

## RESEARCH ARTICLE

# Aberrant Methylation of RASSF1A gene Contribute to the Risk of Renal Cell Carcinoma: a Meta-Analysis

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### Abstract

The aim of this study was to assess the diagnostic value of RASSF1A methylation in renal cell carcinoma. Systematically search were performed using the Pubmed, ProQest and Web of Science for all articles on the association between RASSF1A methylation and renal cell carcinoma before 15 April 2015. After the filtration, 13 studies involving 677 cases and 497 controls met our criteria. Our meta-analysis suggested that hypermethylation of RASSF1A gene was associated with the increased risk of RCC (OR:4.14, 95% CI:1.06-16.1). Stratified analyses showed a similar risk in qualitative detection method (OR:28.4, 95% CI:10.2-79.6), body fluid sample (OR:12.8, 95% CI:5.35-30.8), and American (OR:10.5, 95% CI:1.97-55.9). Our result identified that RASSF1A methylation had a strong potential in prediction the risk of Renal cell carcinoma.

**Keywords:** RAS association domain family 1A, renal cell carcinoma, DNA methylation, meta-analysis

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### Introduction

With the advent of the aging society, cancer has become one of the serious threats to human health and it is the second leading cause of death, only surpassed by heart diseases. Among them, the incidence of kidney cancer ranks third in human urinary system tumors, with more than 61, 000 new cases each year and more than 14, 000 deaths per year (Siegel et al., 2015). Renal cell carcinoma (RCC) is the most common histological type of kidney cancer, accounting for about 85% of all adult kidney epithelial tumors (Young et al., 2006). Because RCC is not sensitive to conventional chemotherapy and radiotherapy, nowadays radical or partial nephrectomy is still the main treatment for patients with non-metastatic RCC. Meanwhile, most of patients with RCC can be symptom-free in the early stage, 20-30% of them have already developed to metastases at the time of diagnosis (Lam et al., 2005). It is necessary for us to seek reliable clinical markers that can identify patients in the earlier stage of RCC.

The tumorigenesis of renal cell carcinoma is still not fully clarified. Numerous researches show that RCC is frequently subjected to LOH (loss of heterozygosity) of chromosome 3p, which has three major deleted regions (Yamakawa et al., 1991; Lubinski et al., 1994). RAS association domain family 1A gene (RASSF1A) is located in 3p21-p22, which is regarded as the most frequent loss region of chromosome 3p (Braga et al., 1999; Dammann et al., 2000). The function of RASSF1A is associated with cell cycle, apoptosis and microtubule (Agathangelou

et al., 2005). RASSF1A is a putative tumor suppressor gene (TSG) and can suppress the tumorigenic properties of different kinds of tumors (Thaler et al., 2012; Feng et al., 2014). It has the potential to be a predictor of RCC.

In the past few decades, more and more evidences showed that aberrant DNA methylation, a typical epigenetic modification, had been observed and regarded as a common mechanism leading to inactivation of TSG in cancers (Cancer Genome Atlas Research, 2013; Asiaf et al., 2014; Fang et al., 2014; Li et al., 2014). However, in the previous studies, methylation frequency of RASSF1A gene had been examined only in limited numbers of kidney tumors. The diagnostic value of RASSF1A methylation in renal cell carcinoma remained controversial. Therefore, we conducted a comprehensive literature search and performed a meta-analysis to estimate the potential that aberrant methylation of RASSF1A would be a promising biomarker for RCC risk prediction.

### Materials and Methods

#### *Searching strategy and study selection*

Databases of pubmed, ProQest and Web of science were searched systematically for studies that investigated the relationship between RASSF1A methylation and RCC risk prediction. The last search updated on 15 April 2015. Various combinations of the following terms were used for searching potentially studies: “kidney neoplasm” or “kidney cancer” or “renal cancer” or “renal neoplasm”, “RASSF1A” or “Ras association domain family 1A”. The inclusion criteria were as follow: (1) they were case-

control studies on the association between RASSF1A methylation and RCC; (2) patients with renal cell carcinoma diagnosed by pathology; (3) the articles had a full-text and provided sufficient data to calculate odd ratios (ORs) and its 95% confidential intervals (CIs). Studies were excluded if they met the following criteria: (1) duplicate records; (2) reviews, letters, animal studies, cell lines or conferences records; (3) lacking enough data to calculate OR and 95%CI.

Reference list of all eligible studies were also manually searched for other potential studies that have been missed in the initial search.

*Data extractions*

The following information of each article was extracted: name of first author, year of publication, country, race, histological type, sample type, the numbers of cases and controls, method for methylation detection and so on. Two reviewers (GY and CL) extracted data independently. If there was disagreement between us, it was resolved by discussion.

