cancer.[4] In recent years, DNA hypomethylation, DNA hypermethylation, loss of imprinting, chromosome inactivation, histone acetylation, histone deacetylation, histone methylation, and microRNA changes were referred in the studies of oral cancer.[4] Meanwhile, these studies often focused on gene methylation which regulated the gene expression without DNA sequence changes. And promoter methylation of many tumor suppressor genes were reported in several cancers like breast cancer, thyroid cancer, liver cancer, and nonsmall cell lung cancer.[5–8] In previous studies, promoter aberrant methylation of many candidate genes, including *p16*, *p15*, *p14*, *DAPK*, *p73*, *APC*, *WIF1*, *RUNX3*, *MGMT*, and *hMLH1*, have been

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GW and HW contributed equally to this work.

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identified in oral cancer.[4,9] It has been reported that the incidence rate of aberrant methylation of these genes in oral cancer tissues were higher than the normal tissues. For example, Don et al[10] have performed a systematic meta-analysis and observed a higher prevalence of methylation of *p16*, *DAPK,* and *MGMT* in OSCC. At the same time, many other case–control or cohort studies have been conducted to explore the potential role of these tumor suppressor genes in the process of oral cancer.[11–13] The results suggested that these genes were often significantly associated with the invasion, metastasis, and differentiation of oral tumor. Thus, these tumor suppressor genes that occurred aberrant methylation might be good biomarkers for the early detection and early treatment of oral cancer.

RASSF1A (RAS association domain family protein 1a), a kind of ras association family (RASSF) proteins, was involved in the Ras/PI3K/AKT signal pathways. RASSF1A played a key role in the cell cycle control, microtubule stabilization, cellular adhesion and motility as well as apoptosis.[14] Moreover, several studies have also found *RASSF1A* promoter hypermethylation contributed a lot to the gene silencing, and therefore leaded to the tumor occurrence.[15] Based on the previous record, the promoter hypermethylation of *RASSF1A* was a common phenomenon in many various tumors.[16] The hypermethylation of *RASSF1A* promoter region was originally detected in lung cancer and breast cancer.[17] Since then, hypermethylation of *RASSF1A* gene was reported in many different cancers and was described as a good prognostic indicator.[18] Several studies have also been performed to evaluate the relationship between *RASSF1A* promoter hypermethylation and oral cancer risk. However, the results remained inconsistent. Moreover, the results of other studies indicated that *CDH1* and *RARβ* promoter hypermethylation frequencies were very high in oral cancer patients.[4] Therefore, in order to systematic assess the associations of *RASSF1A*, *CDH1*, and *RARβ* promoter hypermethylation with oral cancer risk, we conducted this meta-analysis.

2. Methods

2.1. Search strategy for included studies

In this study, 2 researchers independently retrieved PubMed, Web of Science, Embase, and CNKI (Chinese National Knowledge Infrastructure) databases and included the relevant articles. The literature research was up to 15 April 2017. The following keywords or medical subject headings (MeSH) words: "oral cancer," "oral tumor," "oral carcinoma," "oral squamous cell carcinoma," "OSCC," "Salivary Gland Carcinoma," "Buccal Carcinoma," "Salivary Adenoid Cystic Carcinoma," "RASSF1A," "CDH1," "E-cadherin," "RARβ," "methylation," and "hypermethylation" were used to search eligible articles. In addition, references of included articles were reviewed for additional eligible studies. The literature searching was limited to the studies of human disease.

2.2. Study selection criteria

The inclusion criteria were: studies assessing the associations of *RASSF1A*, *RARβ*, and *CDH1* hypermethylation with oral cancer risk; and case–control or cohort studies that contained data of hypermethylation frequency both in control and case group. If studies did not meet the following criteria, they would be removed: no or incomplete relevant data about *RASSF1A*, *RARβ*, and *CDH1* methylation data; duplicate data; meta-analysis and review article; and low-quality studies. All relevant articles were evaluated and selected by 2 investigators. If discrepancies occurred in the process of studies selection, the third researcher would help to resolve this problem through discussion with the 2 reviewers. Furthermore, if duplicate data were showed in different studies, the most complete and latest data were selected.

2.3. Data extraction and methodology quality assessment

Two reviewers independently extracted the data of gene hypermethylation frequency. The following information was extracted from included articles: first author's name, publication year, race, frequency of gene methylation, disease type, detection method of genes methylation, and country of studied population. The methodological quality, including selection of case and control (4 stars), comparability of the groups (2 stars), and ascertainment of exposure (3 stars), were evaluated with the Newcastle–Ottawa scale table. If a study got ≥ 6 stars, the study was considered as high quality and included for this meta-analysis, otherwise it would be removed.

2.4. Statistical methods

STATA software (version 12.0, Stata Corporation, College Station, Texas) was used to conduct all statistical analysis. The associations of *RASSF1A*, *RARβ*, and *CDH1* methylation with oral cancer risk were evaluated with ORs and 95% CI. All P values were 2-sided in which $P<.05$ was considered as statistically significant. Heterogeneity among studies were detected by chi-squared test based on Q-statistic test.[19] If P value was $<.05$ or I^2 value was $>50\%$, which indicated a significant heterogeneity, a random-effects model was used; otherwise, a fixed-effect model would be applied.[20,21] In addition, Z-test was conducted to determine the strength of pooled ORs. In order to assess the publication bias, Egger's test and Begg's test were performed to detect between-study publication bias, in which $P<.05$ indicated a significant publication bias.[22,23] Moreover, a sensitivity analysis was conducted to further detect the stability of overall ORs by sequential deletion of each study. In the meta-analysis of *CDH1* promoter hypermethylation, meta-regression was also performed to explore the source of heterogeneity due to significant heterogeneity.

