Original Paper

The Clinicopathological Significance of Epigenetic Silencing of VHL Promoter and Renal Cell Carcinoma: A Meta-Analysis

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Key Words

VHL • Promoter hypermethylation • Renal cell carcinoma • Meta-analysis

Abstract

Background/Aims: Von Hippel-Lindau gene (*VHL*) has been reported as a tumor-suppressor gene in some cancers. However, the association between *VHL* promoter hypermethylation and renal cell carcinoma (RCC) remains to be clarified. We are the first to systematically integrate published papers to assess the role of hypermethylated *VHL* in RCC. **Methods:** The potential relevant papers were searched via PubMed, Embase, EBSCO, CNKI, and Wanfang databases. The overall odds ratio (OR) and corresponding 95% confidence interval (95% CI) were calculated to evaluate the relationship between *VHL* promoter hypermethylation and RCC. **Results:** Finally, a total of 1,998 RCC patients and 294 controls from 13 eligible articles were included in this meta-analysis. Under the fixed-effects model, the pooled OR from seven studies including 596 RCC and 294 nonmalignant samples showed that *VHL* promoter hypermethylation was significantly higher in cancer than in controls (OR = 7.93, 95% CI = 2.84–22.15, $P < 0.001$). Subgroup analysis based on ethnic population and testing method revealed that hypermethylated *VHL* had a significantly similar OR value in different races and detection methodologies. No significant association was found between hypermethylated *VHL* and tumor grade, tumor stage, tumor size, histological types, and lymph node status in cancer (all $P > 0.05$). In the current study, there was no evidence of publication bias as determined by Egger's test (all $P > 0.05$). **Conclusions:** In the investigated patients, *VHL* promoter hypermethylation, which may play an important role in carcinogenesis of RCC, is significantly associated with an increased risk of RCC. However, *VHL* promoter hypermethylation is not correlated with specific clinicopathological characteristics. Additional future studies are needed to confirm our results.

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Introduction

Renal cell carcinoma (RCC) is the second common cancer after bladder cancer in urinary system malignant tumors, accounting for approximately 62,700 new cases diagnosed, with approximately 14,240 deaths annually in the United States in 2016 [1]. Reportedly, RCC has several histological types, the most common histotypes of whose consist of clear cell RCC (ccRCC) representing 75% of the cases, papillary RCC (pRCC) in 10% of the cases, and chromophobe RCC constituting 5% of the cases, [2]. Generally, RCC is asymptomatic in its early stages, and more than 50% of RCCs are detected incidentally by examinations, such as ultrasound and computed tomography (CT). Approximately 25%–30% of the patients with RCC are defined as cases with advanced or metastatic RCC, and the 5-year survival rate is only 12.3% [3].

Numerous genetic and epigenetic changes are involved in the pathogenesis of renal cancer [4–6]. DNA methylation, an important mechanism of epigenetic alterations, plays a key role in the regulation of gene expression and cancer development [7, 8]. Hypermethylation of the promoter region of tumor-suppressor genes (TSG) and hypomethylation of oncogenes are common events in cancer [9–11]. Von Hippel-Lindau gene (*VHL*), a tumor-suppressor gene, is mapped at chromosome 3p25–26 [12]. *VHL* gene encodes a protein, pVHL, consisting of 213 amino acid residues [13]. It is noteworthy that as a multifunctional protein, pVHL has a close connection with the pathogenesis of familial and sporadic RCC, which is related to its role in the polyubiquitination process [14]. Moreover, due to somatic mutations or promoter methylation, the expression of pVHL is exceedingly common in kidney cancer [15].

However, the statistical power of individual studies with a small sample size remains limited. Therefore, for the first time, we conducted a systematic meta-analysis comprising an extensive number of studies to further identify the association between *VHL* promoter hypermethylation and RCC pathogenesis. In addition, we also evaluated the correlation of *VHL* promoter hypermethylation with clinicopathological characteristics in RCC.

Materials and Methods

Literature search

All articles published up to April 26, 2016 were systematically searched using PubMed, Embase, EBSCO, CNKI, and Wanfang databases without any language restrictions. The subject and search terms strategy were as follows: (kidney OR renal) AND (cancer OR tumor OR neoplasm OR carcinoma) AND (*VHL* OR von Hippel-Lindau) AND (methylation OR epigene*). In addition, references from the relevant review articles published were also manually searched to identify other possible studies. Conference abstracts or case reports were excluded due to the lack of sufficient data for the current meta-analysis.

Inclusion criteria

The eligible articles were selected for analysis if they met the following inclusion criteria: (1) The patients had to be diagnosed with primary renal cancer; (2) The articles had to involve *VHL* gene promote hypermethylation data from tissue, blood, or urine; (3) The studies had to provide sufficient information on the frequencies of *VHL* promoter methylation for estimation of the pooled odds ratio (OR) with 95% confidence interval (CI). If the authors reported multiple papers using the same groups of patients, only the most recent or complete paper was selected to avoid duplicate content.

