Original Research Article

The role of vascular endothelial growth factor (VEGF) in the diagnosis of renal cell carcinoma

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Abstract

Background: Renal cell carcinoma is the most lethal urological cancer. Most patients present with metastasis. Their treatment is limited as the tumor is chemoresistant and hormone resistant. Vascular Endothelial Growth Factor is the key mediator of angiogenesis in cancer. Inactivation of von Hippel-Lindau gene leads to the production of Hypoxia Inducible Factor-1α. Following this VEGF-A becomes highly expressed in the carcinoma tissue. This results in tumorigenesis by promoting distant metastasis, uncontrolled growth, and resistance to apoptosis. **Aim:** The aim of this study was to evaluate the expression of VEGF in renal tumor cells and adjacent normal tissue and to compare the expressions of the marker with the selective risk factors. **Materials and Methods:** This study was conducted among 30 cases of histopathologically proven renal cell carcinoma (RCC). Ethical clearance and the necessary permission from the Pathology department of Sri Ramachandra University were obtained. VEGF was analyzed by Biotin Streptavidin Immunoperoxidase method. Q scoring was done. **Results:** 90% of cases were VEGF positive in the tumor area, whereas the cells in the adjacent normal tissue area expressed weak staining. The clear cell renal cell carcinoma, a subtype of RCC showed strong and diffuse staining. Comparison of the VEGF Q score with the various risk factors and tumor characteristics was done, but no significant difference was found. **Conclusion:** VEGF was strongly expressed in clear cell renal cell carcinoma tissue. VEGF could play a role as a potential diagnostic marker in renal cell carcinoma and could help in assessing prognosis and designing therapy.

Keywords: diagnostic marker, immunohistochemistry, renal cell carcinoma, vascular endothelial growth factor

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Introduction

Renal cell carcinoma (RCC) is the most lethal urological cancer.[1] Nearly 40% of patients die of tumor progression.[1] Most patients present with metastasis and those who are treated also experience recurrence.[1] Despite the recent advances in field of oncology, the prognosis is very poor and the treatment is restricted.[2] These tumors are resistant to chemotherapy

and hormone therapy.[2] Therefore a precise prediction of the results after surgery is necessary for counseling, follow-up and treatment.[3] The risk factors for RCC are smoking, hypertension, obesity, dietary habits, occupational exposure etc.[4]

Clear cell renal cell carcinoma (ccRCC) accounts for nearly 70-80% of the RCC cases.[3] It is graded according to the Furhman's nuclear grading and staged based on the TNM staging.[1] It is described by an increased rate of inactivation of the von Hippel-Lindau (VHL) tumor suppressor gene that leads to a rise in the hypoxia-inducible factor (HIF).[5] This stimulates the expression of hypoxia response genes, vascular endothelial growth factor (VEGF).[5]

Vascular endothelial growth factor is a dimeric glycoprotein with a main role in stimulating vascular permeability and proliferation of the endothelial cell.[6] VEGF is the key mediator of angiogenesis in cancer.[6] VEGF-A is very much expressed in many human cancers, but renal cell carcinoma (RCC) produces extremely high levels, with tumors having a highly vascular histologic appearance.[6] In RCC, the high expression of VEGF is due to the inactivation of the von Hippel-Lindau (VHL) gene.[5] This results in expression of HIF-1α and induction of hypoxia-regulated genes VEGF, transforming growth factor (TGF), and platelet-derived growth factor (PDGF).[7] These gene products result in a highly vascularized tumor with both local and distant metastases through hematogenous spread, uncontrolled growth, and resistance to apoptosis.[8]

VEGF are the signal proteins bound to the extracellular matrix of the tumor cell.[9] The stimulating factors for VEGF are environmental factors, growth factors, oncogenes, cytokines, and hormones.[8] These VEGF proteins when detached and released bind to receptors on the surface of endothelial cells and promote angiogenesis.[10]

With this background, the purpose of our study was to evaluate the expression of VEGF in RCC and to compare the VEGF Q scores (which indicates the intensity and percentage of expression of the inflammatory mediators) with various parameters. The aim of our study was therefore to evaluate the expression of VEGF in renal tumor cells and adjacent normal tissue and to compare the expression of the marker with the selective risk factors.