2.5. Ethics approval

This meta-analysis did not collect clinical sample and applied animals experiment. Moreover, all included studies were approved by relevant Ethics Committee in eligible studies. Therefore, ethical approval was not required.

3. Results

3.1. Characteristics of included studies

The procedure of literature searching was shown in Figure 1. Around 160 potentially relevant articles were identified in the initial searching. After removing repeat articles, 75 relevant records were reviewed and selected. Through reading titles and abstracts, 55 articles were remained, of which 20 irrelevant literatures were excluded. In addition, the full-text of remained 55 articles were read, in which 32 studies did not contained relevant effective data and were eliminated. Finally, 23 articles

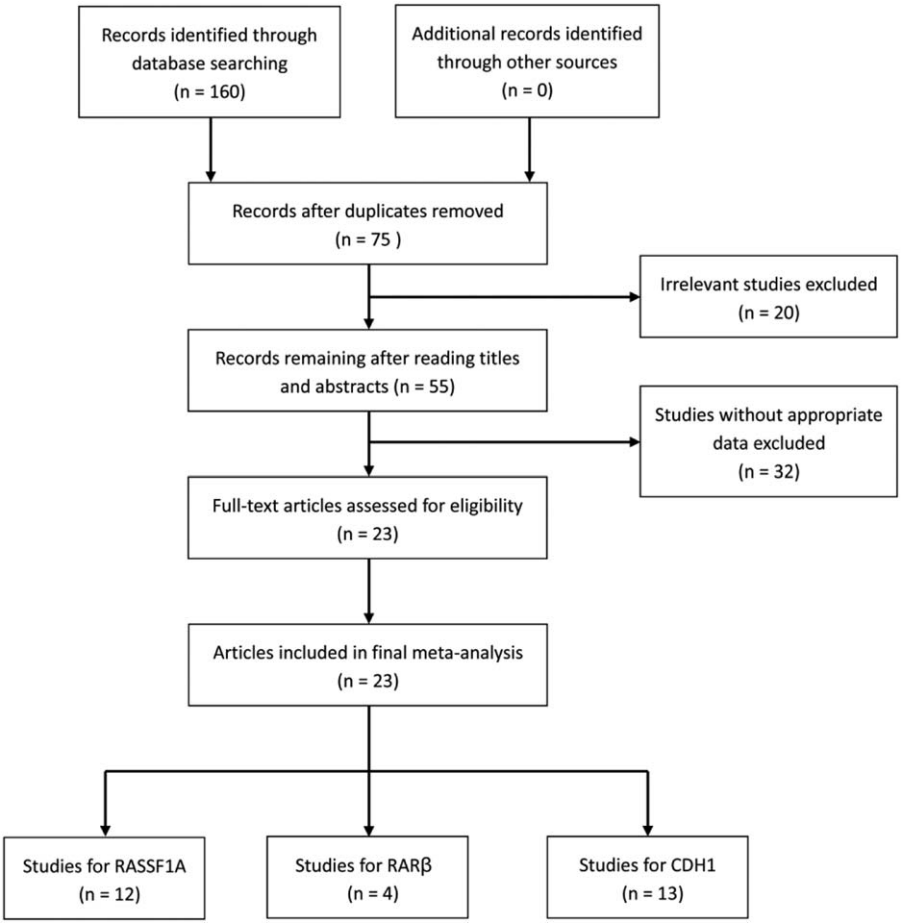

Figure 1. Flowchart of literature selection.

with 29 studies were included for the meta-analysis, in which 12 studies with 254 controls and 1238 cases were about *RASSF1A*, 4 studies with 82 controls and 293 cases were about *RARβ*, and 13 studies with 432 controls and 608 cases were about *CDH1*.[24–43] Moreover, all studies obtained a score of ≥ 6, which indicated high methodological quantity of included studies. The characteristics of eligible studies in the present meta-analysis were shown in Table 1.

3.2. RASSF1A, RARβ, and CDH1 promoter hypermethylation and oral cancer risk

The *RASSF1A* methylation data of 12 case–control studies were pooled together and the ORs were calculated to assess the association between *RASSF1A* promoter hypermethylation and oral cancer risk. On the basis of the results, the overall pooled ORs clarified that *RASSF1A* promoter hypermethylation was significantly associated with oral cancer risk (OR = 11.8, 95% CI = 6.14–22.66). According to the results of *Q*-statistic test, no heterogeneity among studies was found ($P = .337$, $I^2 = 12.0\%$). Then, subgroup analysis based on oral cancer subtype was performed and significant association was detected in OSCC and SGC (salivary gland carcinoma) (OSCC, OR = 6.78, 95% CI = 3.20–14.37; SGC, OR = 18.51, 95% CI = 3.58–95.79). Furthermore, significant associations of *RARβ* and *CDH1* promoter hypermethylation with oral cancer risk were detected in the meta-analysis (*RARβ*, OR = 20.35, 95% CI = 5.64–73.39; *CDH1*, OR = 13.46, 95% CI = 5.31–34.17). In the analysis for *CDH1* methylation, significant heterogeneity among studies was found ($I^2 = 73.1\%$, $P = .000$). In order to explore the source of heterogeneity, we performed a meta-regression, in which the results indicated that ethnicity might be the mainly source of heterogeneity ($P = .028$, 95% CI = 0.275–3.976). In the stratified analysis based on ethnicity, *CDH1* promoter hypermethylation might significantly increase the oral cancer risk in Asians, but not in Caucasians (Asians, OR = 21.79, 95% CI = 8.66–54.82; Caucasians, OR = 2.57, 95% CI = 0.71–9.31). However, only 3 studies for *CDH1* methylation in Caucasians were included to calculate the pooled OR (Table 2, Figs. 2–4).

