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Research Paper

Diagnostic Capacity of *RASSF1A* Promoter Methylation as a Biomarker in Tissue, Brushing, and Blood Samples of Nasopharyngeal Carcinoma

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ABSTRACT

Methylation of the RAS association domain family protein 1A (*RASSF1A*) promoter has been observed in nasopharyngeal carcinoma (NPC). This study investigated the correlation of *RASSF1A* promoter methylation with clinicopathological features and its utility as a diagnostic biomarker in NPC. A total of 926 patients with NPC and 495 non-tumor controls were analyzed in this study. *RASSF1A* promoter methylation was notably higher in NPC compared with non-tumor tissue, brushing and blood samples. *RASSF1A* promoter methylation was associated with clinical stage, lymph node status, distant metastasis, and T classification of patients with NPC, although it was not linked to age and sex. The pooled sensitivity, specificity, and AUC (area under the curve) of *RASSF1A* promoter methylation were determined in NPC samples vs. non-tumor samples (tissue: sensitivity = 0.72, specificity = 0.99, AUC = 0.98; brushing: sensitivity = 0.56, specificity = 1.00, AUC = 0.94; blood: sensitivity = 0.11, specificity = 0.98, AUC = 0.97). Our findings show that *RASSF1A* promoter methylation may be correlated with the development, progression and metastasis of NPC. *RASSF1A* promoter methylation is a promising noninvasive biomarker for the diagnosis of NPC from tissue and brushing samples.

1. Introduction

Nasopharyngeal carcinoma (NPC) is an uncommon malignancy with distinct geographic and ethnic characteristics. GLOBOCAN estimates that approximately 86,700 new cases of NPC have been reported, leading to an estimated 50,800 deaths in 2012 (Torre et al., 2015). NPC occurs frequently, with an incidence rate of 15 to 50 per 100,000 people annually in Southeast Asia. However, the incidence rate is not higher than 1 per 100,000 people in Western countries (Zhou et al., 2007; Yu and Yuan, 2002). Unfortunately, distant metastasis is a main cause of death for NPC patients, which often has an unfavorable prognosis (Chen et al., 2012; Chua et al., 2012; Liu et al., 2003). Although computed tomography (CT) and magnetic resonance imaging (MRI) are effective, they cannot accurately provide a prognosis for NPC or predict the effectiveness of biological therapeutic targets (Lin et al., 2013; Gong et al., 1991). Thus, a novel, noninvasive low-cost biomarker for early detection of NPC is of great importance to clinical practice.

DNA methylation, which is a common mechanism in epigenetic alterations, may be correlated with NPC (Jiang et al., 2015; Nawaz et al., 2015a). Promoter methylation of tumor suppressor genes (TSGs), such as calcium channel voltage-dependent alpha 2/delta subunit 3 (*CACNA2D3*) and cadherin 4 (*CDH4*), may play a crucial role in NPC development and progression (Wong et al., 2013; Du et al., 2011). Localized in human chromosomal region 3p21.3, the RAS association domain family protein 1A (*RASSF1A*) is an important TSG involved in multiple biological functions, including cell cycle regulation, microtubule stabilization, and apoptosis (Allen et al., 2007; Agathanggelou et al., 2005; Burbee et al., 2001). In NPC, *RASSF1A* gene expression is often blocked due to promoter methylation (Wang et al., 2009; Fendri et al., 2009; Lo et al., 2001). *RASSF1A* promoter methylation can be detected in tissue, brushing and blood samples of patients with NPC (Nawaz et al., 2015b; Yang et al., 2015; Hutajulu et al., 2011).

However, there are some inconsistencies in reports on the level of the *RASSF1A* promoter methylation in NPC. For example, Chang et al. reported that the rate of *RASSF1A* promoter methylation in NPC patients was different in tissue (66.7%), blood (3.3%), and brushing samples (33.3%) (Chang et al., 2003). Yang et al. reported that the *RASSF1A* promoter region was frequently methylated in 68.8% of brushing samples from NPC patients (Yang et al., 2015). Therefore, the aim of this study was to assess the relationship between *RASSF1A* promoter methylation and NPC risk in tissue, brushing, and blood samples. Moreover, we

Abbreviations: *RASSF1A*, RAS association domain family protein 1A; NPC, nasopharyngeal carcinoma; OR, odds ratio; 95% CI, 95% confidence interval; AUC, the summary receiver operator characteristic (SROC) curve; TSG, tumor suppressor gene.

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analyzed the correlation of *RASSF1A* promoter methylation with clinicopathological features of patients with NPC. Finally, we determined the diagnostic utility of *RASSF1A* promoter methylation as a noninvasive biomarker in samples of tissue, brushings, and blood.

2. Materials and Methods

2.1. Search Strategy

We conducted a systematic search of online electronic databases (PubMed, Embase, EBSCO, Web of Science, Scopus and the Cochrane Library) to identify eligible literature published prior to January 11, 2017. The following combination of key words and search terms were used to identify studies: 'nasopharyngeal cancer or nasopharyngeal neoplasm or nasopharyngeal carcinoma or nasopharyngeal tumor or NPC', 'RASSF1A or RAS association domain family protein 1A', 'methylation or methylated or epigene*'. We also carefully checked the references of eligible articles to identify other potential studies. This study was conducted based on the preferred reporting items for systematic reviews and meta-analyses (PRISMA) statement criteria (Moher et al., 2009) (Table S1).

2.2. Inclusion Criteria

Studies were included in this meta-analysis if they fulfilled the following selection criteria: 1) patients were diagnosed with primary NPC based on histopathological examination of samples, including tissue, brushing, and blood; 2) articles were published in English; 3) there was sufficient information on the level of *RASSF1A* promoter methylation in NPC and non-tumor samples; 4) there was sufficient data for estimating the relationship between *RASSF1A* promoter methylation and the clinicopathological characteristics of patients with NPC. If multiple papers were published using overlapping sample data, we only included the most appropriate article with the most detailed information.

