Research Article

VHL protein expression in renal cell carcinoma

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Abstract

Introduction: Various studies have been performed to detect VHL gene mutation in renal cell carcinoma (RCCs) but there is paucity of literature analyzing VHL expression at the protein level. Present study was carried out to analyze VHL protein (pVHL) expression in the tissue of RCCs and its correlation with tumor grade & stage. **Material and methods:** Immunohistochemical detection of pVHL was done by using a mouse monoclonal antibody raised against amino acids 54-213 of VHL of human. Statistical analysis was done by using chi-square test and Kruskall Wallis H Test. **Results:** 32 patients of renal cell carcinoma were included in the study. pVHL expression was positive in 84.40% cases . Among all pVHL positive cases, combined cytoplasmic and nuclear expression of pVHL was most common (59.0%). Exclusive nuclear expression alone was rare and was noted in only one case. Chromophobe RCC (1 case) was negative for p VHL. Exclusive cytoplasmic pVHL expression was more frequently noticed in low grade tumors. **Conclusion***:* VHL protein expression and its cytoplasmic and nuclear distribution is of potential relevance for the diagnosis and biological behavior of RCCs. Combined nuclear and cytoplasmic expression of VHL protein is more frequently seen in low grade and early stage of renal cell carcinomas.

Key words: VHL gene, VHL Protein, Renal cell carcinoma, immunohistochemistry, clinical relevance.

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Introduction

Renal cell carcinoma is the most common malignant tumor in the adult kidney, accounting for about 85% of all renal and 3% of all human malignancies [1]. Most renal cell carcinomas are sporadic cases. Nevertheless, about 4% occur within the context of a familial disease, with Von Hippel-Lindau disease being the most common cause of hereditary renal cell carcinomas [2]. Von Hippel-Lindau (VHL) disease is an autosomal dominant disorder predisposing to a variety of malignant and benign tumors of the eye, brain, spinal cord, kidney, pancreas, and adrenal glands. The Von Hippel-Lindau tumor suppressor gene is located on the short arm of the chromosome 3, in the 3p 25-26 locus of the human genome [2,3]. VHL tumor-suppressor gene has been shown to be mutated in both, familial as well as sporadic renal cell carcinoma [3].

The VHL gene encodes a protein known as VHL gene product or VHL protein (pVHL) that appears to play role in regulating several aspects of cellular function [4,5]. The pVHL protein exerts its functions through two domains that allow it to interact with various cellular proteins, such as elongins, fibronectin and hypoxia-inducible factor (HIF-1), almost all of which are functionally related to tumour angiogenesis [4,5,6]. Functional alterations can cause the protein to lose its tumour suppressor capacity, potentially triggering the genesis of renal cell carcinomas. The protein can serve

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as a component of ubiquitin ligase complex that functions in the ubiquitination pathway, contributing to protein degradation. One of the targets of the complex containing VHL protein (pVHL) is hypoxia-inducible factor 1(HIF-1) [6]. When VHL gene is mutated, HIF-1 level remains high, and this constitutively active protein increases the transcription and production of hypoxia-inducible factor, proangiogenic proteins such as VEGF (vascular endothelial growth factor) and TGF-alpha(Transforming growth factor-alpha) [7]. Thus, both cell growth and angiogenesis are stimulated leading to formation of the VHL associated renal cell carcinomas.

The VHL gene acts as a tumor-suppressor gene in both sporadic and familial renal cell carcinomas. Based on correlative cytogenetics, genetics, and histology, both familial and sporadic renal cell carcinomas are classified as- clear cell carcinoma, papillary carcinoma, chromophobe renal cell carcinoma and collecting duct carcinoma. The mutations of the VHL gene are associated with the development of clear cell renal cell carcinomas and chromophobe variety of renal cell carcinomas. Both familial and sporadic forms of papillary renal cell carcinomas are not associated with 3p gene mutation. The chromosome 3p deletion was detected in 98 per cent of non papillary renal cell carcinomas, 25 per cent of chromophobe renal cell carcinomas but none of papillary type renal carcinoma showed VHL gene mutation [8].

Various cytogenetic and molecular studies have been performed to detect VHL gene mutation in sporadic and familial renal cell carcinoma but there are very few studies that analysed VHL expression at the protein level by detecting the cellular localization of pVHL within human tissues [9]. Present study is designed to detect VHL protein (pVHL) in the tissue of renal cell carcinoma by using monoclonal antibodies.