3.3. RASSF1A promoter hypermethylation and development of oral cancer

To determine whether promoter methylation of *RASSF1A* correlates with the development of oral cancer, the statistical analysis of associations of *RASSF1A* promoter methylation with TNM-stage, tumor-stage, differentiation, and lymph node metastasis of oral cancer were conducted. According to the results, no significant associations were detected. However, there was a remarkably high frequency of *RASSF1A* promoter hypermethylation in tongue tumor, which suggested that *RASSF1A* promoter hypermethylation might play an important

Table 1

Basic characteristic of the eligible studies.

First author	Publication year	Country	Ethnicity	Histology	Control		Oral cancer		Methods	Control materials	Case materials	NOS
					U	M	U	M				
RASSF1A												
Zhang et al[24]	2014	China	Asians	SACC	50	0	108	59	MSP	Adjacent tissue	Tumor tissue	8
Lin et al[25]	2013	China	Asians	BC	9	0	34	10	MSP	Normal tissue	Tumor tissue	8
Supic et al[26]	2011	Serbia	Caucasians	OSCC	82	12	29	18	qMSP	Adjacent tissue	Tumor tissue	8
Su et al[27]	2010	China	Asians	OSCC	33	0	22	11	qMSP	Adjacent tissue	Tumor tissue	8
Durr et al[28]	2010	USA	Caucasians	SGC	17	0	45	33	qMSP	Normal tissue	Tumor tissue	6
Lee et al[29]	2008	Korea	Asians	SGC	12	0	45	24	MSP	Normal tissue	Tumor tissue	8
Wan et al[30]	2007	China	Asians	OSCC	10	0	19	13	MSP	Normal tissue	Tumor tissue	7
Williams et al[31]	2006	USA	Caucasians	SGC	29	0	79	23	MSP	Normal tissue	Tumor tissue	6
Huang et al[11]	2009	China	Asians	OSCC	–	–	374	108	PCR-DHPLC	–	Tumor tissue	–
Taioli et al[12]	2009	USA	Caucasians	OPC	–	–	48	8	MSP	–	Tumor tissue	–
Li et al[13]	2005	China	Asians	SACC	–	–	35	25	MSP	–	Tumor tissue	–
Tran et al[32]	2005	Japan	Asians	OSCC	–	–	2	34	MSP	–	Tumor tissue	–
RARβ												
Nagata et al[33]	2012	Japan	Asians	OSCC	22	2	6	28	MSP	Health oral rinse	Cancer patients oral rinse	7
Durr et al[28]	2010	USA	Caucasians	SGC	17	0	55	23	qMSP	Normal tissue	Tumor tissue	6
Lee et al[29]	2008	Korea	Asians	SGC	12	0	37	32	MSP	Normal tissue	Tumor tissue	8
Williams et al[31]	2006	USA	Caucasians	SGC	29	0	102	10	MSP	Normal tissue	Tumor tissue	6
CDH1												
Morandi et al[34]	2015	Italy	Caucasians	OSCC	8	0	7	0	MSP	Normal tissue	Tumor tissue	6
Asokan et al[35]	2014	India	Asians	OSCC	5	0	4	6	MSP	Normal tissue	Tumor tissue	6
Ge et al[36]	2012	China	Asians	SACC	20	0	28	26	MSP	Normal tissue	Tumor tissue	8
Nagata et al[33]	2012	Japan	Asians	OSCC	19	5	2	32	MSP	Healthy rinse	Oral cancer patients rinse	7
Xu et al[37]	2012	China	Asians	OC	48	2	38	22	MSP	Healthy blood	Oral cancer patients blood	6
Supic et al[26]	2011	Serbia	Caucasians	OSCC	82	12	27	20	qMSP	Adjacent tissue	Tumor tissue	8
Tamandani et al[38]	2010	Iran	Caucasians	OSCC	26	31	29	47	MSP	Normal tissue	Tumor tissue	7
Su et al[27]	2010	China	Asians	OSCC	33	0	11	22	qMSP	Adjacent tissue	Tumor tissue	8
Viswanathan et al[39]	2003	Japan	Asians	OSCC	25	0	65	34	PMSRE	Normal tissue	Tumor tissue	6
Chang et al[40]	2002	China	Asians	OTC	11	0	25	45	MSP	Adjacent tissue	Tumor tissue	7
Huang et al[41]	2002	China	Asians	OSCC	40	8	28	20	MSP	Adjacent tissue	Tumor tissue	7
Nakayama et al[42]	2001	Japan	Asians	OSCC	4	1	1	17	MSP	Normal tissue	Tumor tissue	6
Saito et al[43]	1998	Japan	Asians	OSCC	52	0	43	9	MSP	Adjacent tissue	Tumor tissue	8

BC = buccal carcinoma, CDH1 = E-cadherin, MSP = methylation-specific PCR, NOS = Newcastle–Ottawa scale, OPC = oral and pharyngeal cancer, OSCC = oral squamous cell carcinoma, OTC = oral tongue carcinoma, PCR-DHPLC = polymerase chain reaction-denaturing high performance liquid chromatography, PMSRE = PCR based on methylation-sensitive restriction enzyme, qMSP = quantitative real-time methylation-specific PCR, RARβ = retinoic acid receptor beta, SACC = salivary adenoid cystic carcinoma, SGC = salivary gland carcinoma.