*Statistical analyses*

The pooled OR and its 95%CI were conducted to assess the relationship between RASSF1A methylation and the risk of RCC. We considered heterogeneity presently if the  $I^2 \geq 50\%$  and  $P < 0.05$  for the Q-statistics. The pooled effect was calculated using either a fixed-effects model or a random-effects model on the basis of heterogeneity among studies. Moreover, we carried out stratified analysis and meta-regression to seek the sources of heterogeneity. A sensitivity analysis was performed to investigate the stability of our result and to determine the influence of each individual study on the overall pooled OR. Publication bias was sought by Egger's test, Begg's test and funnel plots.  $P < 0.05$  was considered statistically significant. All the statistical analyses were performed using STATA12.0 (STATA Corporation, College Station, TX, USA).

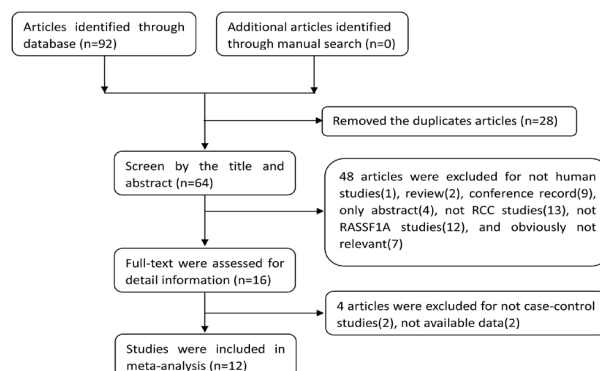
**Results**

*Study characteristics*

The process of study selection was summarized in the flow diagram (Figure 1). According to our strategies, 64 studies were identified in the preliminary search after duplicates removed. After the filtration, 12 articles met our criteria (Dreijerink et al., 2001; Morrissey et al., 2001; Yoon et al., 2001; Battagli et al., 2003; Dulaimi et al., 2004; Gonzalzo et al., 2004; Hoque et al., 2004; Tokinaga et al., 2004; Costa et al., 2007; Peters et al., 2007; Ellinger et al., 2011; Hauser et al., 2013). Among them, Mohammad et al investigated in both serum and urine sample. We considered that it was two studies. Finally, 13 studies with a total of 677 patients with RCC and 497 controls were enrolled in the pooled analyses. The main characteristics of included studies were summarized in Table 1.

*Association between RASSF1A methylation and the risk of RCC*

Our result indicated that hypermethylation of RASSF1A was associated with the increased risk of RCC (OR:4.14, 95%CI:1.06-16.1) (Figure 2). It showed significant heterogeneity among the 13 studies ( $I^2=82.1\%$ ). In the stratified analysis, significantly increased risk was found



**Figure 1. Flow chart of Literature Search and Selection**

**Table 1. Characteristics of Studies Included in this Meta-Analysis**

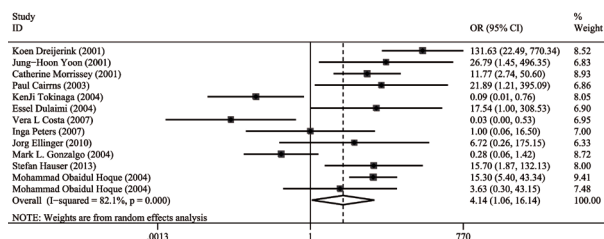
| Author                 | Year | Country        | Method | Sample Type | Histology Type | Tumor |     | Control |    |
|------------------------|------|----------------|--------|-------------|----------------|-------|-----|---------|----|
|                        |      |                |        |             |                | M     | U   | M       | U  |
| Koen Dreijerink        | 2001 | America        | MSP    | Tissue      | RCCs           | 39    | 4   | 2       | 27 |
| Jung-Hoon Yoon         | 2001 | America        | MSP    | Tissue      | RCCs           | 18    | 14  | 0       | 10 |
| Catherine Morrissey    | 2001 | United Kingdom | MSP    | Tissue      | ccRCC          | 32    | 106 | 2       | 78 |
| Paul Cairns            | 2003 | America        | MSP    | Tissue      | RCCs           | 24    | 23  | 0       | 10 |
| Kenji Tokinaga         | 2004 | Japan          | COBRA  | Tissue      | ccRCC          | 39    | 11  | 38      | 1  |
| Essel Dulaimi          | 2004 | America        | MSP    | Tissue      | RCCs           | 40    | 48  | 0       | 10 |
| Mark L. Gonzalzo       | 2004 | America        | QMSP   | Tissue      | RCCs           | 28    | 10  | 20      | 2  |
| Mohammad Obaidul Hoque | 2004 | America        | QMSP   | Urine       | RCCs           | 17    | 9   | 10      | 81 |
| Mohammad Obaidul Hoque | 2004 | America        | QMSP   | Serum       | RCCs           | 2     | 16  | 1       | 29 |
| Vera L Costa           | 2007 | Portugal       | QMSP   | Tissue      | RCCs           | 68    | 17  | 62      | 0  |
| Inga Peters            | 2007 | Germany        | COBRA  | Tissue      | ccRCC          | 44    | 1   | 44      | 1  |
| Jorg Ellinger          | 2010 | Germany        | QMSP   | Tissue      | pRCC           | 32    | 0   | 14      | 1  |
| Stefan Hauser          | 2013 | Germany        | QMSP   | Serum       | RCCs           | 8     | 27  | 1       | 53 |