Material and Methods

The present study was carried out in the Department of Pathology, King George Medical University, Lucknow**.** Total 32 cases were included in the study, among these 22 cases were studied prospectively from year 2012 to 2013 and 10 cases retrospectively. Clinical data of all retrospective cases were obtained by reviewing hospital records and direct communication with treating surgeon. For all prospective cases, clinical data were collected by taking detailed history, including present, past and family history and by conducting through local and systemic examination. Histological grading of tumors were done by using Fuhrman grading system [10]. Staging of all cases were done by using as per criteria defined by The American Joint Committee on Cancer (AJCC) by TNM classification of the tumor [11].

Immunostaining was performed by using Mouse IgG monoclonal anti human VHL antibody which is recommended for detection of VHL of human origin by immunohistochemistry (dilution1:100) as per standard protocol. Correlation of the pVHL with histopathology of renal cell carcinoma was done. Presence and staining pattern of VHL protein was evaluated in test tissues and cell types. Sub cellular localization of VHL protein , whether cytoplasmic, nuclear, combined nuclear and cytoplasmic, were studied. Interpretation of results was done according to the criteria defined by Fan Lin et al. in 2008 [12]. They used following scoring system-

Tumors were considered VHL protein positive if cytoplasmic and/or nuclear expression was found in more than or equal to 6% of neoplastic cells. The statistical analysis was done using SPSS (Statistical Package for Social Sciences) Version 15.0 statistical Analysis Software. The values were represented in Number (%) and Mean±SD.

Qualitative criteria for pVHL immunoreactivity in renal tumor cells

Score	Staining intensity
0	Weak
1	Light brown
2	Dark brown

Results

Age of patients ranged from 5 to 70 years with a mean age of 54.5±12.0 years. Out of total 32 patients, 84.4% were males (n=27) and 15.65 females (N=5). Majority of patients were diagnosed as clear cell RCC (n=23; 71.9%) followed

by those diagnosed as papillary RCC (n=8; 25%). There was 1 (3.1%) patient with chromophobe RCC. 71.9% cases were classified as Furham's grade II tumor followed by those diagnosed as Grade III (n=7; 21.9%). Two (6.3%) patients were categorized as Grade I tumor. Maximum number of patients were diagnosed Stage I (n=14;43.8%) followed by Stage II (n=9; 28%), stage III (n=6; 18.8%) and stage IV (n=3; 9.4%).

Positive VHL protein expression was found in majority of cases (n=27; 84.4%) except 5 (15.6%) cases who did not show VHL protein expression [Table 1]. Pattern of pVHL expression was studied among positive cases. Majority tumors had combined nuclear cytoplasmic expression (n=16; 59.3%), 10 (37%) cases with cytoplasmic expression and 1 (3.7%) had only nuclear expression [Table 2].

Table 1: Distribution of patients according to VHL protein expression

SN	VHL Protein expression	No. of cases	Percentage
1.	Negative	5	15.6
2.	Positive	27	84.4
	Total	32	100

Table 2: Pattern of VHL protein expression in positive cases (n=27)

SN	Pattern of VHL protein expression	No. of cases	Percentage
1.	Only cytoplasmic	10	37.0
2.	Only Nuclear	1	3.7
3.	Nuclear + Cytoplasmic	16	59.3

Nuclear and cytoplasmic distribution of pVHL in different histological subtypes of RCC was also analysed. Combined nuclear-cytoplasmic, cytoplasmic and nuclear expression was 47.8%,

39.1% and 4.3% respectively among clear cell carcinomas while 8.7% clear cell RCCs were negative for pVHL [Table 3]. Similarly, 62.5% papillary RCCs showed combined nuclear and cytoplasmic expression while 25 % papillary RCCs were negative for pVHL [Table 3].