Table 2

Subgroup analysis of the association between *RASSF1A*, *RARβ*, and *CDH1* promoter methylation and oral cancer.

Variables	N	Test of associations		Heterogeneity		Begg test		Egger test	
		OR	95%CI	I^2	P	Z	P	t	P
RASSF1A									
Total	7	11.80	6.14–22.66	12.0%	0.337	0.37	0.711	4.17	.006
Asians	5	22.99	6.41–82.43	0.00%	0.821	1.22	0.221	−2.55	.084
Caucasians	2	7.24	3.35–15.62	25.2%	0.263	1.04	0.296	7.82	.081
Normal tissue	5	22.80	6.43–80.88	0.00%	0.851	1.22	0.221	−1.96	.145
Adjacent tissue	2	6.82	3.20–14.53	24.1%	0.268	0.00	1.00	7.51	.084
OSCC	2	6.78	3.20–14.37	24.8%	0.264	0.00	1.00	3.46	.179
SGC	3	18.51	3.58–95.79	0.00%	0.951	0.00	1.00	−0.61	.652
RARβ									
Total	4	20.35	5.64–73.39	0.00%	0.597	1.02	0.308	−2.63	.12
CDH1									
Total	13	6.80	4.47–9.74	73.10%	0.00	1.71	0.086	5.13	.00
Asians	10	16.35	9.16–29.19	43.20%	0.07	1.07	0.283	3.77	.005
Caucasians	3	2.30	1.36–3.89	82.10%	0.018	0.00	1.00	–	–
Normal tissue	6	3.84	2.23–6.64	76.70%	0.002	0.73	0.462	8.37	.004
Adjacent tissue	5	8.29	4.81–14.29	58.10%	0.049	1.71	0.086	4.03	.027
OSCC	10	5.49	3.75–8.04	76.20%	0.00	1.15	0.251	3.95	.006

BC = buccal carcinoma, CDH1 = E-cadherin, CI = confidence interval, OR = odds ratio, OSCC = oral squamous cell carcinoma, SGC = salivary gland carcinoma.

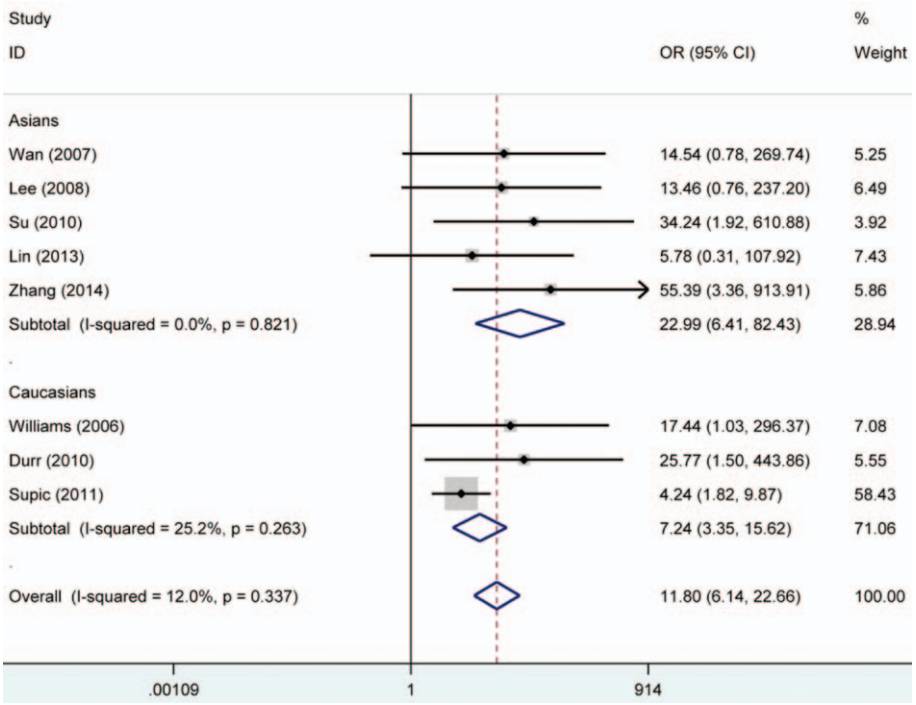

Figure 2. Forest plot on association between *RASSF1A* promoter hypermethylation and oral cancer risk. The forest plot is drawn to calculate a pooled estimated value of ORs and 95%CIs with stata 12.0 software. For each study, the estimation of ORs and its 95% CI are plotted with a box and a horizontal line which cross the imaginary line. The length of the black line which crosses the imaginary line is proportional to the 95%CIs of included studies. The weight represents the number of elements that give rise to the overall value. According to the chi-squared test based on *Q*-statistic test, the value of *I*-squared and *P* value were used to evaluate the heterogeneity among studies. And no heterogeneity is found in the forest plot. The results indicated that people with *RASSF1A* promoter hypermethylation were 11.8 times higher risk than those without *RASSF1A* promoter hypermethylation to suffer from oral cancer. In addition, subgroup analysis based ethnicity was performed. ORs = odds ratios, RASSF1A = RAS association domain family protein 1a.

