

Research Article

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Correlation analysis of VHL and Jade-1 gene expression in human renal cell carcinoma

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Abstract: Objective: The aim of this study was to investigate the correlation of von Hippel-Lindau tumor suppressor (VHL) mRNA expression and jade family PHD finger 1 (Jade-1) gene expression in patients with renal cell carcinoma (RCC). Another aim of this study was to analyze the relationship of these two genes with clinical/pathological features of the RCC patients. Methods: A total of 75 RCC patients who received surgically therapy in our hospital were included. All patients had complete pathological data. The expression of VHL/Jade-1 was determined by real-time polymerase chain reaction (RT-PCR). Results: VHL and Jade-1 were both obviously downregulated in RCC tissues than that of the matched normal tissues, and both negatively correlated with tumor size as well as tumor grade. And we found a fine association of VHL gene expression with Jade-1. Conclusion: VHL/Jade-1 exhibited significantly decreased expression in RCC tissues and was closely related to the clinical prognosis of patients. The finding of VHL expression positively correlated with Jade-1 expression shed light and provided crucial evidence on the connection of VHL protein with Wnt/b-catenin pathway.

Keywords: VHL, Jade-1, RCC, correlation, Wnt/b-catenin

1 Introduction

Renal cell carcinoma (RCC) ranks as the most frequent type of all kidney cancers, which represents more than 85% incidence among kidney cancers [1,2]. It is also the ninth most common cancer in developed countries [3]. In recent years, the incidence of RCC shows a rising trend. Recent progress in antiangiogenic therapy has increased the median survival of RCC patients at advance stages from 10 months to more than 40 months [4]. However, because of the highly expression of drug-resistant genes, general chemotherapy method has limited or absolutely no significant effect [5,6]. Therefore, deeper understanding of the pathogenesis and progression of RCC is of highly importance for developing more therapy targets and improving prognosis of RCC patients.

Von Hippel-Lindau (VHL) disease is a hereditary cancer syndrome caused by inherited mutations that inactivate the VHL tumour suppressor gene [7]. Patients with VHL disease are with relative higher risk for a variety of cancers, including RCC of the clear cell histology and other kinds of cancers [8]. The best-characterized function of VHL refers to targeting of the hypoxia-inducible factor (HIF) transcription factor for proteolytic degradation [9,10]. However, VHL inactivation mediates both HIF-dependent and -independent pathways [11-14]. Notably, the majority of the HIF-independent functions were discovered through biochemical interactions, among which, VHL had been reported to interact with and stabilize Jade-1.

Jade-1, a short-lived protein most highly expressed in renal proximal tubules, is a candidate renal tumor suppressor. It was initially identified by yeast two-hybrid analysis as a VHL-interacting protein [15]. Jade-1 could suppress renal cancer cell growth in part by increasing apoptosis [16]. It functions as: 1) a ubiquitin ligase to inhibit canonical Wnt/b-catenin signaling [17]; 2) a transcription factor associated with histone acetyltransferase activity [18]; 3) with increased abundance of cyclin-dependent kinase inhibitor p21 [19]. The relationship between Jade-1 and VHL has not yet to be elucidated fully, and may have important implications for RCC development [16]. Recent findings of ubiquitylation followed by

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degradation of b-catenin by Jade-1 provided evidence that loss of VHL could lead to tumor formation through b-catenin de-repression [20].

However, the connection of VHL expression with Jade-1 expression, and their clinical significance in RCC remain unclear. Here we sought to give direct evidence to demonstrate their correlations and characterize their clinical value in RCC patients.

2 Materials and methods

2.1 Patients and tissues

A total of 75 RCC tissues, as well as adjacent normal tissues from patients with RCC pathologically confirmed were collected from 2012 to 2015. Characteristic data for sex, age, tumor size, lymph node (LN) metastasis and tumor grade of patients were obtained from patients' medical records. The study protocol was complied with the ethical guidelines of The Central Hospital of Lishui City.

Informed consent: Informed consent has been obtained from all individuals included in this study.