Data extraction

The relevant data were extracted independently by two authors. The following information was collected for the eligible studies: surname of the first author, year of publication, original country, ethnicity, testing method, sample type, number of cases and control treatments, number of *VHL* promoter hypermethylation, clinical staging, tumor grade, histologic type, tumor size, and lymph node status. Lymph nodes metastases were defined as lymph node-positive status. Tumor grades of ≤ 2 were defined as low-grade, and tumor grades of 3–4 were defined as high-grade. Tumor stages of ≤ 2 were referred to as early stage, whereas tumor stages of 3–4 were defined as advanced stage.

Statistical analysis

Meta-analysis was conducted using the STATA software (version 12.0, Stata Corporation, College Station, TX, USA). The pooled odds ratio (OR) and corresponding 95% confidence interval (95% CI) were calculated to determine the correlation of *VHL* promoter hypermethylation with renal cancer. The Cochran's Q test and I^2 statistic were used to evaluate the between-study heterogeneity [16]. If the values of $I^2 < 50\%$ and $P \geq 0.1$, representing a measure of lack of heterogeneity, the fixed-effects model was used; otherwise, the random-effects model was applied in the cases with significant heterogeneity [17, 18]. Egger's linear regression test was performed to detect the potential publication bias [19].

Results

Basic characteristics of the included studies

A total of 504 potentially relevant articles were initially identified through the searches in the electronic databases as described above, and five further studies were obtained by manual searching. The abstracts or full texts of these papers were carefully reviewed for compliance with the inclusion criteria. Eventually, thirteen eligible studies with 1,998 RCC patients and 294 controls were included in our study (Fig. 1) [20-32]. Seven of these studies, including 596 RCC patients and 294 nonmalignant samples, evaluated the association of *VHL* promoter hypermethylation with RCC risk. In addition, eight studies, involving 967 ccRCC and 259 non-ccRCC, assessed the relationship between *VHL* promoter hypermethylation and tumor histologic type. Other seven studies with 402 low-grade and 299 high-grade patients estimated the correlation between *VHL* promoter hypermethylation and tumor grade. In five of the studies, including 53 lymph node-positive patients and 379 lymph node-negative patients, assessed the correlation between *VHL* promoter hypermethylation and lymph node status. Further, three studies evaluated the association of *VHL* promoter hypermethylation with tumor stage, and three studies investigated the relationship between *VHL* promoter hypermethylation and tumor size. The general characteristics of the included studies are summarized in Table 1.

Fig. 1. Flow diagram of the strategy of literature search among the associated studies.

VHL hypermethylation status in RCC and control groups

No statistically significant heterogeneity was detected in the comparison of cancer vs. control groups ($I^2 = 0.0\%$ and $P = 0.859$), and the fixed-effects model was conducted. Figure 2 depicts the *VHL* promoter hypermethylation status which was significantly higher in the cancer samples than in the nonmalignant samples (OR = 7.93, 95% CI = 2.84–22.15, $P < 0.001$); seven studies with 596 RCC patients and 294 nonmalignant samples were included in the comparative analysis.

Subgroup analyses based on ethnic population (Asian population or Caucasian population) and testing method, methylation-specific polymerase chain reaction (MSP) and Non-MSP, were conducted to investigate the difference between the pooled OR in *VHL* promoter hypermethylation (Table 2). Our findings showed that *VHL* promoter hypermethylation was significantly associated with RCC risk in both the Asian population and the Caucasian population, with a similar OR values (OR = 7.76, 95% CI = 1.75–34.43, $P = 0.007$ vs. OR = 8.05, 95% CI = 1.98–32.74, $P = 0.004$, respectively). Subgroup analysis based on the testing method suggested that MSP detection and Non-MSP were susceptible to hypermethylated *VHL*, with similar OR values (OR = 7.91, 95% CI = 1.83–34.28, $P = 0.006$ vs. OR = 7.95, 95% CI = 1.89–33.50, $P = 0.005$, respectively).

Correlation of VHL hypermethylation with clinicopathological characteristics

The association of *VHL* promoter hypermethylation and clinicopathological characteristics was further determined to evaluate the clinical significance of *VHL* promoter hypermethylation (Table 2). In the cases without significant heterogeneity, the fixed-effects model was used in relation to tumor grade, lymph node status, tumor size, and clinical staging ($I^2 = 14.1\%$, $P = 0.322$; $I^2 = 0.0\%$, $P = 0.736$; $I^2 = 0.0\%$, $P = 0.853$; $I^2 = 0.0\%$, $P = 0.763$, respectively). Substantial heterogeneity related to the tumor histotype ($I^2 = 58.0\%$ and $P = 0.020$) was found in the cases of cancer; thus, the random-effects model was employed in these conditions.