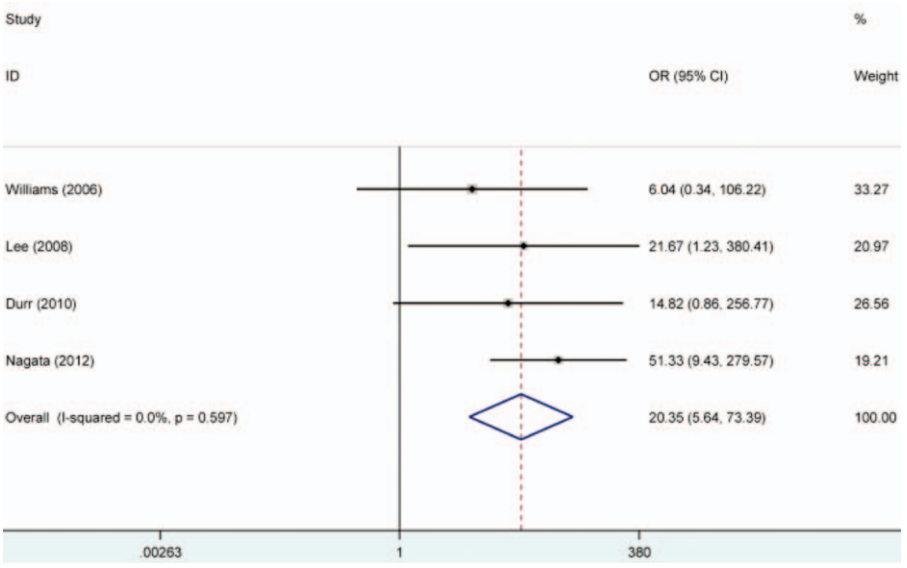

Figure 3. Forest plot on association between *RARβ* promoter hypermethylation and oral cancer risk. The forest plot is drawn to calculate a pooled estimated value of ORs and 95%CIs with stata 12.0 software. For each study, the estimation of ORs and its 95% CI are plotted with a box and a horizontal line which cross the imaginary line. The length of the black line which crosses the imaginary line is proportional to the 95%CIs of included studies. The weight represents the number of elements that give rise to the overall value. According to the chi-squared test based on *Q*-statistic test, the value of *I*-squared and *P* value were used to evaluate the heterogeneity among studies. And no heterogeneity is found in the analysis. In this forest plot, the results showed that people with *RARβ* promoter hypermethylation were 20.35 times higher risk than those without *RARβ* promoter hypermethylation to suffer from oral cancer. ORs = odds ratios, RARβ = retinoic acid receptor beta, RASSF1A = RAS association domain family protein 1a.

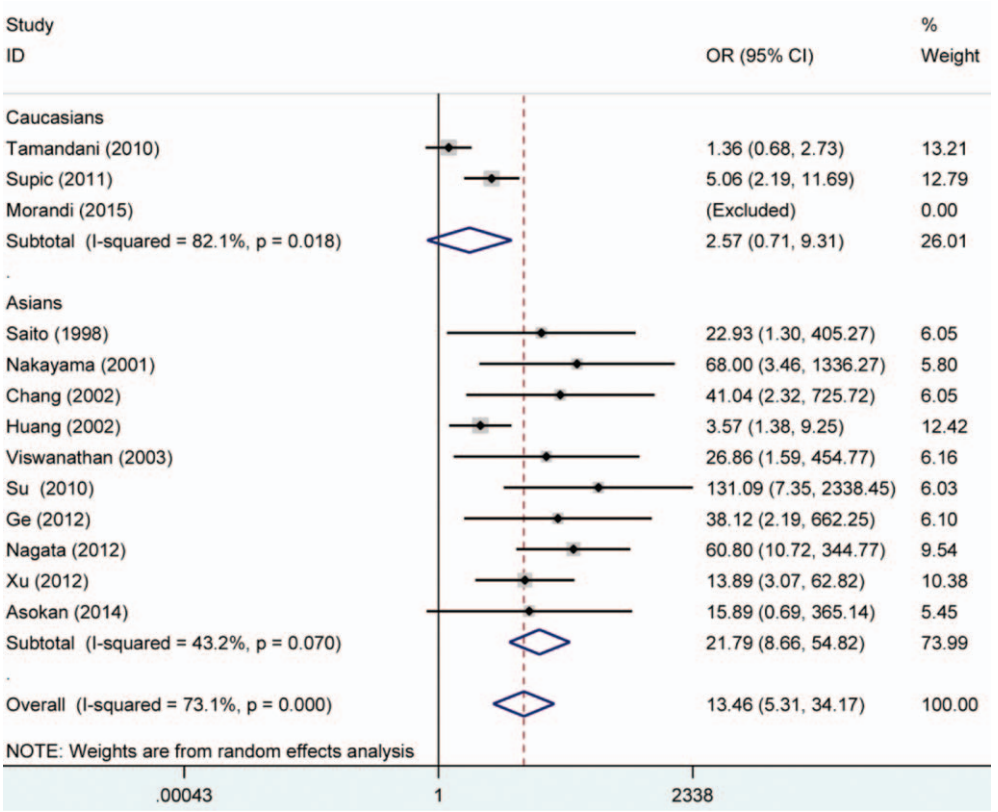

Figure 4. Forest plot on association between *CDH1* promoter hypermethylation and oral cancer risk. The forest plot is drawn to calculate a pooled estimated value of ORs and 95%CIs with stata 12.0 software. For each study, the estimation of ORs and its 95% CI are plotted with a box and a horizontal line which cross the imaginary line. The length of the black line which crosses the imaginary line is proportional to the 95%CIs of included studies. The weight represents the number of elements that give rise to the overall value. According to the chi-squared test based on *Q*-statistic test, the value of *I*-squared and *P* value were used to evaluate the heterogeneity among studies. Although heterogeneity is found in the overall analysis, no significant heterogeneity is found in the subgroup-analysis. In the forest plot, the results demonstrated that people with *CDH1* promoter hypermethylation were 13.46 times higher risk than those without *CDH1* promoter hypermethylation to suffer from oral cancer. CDH1 = E-cadherin, ORs = odds ratios.