Table-3: pVHL expression in different subtypes of renal cell carcinoma

Tumor type	Negative (%)	Cytoplasmic %)	Nuclear (%)	Nuclear+ Cytoplasmic
cCRCC	2 (8.7%)	9 (39.1%)	1 (4.3%)	11 (47.8%)
PRCC	2 (25%)	1 (12.5%)	0 (0%)	5 (62.5%)
Chromophobe RCC	1 (100%)	0 (0%)	0 (0%)	0 (0%)

Association of pVHL expression with tumor stage in renal cell carcinoma was also analyzed. VHL protein expression showed a nuclear plus cytoplasmic pattern in majority of cases of early stage I & II (52.2%) of renal cell carcinoma [Table 4]. Statistically, there was no significant association between tumor stage and pattern of VHL protein expression (p=0.347).

Discussion

VHL is a tumor-suppressor gene located on the short arm of chromosome number 3 [2, 3]. It is widely expressed in normal fetal and adult tissues, yet mutations in VHL leads to development of various human neoplasms including renal cell carcinomas [5,13]. It has been proven that familial as well as sporadic renal cell carcinomas are associated with VHL gene mutations.

The VHL gene produces proteins of 213 and 160 amino acids, respectively [14,15]. These proteins display similar biochemical properties and both forms are referred to as pVHL, VH protein or VHL gene product. This VHL protein plays important role in cell cycle regulation and acts as tumor suppressor product of VHL gene. Mutations in VHL gene leads to structural alteration in VHL protein predisposing to development of renal cell carcinomas. VHL gene mutations in renal cell carcinoma have been studied widely at cytogenetic and molecular level in both familial as well as sporadic renal cell carcinomas but there are very few studies that analyzed association of VHL and renal cell carcinomas at protein level. To examine VHL expression at the protein level, we have performed an immunostaining of renal cell carcinoma specimen tissues using monoclonal antibodies to pVHL.

In present study we evaluated 32 cases of renal cell carcinoma for pVHL expression and We found that pVHL expression was positive in 84.40% (n=27) of cases of renal cell carcinoma and rest of 15.60% (n=5) cases were found to be negative for it. Our findings are in agreement with a recent study by Schraml et al [17] on analysis of pVHL expression in renal cell carcinoma by immunohistochemistry using poly and monoclonal antibodies against VHL protein [16]. They found that immunohistochemically, pVHL was expressed in 70% of cases of RCCs under their study and rest 30% was found to be negative for VHL protein.

Lin et al immunohistochemically evaluated renal and nonrenal tumors for usefulness of pVHL by using rabbit polyclonal antibodies against VHL protein [12]. They concluded strong positive immunoreactivity for pVHL and nearly 100% primary renal cell carcinomas and 95% of metastatic RCCs showed positive immunoreactivity for VHL protein. Our results are supported by study of Corless et al who had immunohistochemically detected VHL protein in 80% cases of RCCs and rest were negative for it [9]. They also gave a very important statement regarding negative staining of pVHL in renal tumors. They stated that mutated VHL gene is predicted to produce altered forms of VHL protein that is truncated or elongated with respect to Elongin BoC binding region. These mutant forms of VHL protein should be remain detectable by immunostaining provided that the appropriate epitopes remain intact [9]. Schraml et al [17], also reported that allelic deletion of the *VHL* gene was not associated with pVHL expression. Schumeli et al [18] stated that loss of function mutations in the Von Hippel-Lindau (pVHL) tumor suppressor protein are tumorogenic and by using biophysical methods confirmed that mutant pVHL proteins have lower stability than the wild type, distorted core domain and as a result reduce the ability of the protein to bind its target HIF-1α.

Importantly, all recent studies of human tissues have exclusively described cytoplasmic pVHL expression. Only some immune fluorescence analyses have shown that pVHL can be expressed, to a lesser extent, in the nucleus or in association with cell membranes [17,19,20.21]. Only few recent studies have examined the combined cytoplasmic and nuclear localization of VHL protein in RCCs and its clinical relevance [20,22]. In present study, among all pVHL positive cases, combined cytoplasmic and nuclear expression of pVHL was most common (59.0%) followed by only cytoplasmic expression (37%). Nuclear expression alone was rare and was noted in only one case. Our this finding is in agreement to the study of Schraml et [17] who found combined cytoplasmic and nuclear expression of pVHL was commonest and nuclear alone was rarest pattern in RCCs.