2.3. Data Extraction

Two authors independently scanned and abstracted the following information from available studies: surname of first author, year of publication, country, population by race, sample types, number of cases and non-tumor controls, methodology for the detection of methylation, rate of *RASSF1A* promoter methylation, expression status of the *RASSF1A* gene, and clinicopathological parameters, such as age (>50 years vs. ≤ 50 years), sex (male vs. female), clinical stage (stage 3–4 vs. stage 1–2), lymph node status (positive vs. negative status), distant metastasis (yes vs. no), and T classification (T3–4 vs. T1–2). Any inconsistent data or information was resolved by a discussion including all authors.

2.4. Statistical Analysis

Pooled data in this meta-analysis were analyzed using Stata software, version 12.0 (STATA Corp., College Station, TX, USA). The strength of the correlation between *RASSF1A* promoter methylation and NPC was estimated by the combined odds ratios (ORs) with 95% confidence intervals (95% CIs). The pooled ORs and corresponding 95% CIs were also used to analyze the relationship between *RASSF1A* promoter methylation and the clinicopathological features of NPC patients, including age, sex, clinical stage, lymph node status, distant metastasis, and T classification. Potential heterogeneity among studies was detected using Cochran's Q test (Coory, 2010). The random-effects model was applied when Q-test P values were <0.1, indicating obvious heterogeneity. A fixed-effect model was applied to the data when the P values were >0.1, indicating no evidence of heterogeneity (Higgins et al., 2003; DerSimonian, 1996). Meta-regression analyses were performed to assess the sources of heterogeneity. Sensitivity analyses were conducted to determine whether removing individual studies with substantial heterogeneity changed the overall OR (Lau et al., 1997). Egger's test was used to evaluate potential publication bias for results with more than nine studies (Egger et al., 1997). Based on the bivariate analysis, we generated the combined sensitivity, specificity, and the summary receiver operator characteristic (SROC) curve (AUC) to evaluate the diagnostic capacity of *RASSF1A* promoter methylation in tissue, blood, and brushing samples from NPC patients in the meta-analysis (Reitsma et al., 2005; Jones and Athanasiou, 2005).

3. Results

3.1. Study Characteristics

Fig. 1 lists a detailed procedure for our literature search in a range of online electronic databases. After a careful screen based on the inclusion criteria described above, we identified 16 studies, including 926 patients with NPC and 495 non-tumor controls, with sufficient data in the final meta-analysis (Nawaz et al., 2015b; Yang et al., 2015; Tian et al., 2013; Challouf et al., 2012; Hutajulu et al., 2011; Wang et al., 2009; Fendri et al., 2009; Zhou et al., 2005; Pan et al., 2005; Qiu et al., 2004; Wong et al., 2004; Chang et al., 2003; Wong et al., 2003; Tong et al., 2002; Kwong et al., 2002; Lo et al., 2001). Of the 16 eligible studies, 11 investigated the correlation between *RASSF1A* promoter methylation and NPC in tumor versus non-tumor tissues (Nawaz et al., 2015b; Challouf et al., 2012; Wang et al., 2009; Fendri et al., 2009; Zhou et al., 2005; Qiu et al., 2004; Chang et al., 2003; Wong et al., 2003; Tong et al., 2002; Kwong et al., 2002; Lo et al., 2001). Four studies determined the relationship between *RASSF1A* promoter methylation and NPC in tumor versus non-tumor blood samples (Yang et al., 2015; Tian et al., 2013; Wong et al., 2004; Chang et al., 2003). Four studies analyzed the association between *RASSF1A* promoter methylation and NPC in tumor versus non-tumor brushing samples (Yang et al., 2015; Hutajulu et al., 2011; Chang et al., 2003; Tong et al., 2002). Eight studies involving 502 NPC patients assessed the relationship between *RASSF1A* promoter methylation and the clinicopathological characteristics of patients with NPC (Yang et al., 2015; Tian et al., 2013; Wang et al., 2009; Fendri et al., 2009; Zhou et al., 2005; Pan et al., 2005; Wong et al., 2004; Tong et al., 2002). Table 1 and Table S2 present the general characteristics of the studies included in the meta-analysis.

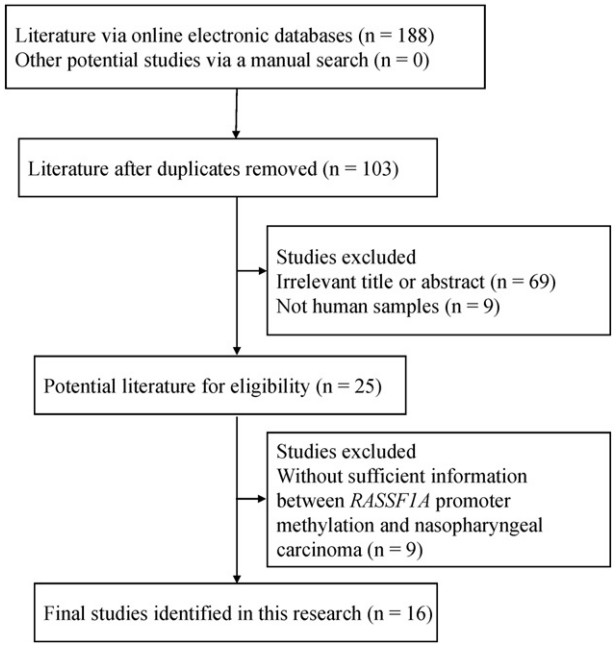

Fig. 1. PRISMA flow chart of the procedure for selecting literature.