role in the risk of tongue tumor (tongue tumor vs other site tumor in mouth, OR = 0.65, 95% CI = 0.44–0.98). Furthermore, we discussed the correlation of promoter hypermethylation of *RASSF1A* with smoking and drinking in oral cancer. Similarly, no significant associations of *RASSF1A* promoter hypermethylation with smoking and drinking in oral cancer were found. All results were shown in Table 3 (Fig. 5).

3.4. Sensitivity analysis and publication bias

Begg's test and Egger's test were all conducted to observe the publication bias in the meta-analysis of *RASSF1A*, *RARβ*, and *CDH1* hypermethylation. Significant publication bias was found ($P < .05$) in the analysis of *CDH1* aberrant methylation. So the subgroup analysis based on ethnicity was performed. Moreover, the results of funnel plots shown that the most dots

Table 3

Quantitative analyses of *RASSF1A* promoter hypermethylation and clinicopathological variables of oral cancer.

		Test of associations		Heterogeneity		Begg's test		Egger's test	
Variables	**N**	**OR**	**95%CI**	I^2	***P***	***Z***	***P***	***t***	***P***
RASSF1A									
TNM-stage	3	1.89	0.66–5.39	0.00	.816	0.00	1.00	0.57	.671
Smoking	4	0.81	0.46–1.41	0.00	.478	−0.34	1.00	−0.17	.88
Drinking	4	1.22	0.82–1.82	0.00	.438	−0.34	1.00	0.65	.582
Tongue tumor	3	0.65	0.44–0.98	51.7	.126	1.04	.296	21.59	.029
Lymph node metastasis	5	1.21	0.82–1.78	11.2	.337	1.02	.308	−1.37	.304
Differentiation	3	0.73	0.14–3.87	73.5	.023	0.00	1.00	−0.39	.763
Tumor stage	5	0.89	0.62–1.27	26.7	.244	−0.24	1.00	0.60	.592

OR = odds ratio, RASSF1A = RAS association domain family protein 1a.

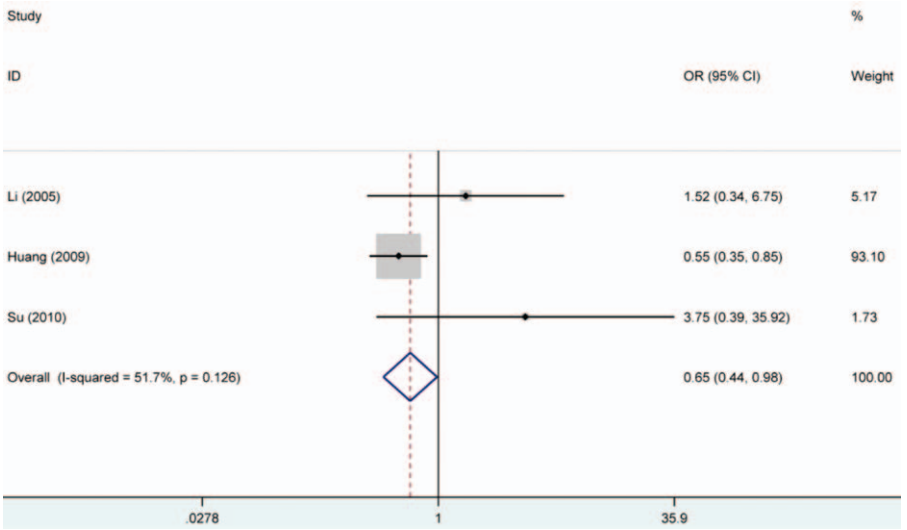

Figure 5. Forest plot on association between *RASSF1A* promoter hypermethylation and tongue tumor risk. The forest plot is drawn to calculate a pooled estimated value of ORs and 95%CIs with stata 12.0 software. For each study, the estimation of ORs and its 95% CI are plotted with a box and a horizontal line which cross the imaginary line. The length of the black line which crosses the imaginary line is proportional to the 95%CIs of included studies. The weight represents the number of elements that give rise to the overall value. According to the chi-squared test based on *Q*-statistic test, the value of *I*-squared and *P* value were used to evaluate the heterogeneity among studies. And no heterogeneity is found in the analysis. Interestingly, in this forest plot, the results showed that people with *RASSF1A* promoter hypermethylation had a lower risk than those without *RARβ* promoter hypermethylation to suffer from tongue cancer. ORs = odds ratios, RASSF1A = RAS association domain family protein 1a.

were symmetric other than the funnel plot for *CDH1* hypermethylation. At the same time, sensitivity analysis was also conducted and the overall pooled ORs did not significantly changed (Figs. 6–8).