We analyzed pVHL expression in different histological subtypes of RCCs. Positive pVHL expression was found in 91.30 % of clear cell RCCs, 75 % of papillary RCCs .Only one case of chromophobe RCC was found in our study, that came to be negative for VHL protein expression. Schraml et detected VHL protein expression in 69 % clear cell carcinoma, 68 % papillary carcinoma and 76% of chromophobe carcinomas while Lin et [12] found immunohistochemica positivity for VHL protein in 99% clear cell RCC, 100 % papillary and 100 % chromophobe RCCs. This discrepancy in findings

may be attributed to different methods of immunostaining and different kind of antibodies used for detection of pVHL expression.

Among clear cell RCCs, 47.80% showed combined cytoplasmic and nuclear pVHL expression, 39.10% showed only cytoplasmic expression and 4.30% showed only nuclear expression. Out of all pVHL positive papillary RCCs, 62.50% showed combined cytoplasmic and nuclear expression, 12.50 % showed cytoplasmic expression and no isolated nuclear expression was seen. Hence, we concluded that combined cytoplasmic and nuclear expression of pVHL is most common pattern of expression in RCCs. Schraml et al [17] found 44% clear cell RCCs and 44 % of papillary RCCs were positive for combined cytoplasmic and nuclear expression. We observed that exclusive nuclear expression of pVHL was the least common pattern but in contrast to our finding they observed exclusive cytoplasmic expression as least common pattern.

We analyzed association between pVHL expression and tumor stage. Combined cytoplasmic and nuclear pVHL expression was more frequently seen in early stage RCCc, stage I & II (52.20%) than stage III & IV (44.40%). Similarily, only cytoplasmic expression was noted in 38.1% of early stage tumors and 18.2% of stage III & stage IV tumors but this association between stage of tumor and pattern of pVHL expression could not reach statistical significance *(χ^2=3.308; p=0.347)*. This statistical insignificance may be attributed to relatively small study population size. Notably, exclusive nuclear expression was noted in only advance stage RCCs (stage III & stage IV). In terms of combined cytoplasmic and nuclear expression, our results are supported by study of Schraml et al (17) who found that combined cytoplasmic and nuclear expression was associated with early tumor stage and this association was statistically significant(*p value=0.01*.

In present study, we also evaluated pVHL expression in different histological grades of RCCs. We observed, exclusive cytoplasmic pVHL expression was more frequently noticed in low grade tumors compare to high grade tumors (100% in grade-I, 30.4% in grade-II and 14.3% in grade - III) while exclusive nuclear expression of pVHL was found only in grade-III tumors [Table 5]. Combined cytoplasmic and nuclear expression was also more frequent in grade II tumors (56.5%) than grade III tumors (42.9%) [Table 5].

Again this association could not reach statistical significance *(χ^2=2.965; p=0.397)*. Similar to our study, Schraml et al [17] found that combined cytoplasmic and nuclear p VHL expression was more common in low histological grade and this correlation was statistically significant in their study.

Table 4: Association of pVHL expression with tumor stage in renal cell carcinoma

Grade	Negative (%)	Cytoplasmic %)	Nuclear (%)	Nuclear+Cytoplasmic
I	0 (0%)	2 (100%)	0 (0%)	0 (0%)
II	3 (13%)	7 (30.4%)	0 (0%)	13 (56.5%)
III	2 (28.6%)	1 (14.3%)	1 (14.3%)	3 (42.9%)

Table 5: Association of pVHL expression with Fuhrman grade of renal cell carcinoma

Stage	Negative (%)	Cytoplasmic %)	Nuclear (%)	Nuclear+ Cytoplasmic
I & II (n=23)	3 (14.3%)	8 (38.1%)	0 (0%)	12 (52.2%)
III & IV (n=9)	2 (18.2%)	2 (18.2%)	1 (9.1%)	4 (44.4%)

Conclusion

A relatively limited number of studies evaluating localization and distribution of the pVHL in RCCs were available for review and compare our data. Among these studies, different anti-VHL antibodies produced in each individual laboratory, different antibody retrieval methods, and different immunohistochemical staining methods were used. As a result, data were somewhat inconsistent and difficult to compare. We adopted material methods in accordance to few recent studies and compared our results to these studies. Our results along with supportive literature imply that VHL protein expression and its cytoplasmic and nuclear distribution is of potential relevance for the diagnosis and biological behavior of RCCs. Although our data could not attain statistical significance at many instances, we recommend that further studies with relatively larger study population should be conducted in this context.

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