Table 1. General characteristics of the included studies. “*” stands for a detection method using restriction enzyme digestion and Southern blotting; “-” stands for “data not available”; MSP: methylation specific polymerase chain reaction; MPCR: multiplex polymerase chain reaction; “#” denotes a detection method using nested polymerase chain reaction nested and Sanger sequencing; M: methylation; N: number of the total samples; T: tumor; CCRCC: clear cell renal cell carcinoma

[illegible]

Fig. 2. The pooled OR showing the association between *VHL* promoter hypermethylation and RCC in cancer vs. control group.

Table 2. Pooled OR of the studies evaluating *VHL* promoter hypermethylation. MSP: methylation specific polymerase chain reaction; T: tumor; CCRCC: clear cell renal cell carcinoma; OR: odds ratio; 95% confidence interval (95% CI)

	Studies	OR (95% CI %)	I ² ; p	P value	Cases	Controls	p (Egger's test)
Total	7	7.93 (2.84–22.15)	0.0%; 0.859	< 0.001	596	294	0.794
Asians	3	7.76 (1.75–34.43)	0.0%; 0.829	0.007	135	128	
Caucasians	4	8.05 (1.98–32.74)	0.0%; 0.528	0.004	461	166	
Other	3	7.95 (1.89–33.50)	0.0%; 0.409	0.005	353	140	
MSP	4	7.91 (1.83–34.28)	0.0%; 0.850	0.006	243	154	
Clinicopathological features					Cancer patients		
Tumor grade	7	0.88 (0.53–1.46)	14.1%; 0.322	0.616	Low-grade 402	High-grade 299	0.738
Tumor stage	3	3.69 (0.96–14.17)	0.0%; 0.763	0.057	Stage I–II 155	Stage III–IV 59	0.672
Tumor size	3	0.91 (0.35–2.39)	0.0%; 0.853	0.854	T ≥ 5cm 81	T < 5cm 102	0.837
Tumor histology	8	1.41 (0.56–3.51)	58.0%; 0.020	0.464	CCRCC 967	Non-CCRCC 259	0.175
Lymph node status	5	0.91 (0.43–1.93)	0.0%; 0.736	0.805	Node+ 53	Node- 379	0.127

As seen in Table 2, no significant correlation was observed between *VHL* promoter hypermethylation and clinicopathological features (all $P > 0.05$).

Publication bias

Egger's test was applied to determine the possible publication bias. The results indicated the absence of statistically significant publication bias in the current meta-analysis (Table 2; all $P > 0.05$).

Discussion

CpG islands methylation of the promoter region is an important mechanism of reduction of gene expression that can cause inhibition of gene transcription [33]. The hypermethylation of tumor-suppressor genes (TSG) is significantly associated with tumor cell proliferation, cell migration, and cell invasion [34]. The condition of *VHL* promoter methylation has been reported in various cancers, including papillary thyroid carcinoma [35], RCC [36], and colorectal cancer [37]. However, we found that the reported frequency of *VHL* promoter methylation in RCC is still controversial, with a range from 0% [38] to 35.3% [20]. Therefore, the present meta-analysis was performed to further validate the relationship between *VHL* promoter hypermethylation and RCC.

Under a fixed-effects model, our findings combining seven published articles revealing that *VHL* promoter hypermethylation was significantly higher in RCC than in nonmalignant controls (OR = 7.9, $P < 0.001$). These results suggested that *VHL* inactivation through promoter

hypermethylation may play a key role in the carcinogenesis of RCC. We also conducted subgroup analyses to investigate the difference of the pooled OR for two ethnic populations (Asians and Caucasians) and testing method (MSP and Non-MSP). The results indicated that no significant difference was present in terms of hypermethylated *VHL* related to method and race. According to Egger's test results, no publication bias was detected, indicating that the result was stable.

We further determined whether the *VHL* promoter hypermethylation status was associated with clinicopathological features. The findings of the current analysis indicate that the frequency of hypermethylated *VHL* was similar in different tumor grades, tumor stages, tumor sizes, pathological types, and lymph node statuses, suggesting that the *VHL* promoter hypermethylation status was correlated with clinicopathological features. In addition, there was no evidence of publication bias as established by Egger's test, indicating that our results were reliable.

Several potential limitations are present in the present study. First, the main ethnic populations investigated were Asian and Caucasian, while other ethnicities, such as Africans, were not included in the analysis. Second, the fluid samples from cell-free DNA in the serum and plasma or urine were limited, and thus more studies will be necessary to validate the value of fluid detection in the future. Third, our research included only articles published in English or Chinese, which might have led to selection bias.

In conclusion, our study showed that *VHL* promoter hypermethylation is significantly associated with an increased risk of RCC. In addition, no significant association was observed between hypermethylated *VHL* and clinicopathological features. Further large-scale studies are essential to confirm the role of hypermethylated *VHL* in tumorigenesis and its clinical significance.

Disclosure Statement

The authors declare that they have no competing or conflict of interests.

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