4. Discussion

DNA mutations and gene epigenetic inactivation affected the function of many genes, which they were commonly detected in tumor suppressor genes. These changes affected the normal growth control of cells in which cell cycle was disturbed and cell

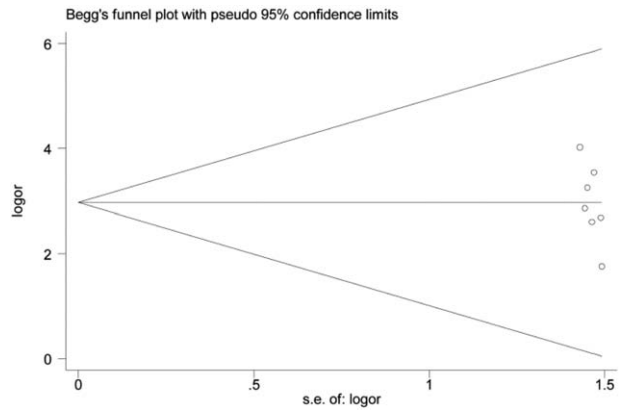

Figure 6. Funnel plot on association between *RASSF1A* promoter hypermethylation and oral cancer risk (control vs case, unmethylation vs methylation). s.e.of: logOR, standard error of log odds ratio. Log OR, log odds ratio. The funnel plot is used to assess the publication bias among included studies. The horizontal axis represents the effect size of each included study. The area enclosed by the 2 diagonal lines simulates the 95% CIs. If the dot is located out the 95%CIs, publication bias may exist among studies. In addition, these dots that represent included studies should be symmetrical and be distributed on both sides of the angular bisector, which demonstrates no publication bias is found. From the funnel plot of Begg's test, we will acquire a *P* value. According to the results, the *P* value of the funnel plot was > 0.05. Thus, no significant publication bias was found in this analysis. Log OR = standard error of log odds ratio, RARβ = retinoic acid receptor beta, ORs = odds ratios, RASSF1A = RAS association domain family protein 1a, s.e. of: log OR = standard error of log odds ratio.

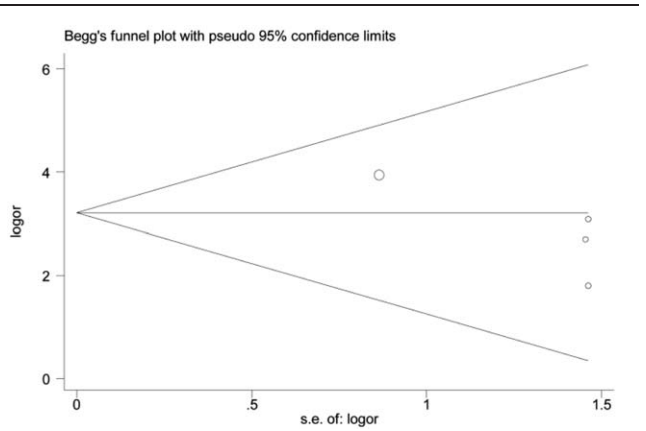

Figure 7. Funnel plot on association between *RARβ* promoter hypermethylation and oral cancer risk (control vs case, unmethylation vs methylation). s.e.of: logOR, standard error of log OR. The funnel plot is applied to evaluate the publication bias among included studies. The horizontal axis represents the effect size of each included study. The area enclosed by the 2 diagonal lines simulates the 95%CIs. If the dot is located out the 95%CIs, publication bias may exist among studies. In addition, these dots that represent included studies should be symmetrical and be distributed on both sides of the angular bisector, which demonstrates no publication bias is found. From the funnel plot of Begg's test, we will acquire a *P* value. From the results, the *P* value of the funnel plot was > 0.05. Therefore, no significant publication bias was found in this analysis. Log OR = standard error of log odds ratio, RARβ = retinoic acid receptor beta, s.e.of: log OR = standard error of log odds ratio.

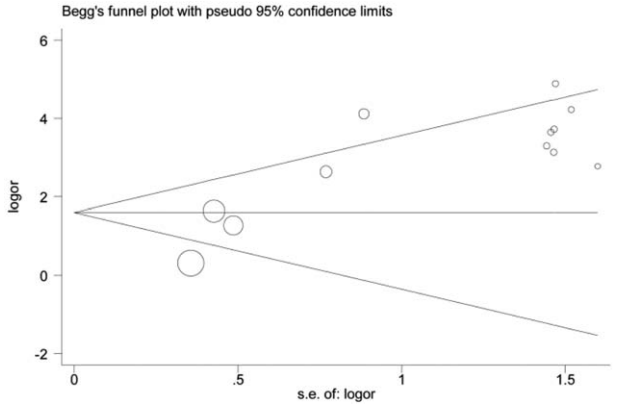

Figure 8. Funnel plot on association between *CDH1* promoter hypermethylation and oral cancer risk (control vs case, unmethylation vs methylation). s.e.of: logOR, standard error of log OR. The funnel plot is applied to evaluate the publication bias among included studies. The horizontal axis represents the effect size of each included study. The area enclosed by the 2 diagonal lines simulates the 95%CIs. If the dot is located out the 95%CIs, publication bias may exist among studies. In addition, these dots that represent included studies should be symmetrical and be distributed on both sides of the angular bisector, which demonstrates no publication bias is found. From the funnel plot of Begg's test, we will acquire a *P* value. From the funnel plot, the *P* value was < 0.05. Therefore, publication bias may exist among these studies involving the analysis of association between *CDH1* promoter hypermethylation and oral cancer risk. CDH1 = E-cadherin, log OR = log odds ratio, s.e.of: log OR = standard error of log odds ratio.