\*RCCs: renal cell carcinoma that unclassified; ccRCC: clear cell renal cell carcinoma; pRCC: Papillary renal cell carcinoma; MSP: Methylation-specific polymerase chain reaction; QMSP: quantitative methylation-specific polymerase chain reaction; COBRA: combined bisulfite restriction analysis

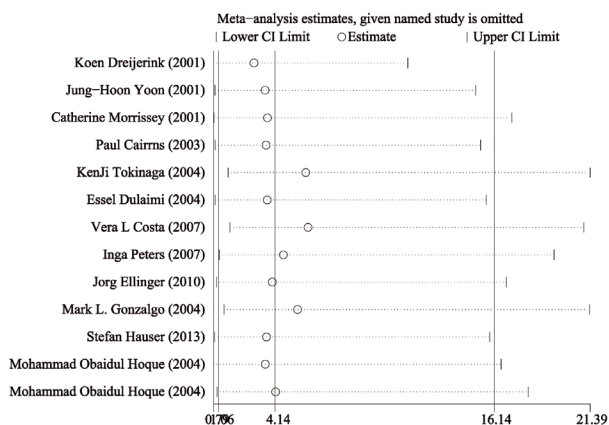
**Table 2. Stratification Analyses of RASSF1A Methylation and Renal Cell Carcinoma Risk**

| Variables              | Studies | Tumor |     | Control |     | Pooled Effect    |      |       | Heterogeneity      |       |
|------------------------|---------|-------|-----|---------|-----|------------------|------|-------|--------------------|-------|
|                        |         | M     | U   | M       | U   | OR (95%CI)       | Z    | P     | I <sup>2</sup> (%) | P     |
| Total                  | 13      | 391   | 286 | 194     | 303 | 4.14(1.06-16.1)  | 2.05 | 0.041 | 82.1               | 0     |
| Method                 |         |       |     |         |     |                  |      |       |                    |       |
| Qualitative detection  | 5       | 153   | 195 | 4       | 135 | 28.4(10.2-79.6)  | 6.37 | 0     | 12.9               | 0.332 |
| Quantitative detection | 8       | 238   | 91  | 190     | 168 | 1.27(0.20-8.09)  | 0.25 | 0.803 | 84.7               | 0     |
| Sample Type            |         |       |     |         |     |                  |      |       |                    |       |
| Tissue                 | 10      | 364   | 234 | 182     | 140 | 3.10(0.51-18.8)  | 1.23 | 0.219 | 84.2               | 0     |
| Body fluid             | 3       | 27    | 52  | 12      | 163 | 12.8 (5.35-30.8) | 5.72 | 0     | 0                  | 0.564 |
| Ethnicity              |         |       |     |         |     |                  |      |       |                    |       |
| America                | 7       | 168   | 124 | 33      | 169 | 10.5(1.97-55.9)  | 2.76 | 0.006 | 79.5               | 0     |
| Europe                 | 5       | 184   | 151 | 123     | 133 | 2.35(0.24-22.9)  | 0.74 | 0.462 | 78.4               | 0.001 |
| Asia                   | 1       | 39    | 11  | 38      | 1   | 0.09(0.01-0.76)  | 2.22 | 0.027 | NA                 | NA    |

\*OR: Odds Ratio; CI: Confidence Interval; NA: Not Applicable



**Figure 2. Forest Plot of odd Ratios (ORs) for Association between RASSF1A Methylation and Risk of Renal Cell Carcinoma**