2.2 RNA extraction, Reverse transcription and Real-Time PCR

Total RNA of tissues was extracted with TRIzol reagent (Invitrogen, Carlsbad, CA). The quantitation and quality of RNA was determined using Nanodrop 2000c spectrophotometer. Reverse transcription of RNA was done. Real-Time PCR analysis was performed with SYBR Green qPCR mix (TOYOBO). Samples were normalized to β -actin. Relative VHL or Jade-1 expression was calculated using the power formula: $2^{-\Delta Ct}$ ($\Delta Ct = Ct_{Gene} - Ct_{\beta-actin}$).

2.3 Statistical analysis

All statistical analyses were performed using SAS software v8. The significance of differences between cancer and normal tissues was estimated by Paired Student's t-test. κ^2 test was performed to test the relationship between gene expression and clinical parameters. Correlation between expression levels of VHL and Jade-1 genes in RCC tissues was analyzed using Spearman's linear correlation. Two-sided P-values were calculated, and a probability level of 0.05 was chosen for statistical significance.

3 Results

3.1 VHL and Jade-1 expressions were decreased in RCC and negatively correlated with advanced clinical stage

Real-time PCR was performed to detect the expression of VHL and Jade-1 in 75 pairs of human RCC tissues. We found that 62.7% (47/75) of VHL expression was downregulated in RCC tissues when compared with adjacent normal tissues (Figure 1A, t-test, $P < 0.01$). Also, 60% (45/75) of Jade-1 expression in RCC tissues was significantly downregulated (Figure 1B, t-test, $P < 0.01$).

Then, patients with RCC were classified into two groups based on the DDCT of relative VHL expression or Jade-1 expression (positive or negative expression). As showed in Table 1, VHL expression was significantly associated with tumor size and tumor grade (Table 1, κ^2 test, $P < 0.01$), but no significant association with patients' sex, age or LN metastasis (Table 1, κ^2 test, $P > 0.05$). However, Jade-1 expression was not only significantly associated with tumor size and tumor grade (Table 1, κ^2 test, $P < 0.01$), but also associated with LN metastasis (Table 1, κ^2 test, $P < 0.01$). Taken together, these results indicated that VHL and Jade-1 were downregulated in RCC, and reduced expression of them may play tumor suppressive roles in the progression of RCC.

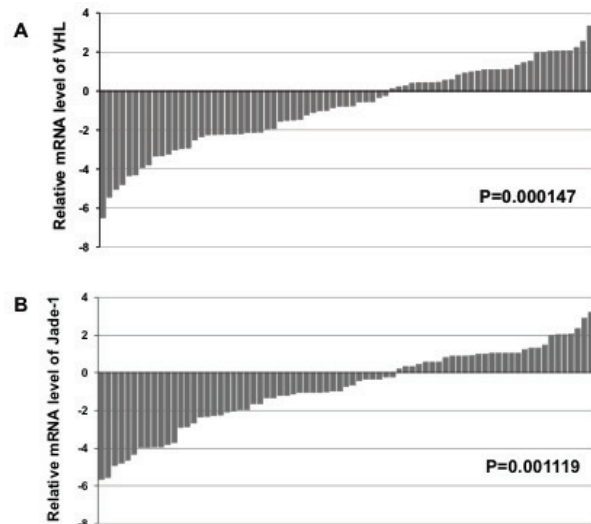


Figure 1: The status and expression levels of VHL and Jade-1 in RCC and adjacent normal tissues. Comparison of relative VHL (A) and Jade-1 (B) expression levels as assessed by real-Time PCR between 75 RCCs and adjacent normal tissues. The relative mRNA expression levels in kidney tumors are presented as fold change = $2^{ACIN-ACIT}$ of tumors versus matched normal tissues.

Table 1: Relationship between mRNA expression of both VHL and Jade-1, and age, sex, tumor size, lymph node metastasis and tumor grade in RCC.

Variables	Total number of cases	VHL positive n (%)		P	Jade-1 positive n(%)		P
		28 (37.3%)	47 (62.7%)		30 (40.0%)	45 (60.0%)	
Age							
<=50	29	13	16	0.3324	12	17	1.0000
>50	46	15	31		18	28	
Sex							
Male	44	16	28	1.0000	19	25	0.6331
Female	31	12	19		11	20	
Tumor Size							
<=4cm	33	19	14	0.0018	19	14	0.0088
>4cm	42	9	33		11	31	
LN metastasis							
Yes	35	15	20	0.4734	8	27	0.0053
No	40	13	27		22	18	
Tumor Grade							
I	15	12	3	0.0005	13	2	0.0001
II	25	8	17		9	16	
III+IV	35	8	27		8	27	

3.2 VHL was positively correlated with Jade-1 expression in RCC

To further explore the relationship between VHL and Jade-1, Spearman's correlation analysis was performed by SAS software. The results indicated that there was a positively correlation between VHL and Jade-1 expression (Figure 2, $P < 0.0001$). Our data demonstrated that Jade-1 might be positively regulated by VHL, or they might be involved in one pathway.