abnormal proliferation was driven. Although several changes of chromosome and genes were described in OSCC, the fundamental cause was the inactivation of tumor suppressor genes.[44] For example, one of the most important tumor suppressor genes was p16, as we all known, it regulated the cell growth just like p53.[45] Much of the work of genetic and epigenetic changes in oral cancer have been done, however, complex interactions between the gene products and signal pathways were very complicated.[46] MDM (murine double minute), class of oncogenes, had a multiple role in tumor inhibition, in which MDM2 (murine double minute 2) inhibited p53 if p53 mutations were absent. Moreover, the tumor cell cycle was regulated by p16 and p14 which stabilized p53 protein by MDM2.[45] Although a lot of studies about tumor suppressor genes in OSCC were conducted, there might be some different patterns of gene silencing among oral cancer patients. Therefore, more tumor suppressor genes might be studied to discuss the potential role in the development of oral cancer.

According to the results of literature search, this was the first meta-analysis to evaluate the associations of *RASSF1A*, *RARβ*, and *CDH1* promoter hypermethylation with oral cancer risk. In the present study, 12 studies were combined to assess the association between *RASSF1A* promoter hypermethylation and oral cancer risk, in which the results indicated a significant association. In the included studies, these results were consistent with the results of Su et al, Zhang et al, Williams et al, and Durr et al.[24,27,28,31] At the same time, no significant heterogeneity among studies was detected in which *P* value was $> .05$ and I^2 value was $< 50\%$. So although oral cancer included many subtypes, theses studies could be put together to calculate the pooled ORs and assess the association between levels of genes methylation and oral cancer risk. Meanwhile, subgroup analysis was still conducted to obtain more accurate results in different races. From the results, we found that *RASSF1A* promoter hypermethylation was significantly associated with oral cancer risk in Asians and Caucasians. However, the number of studies was very small after subgroup analysis based on oral cancer subtype was performed. In addition, the significant correlation between *RASSF1A* promoter hypermethylation and oral cancer risk was observed both in normal tissue and adjacent tissue of control group. But the results should be carefully taken into consideration due to the small sample size. Furthermore, this study demonstrated that *RARβ* and *CDH1* promoter hypermethylation were significantly associated with the oral cancer risk. In previous studies, Williams et al and Durr et al believed that there were no association between *RARβ* methylation and oral cancer risk, however, according to the results of the meta-analysis, the frequency of *RARβ* promoter hypermethylation in oral cancer group was higher than control group.[28,29,31,33] In addition, we discussed the relationship between *RASSF1A* promoter hypermethylation and clinicopathological features in oral cancer patients. To achieved accurate statistic data, we conducted heterogeneity analysis for all clinicopathological variables of oral cancer and environmental factors such as: smoking and drinking. Significant interstudy heterogeneity was only detected among studies for differentiation of oral cancer ($I^2 = 73.5\%$, $P = .023$), and random effects model was therefore applied to calculate the pooled ORs and 95% CI. Based on the ORs and 95% CI derived from the present meta-analysis, a significantly increased risk of tongue cancer with *RASSF1A* promoter hypermethylation was found. However, no significant associations were found between *RASSF1A* promoter hypermethylation and tumor stage, lymph node metastasis, differentiation, and TNM-stage of oral cancer. Although there was no clear evidence of significant associations between *RASSF1A* promoter hypermethylation and clinicopathologic features of oral cancer, we still needed to explore these associations due to the small sample size. Furthermore, more larger scale, multicenter, and more reasonable study should be performed to confirm the predictive value of *RASSF1A* promoter hypermethylation in the development of oral cancer.

Additionally, significant heterogeneity among studies was detected in the meta-analysis for *CDH1* promoter hypermethylation. Thus, the subgroup analysis based on ethnicity and meta-regression was carried out. The effects of publication year, country, disease type, control type, detection method of methylation, and ethnicity on the association between RASSF1A promoter methylation and oral cancer risk were evaluated by meta-regression. The results showed these factors were not the mainly cause of significant heterogeneity other than ethnicity in which *P* value was $< .05$. The same results were found in the stratified analysis based on race which no significant heterogeneity was detected in the subgroup analysis. Finally, the pooled ORs of *CDH1* and *RARβ* were stable on the basis of the results of sensitivity analysis. However, significant publication bias for the meta-analysis of *RASSF1A* promoter hypermethylation was detected according to the results of Egger's test ($P = .006 < .05$), while sensitivity analysis results of *RASSF1A* promoter hypermethylation indicated that the ORs were significantly changed after the study of Supic et al[26] was removed. So the study of Supic et al was eliminated and the heterogeneity and publication bias were significantly reduced.

Notably, although many studies were included to explore these associations, several limitations should be noted in this meta-analysis: the studied population only included Asians and Caucasians; all eligible studies in this meta-analysis were retrospective, some biases might exist in the selection of samples; the cut-off value or evaluation criteria of genes methylation

detection was unclear which might bring heterogeneity among different studies; the clinical information of oral patients was too small to get more accurate results; few negative and unpublished studies were included and a tendency for positive results might increase the publication bias.

5. Conclusion

In summary, our meta-analysis demonstrated that *PASSF1A*, *RARβ*, and *CDH1* promoter hypermethylation were significantly associated with the oral cancer risk. Considering limitations in this meta-analysis, additional multicenter validation studies were still needed to evaluate the associations between *RASSF1A*, *RARβ*, and *CDH1* promoter hypermethylation and oral cancer risk in the future.

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