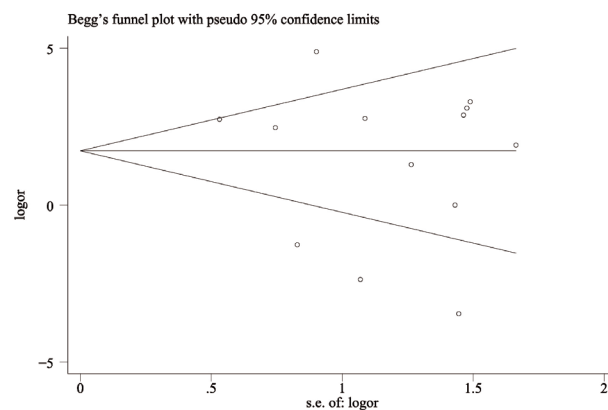


**Figure 3. Sensitivity Analysis of Overall odd Ratios (ORs) Coefficients for RASSF1A**

in qualitative detection method (OR:28.4, 95%CI:10.2-79.6), body fluid sample (OR:12.8, 95%CI:5.35-30.8) and American (OR:10.5, 95%CI:1.97-55.9). Detailed results were summarized in Table 2. The result of meta-regression indicated that the trend in ORs correlated with the detection methods for RASSF1A methylation, which accounted for part of the heterogeneity (coefficient=-2.39,  $P=0.009$ , adjusted  $R^2=53.8\%$ ). However, other factors such as sample type ( $P=0.485$ ), sample size ( $P=0.169$ ) and the race of the patients ( $P=0.065$ ) could not explain the heterogeneity.

#### Sensitivity analyses

Sensitivity analysis was conducted to assess the influence of each individual study on the pooled ORs by



**Figure 4. Funnel Plot For Publication Bias Test**

sequential omission of each eligible study. The analysis results showed that the pooled ORs were not significantly affected by any individual study (Figure 3). Thus it indicated a robust result of the analysis.

#### Publication biases

We performed Egger's test, Begg's test and funnel plots to assess publication bias in the meta-analysis. Both Egger's test ( $t=-0.83$ ,  $P=0.427$ ) and Begg's test ( $Z=0.43$ ,  $P=0.669$ ) indicated no significant publication bias. Moreover, we implemented a trim-and-fill method to confirm this observation. Meta-analysis with or without the trim-and-fill method did not draw different conclusions, indicating that our results were statistically robust (data not showed). The shapes of the funnel plots show no significant asymmetry (Figure 4).

#### Discussion

DNA methylation is a typical epigenetic modification which regulates genes expression without changes in DNA sequences (Waddington, 1939). If hypermethylation occur in a tumor-suppressor gene promoter, its downstream genes are consistently inactivated which leading to tumor development and progression (Ushijima, 2005). Inactivation of the RB gene by hypermethylation of its promoter was the first reported evidence that involved in the progression of cancer (Ohtani-Fujita et al., 1993). Subsequently, more and more researches about

the association between aberrant DNA methylation of different genes and cancer progression were also reported. It implicated a potential role of aberrant DNA methylation in the prognostication for RCC.

Previous reports had demonstrated methylation of RASSF1A in many solid tumors and its frequency was significantly higher in patients group compared with controls. To further confirm the diagnostic value of RASSF1A methylation in RCC, we conducted a detail meta-analysis in 13 eligible studies involving 677 cases and 497 controls. It suggested that hypermethylation of RASSF1A might be a risk factor in renal cell carcinoma (OR:4.14, 95%CI:1.06-16.1). Part of heterogeneity could be explained by method for methylation detection. In the eligible studies using qualitative detection method (MSP), RASSF1A promoter methylation was rarely observed in the control group. Nevertheless, RASSF1A methylation was widespread in "normal tissue" when used quantitative detection method (QMSP, COBRA). The morphologically normal tissues in control group were collected from specimens being adjacent to neoplasm. we were tempted to speculate that these "normal tissue" had acquired genetic or epigenetic changes trending to tumorigenesis. Recently, professor Eeles suggested that mutations were present at high levels in morphologically normal tissue distant from the cancer using genome-wide DNA sequencing in prostate cancer (Cooper et al., 2015). These genetic mutations would promote carcinogenesis. These findings supported our hypothesis to some extent. It indicated that RASSF1A promoter methylation might be a relatively early event in RCC tumorigenesis. Quantitative detection method was more sensitive and specific compared to MSP (Eads et al., 2000). If we contrast the RASSF1A methylation degree instead of frequency in the studies using quantitative detection method, methylation of RASSF1A was significantly higher in tumor samples compared to control group. So the test method would not affect RASSF1A methylation as a favourable biomarkers in the early diagnosis of RCC.

Several limitations in our meta-analysis should be clarified. First, because we restricted to articles published in English, selection bias was inevitable. Second, this analysis was performed at the study level, it was difficult for us to evaluate the potential confounding, such as demographic characteristic. Finally the impact of bias in the present analysis could not be completely excluded because positive results were more likely to be accepted by journals while negative results were often rejected or not submitted.

In conclusion, our meta-analysis indicate that RASSF1A methylation has a strong potential in prediction the risk of RCC. Our finding is necessary to clearly confirmed by well-designed prospective controlled trials with sufficient sample size.

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