4 Discussion

The familial cancer syndrome, VHL disease, occurs as a consequence of inheriting a mutation in the VHL tumour-suppressor gene located on chromosome locus 3p257. VHL is frequently inactivated in sporadic cases of clear-cell RCC. Its genetic alterations, including gene mutations, hypermethylation of the promoter region, and loss of heterozygosity at the VHL locus are common genetic events in sporadic cases of clear-cell RCC. Clear-cell RCC is the

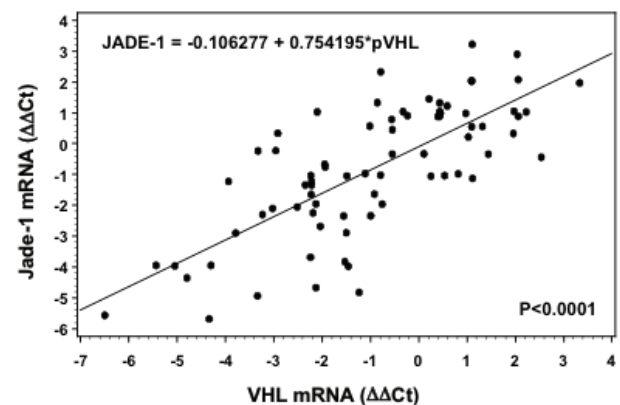


Figure 2: The positive correlation between VHL and Jade-1 expression in 75 RCC samples was determined using Spearman's correlation analysis ($P < 0.0001$).

major subtype of RCC. The findings in clear-cell RCC might be applied into the total saturation of RCC.

In our study, we determined the mRNA expression of VHL as well as its partner Jade-1 in RCC tissues, matched with paired normal tissues as controls. We found that both of them exhibited decreased expression in RCC tissues. And the expression level of VHL positively correlated with that of Jade-1, indicating a positive regulation of Jade-1

by VHL in RCC cells. This phenomenon was ideally and in line with our expectation, since it was already known that VHL could interact with and stabilize Jade-1 [15]. As the upstream regulator, VHL to some extent would decide at least protein levels of Jade-1. However, why VHL could also regulate the mRNA expression of Jade-1 remains unclear. Some other mechanisms should be involved in this process. Nevertheless, we do believe that, VHL might function as a tumor suppressor in RCC through Jade-1 to regulate the Wnt/b-catenin signaling. Actually, only one previous report has discussed the interplay between VHL/HIF-1 α and Wnt/b-catenin pathways during colorectal tumorigenesis [21]. Therefore, our results provide another possibility to interpret the crosstalk of VHL with Wnt/b-catenin pathway in RCC.

We also analyzed the relationships of VHL and Jade-1 with clinical parameters of the collected RCC patients. We surprisingly found that both VHL and Jade-1 significantly correlated with tumor size and tumor grade, which further confirmed their tumor suppressor roles in RCC. Additionally, we found that only Jade-1 was correlated with lymph node metastasis parameters, indicating that besides promoting apoptosis, Jade-1 might also participate in metastasis processes in RCC cells.

The limited aspect of our study is that, we merely detect the mRNA expression of the two molecules. Indeed, it is more important to address the gene mutation, DNA methylation or protein expression of VHL in RCC tissues. Considering the huge workload, we just measured the mRNA levels of VHL. Hence, for the follow-up studies, we will focus more on genetic and epigenetic levels of VHL regulation. It is the same for Jade-1 studies.

In summary, our results reported the correlation of VHL with Jade-1 in RCC tissues, both of which were decreased, and accessed their clinical values by analyzing the relationship between mRNA expression of VHL and/or Jade-1 and clinical parameters of RCC patients. Our findings provide another clue to connect VHL and Wnt/b-catenin pathway in RCC.

Conflict of interest statement: Authors state no conflict of interest

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