Materials and Methods

The study proposal was submitted to the ethics committee of Sri Ramachandra University and was approved. All renal cell carcinoma patients who underwent nephrectomy in Sri Ramachandra Hospital from 2011 to 2013 in the age group 31 to 76 years were included in the study.

The study was started after getting the required permissions from the Medical Director and the Medical Superintendent, Sri Ramachandra University and the Head of the Department, Department of Pathology. The study involved a histopathological procedure using Immunohistochemistry.

Thirty cases of histopathologically proven renal cell carcinoma diagnosed in the Department of Pathology were identified and the corresponding tissue blocks collected. Their detailed history was obtained from the records available in the medical records department.

The inflammatory mediator studied here was Vascular Endothelial Growth Factor and it was analyzed by Biotin Streptavidin Immunoperoxidase method.[11] The intensity and percentage of expression of the inflammatory mediators were studied using Q scoring. Q scoring was done by multiplying the grade and intensity of the stain taken by the tissue and the results range from 0 to 12.[12] Values 0 and 1 are considered immunoscoring negative.[12] Values 2 to 12 are considered immunoscoring positive.[12] The immunoscoring values were compared with the various parameters (age, sex, smoking,

obesity, hypertension, type of RCC, grade and stage) and analyzed.

Statistical Analysis: Statistical analysis was done by using SPSS software version 16. Data was expressed as Mean ± SD. Independent Student t test was done to compare the VEGF Q score with the parameters (age, sex, BMI, smoking, hypertension). One-way ANOVA test followed by post hoc test was done to compare VEGF Q score with the tumor characteristics. A p value < 0.05 was considered as statistically significant.

Results

Our study showed that 22 (73%) of the renal cell carcinoma patients were more than 50 years of age and 21 (70%) cases were males.

Among these smoking (57%), obesity (63%) and hypertension (57%) were found to be associated with renal cell carcinoma.

Most of the renal cell carcinomas were the clear cell types (77%) followed by papillary (10%), chromophobe (10%) and collecting duct (3%).

They were graded according to the Furhman's nuclear grading. It was found that most of the RCC belong to grade II (77%), 12% cases belong to grade I, 7% cases grade III and 3% cases grade IV. Based on the TNM staging system, the RCC were staged as 43% in stage I, 37% in stage II, 13% in stage III and 7% in stage IV.

Immunostaining with VEGF (A-20) using the Biotin Streptavidin Immunoperoxidase method revealed that VEGF was strongly expressed in the tumor tissue. 90% of cases were VEGF positive in the tumor area, whereas in the adjacent normal tissue area, the cells expressed weak staining.

CcRCC showed strong and diffuse staining while papillary, chromophobe RCC and collecting duct carcinoma expressed weak and moderate staining.

Q scoring was done to find the strength of the VEGF antigen present in the tumor tissue.

The comparison of VEGF Q scoring with the patient details (p value for age = 0.58, sex = 0.65, BMI = 0.22, smoking = 0.4, hypertension-0.44) and tumor characteristics (p value for type = 0.74, grade = 0.19, stage = 0.58) was done but no significant difference was found as shown in Table 1 and Table 2 respectively. Neither risk factors nor the tumor characters were found to have a significant role in influencing the expression of molecular markers in the tumor tissue.

Table 1: Comparison of VEGF Q scores with the patient details

Parameter		Subjects n (%)	Q score	p value
Age	>50	22 (73%)	5.68± 3.12	0.58
	<50	8 (7%)	6.5± 4.78	
Sex	M	21 (70%)	6.1± 3.40	0.65
	F	9 (30%)	5.4± 4.03	
Smoker	Yes	17 (57%)	6.29± 3.25	0.40
	No	13 (43%)	5.07± 4.25	
Body mass index	>25	19 (63%)	5.1± 3.44	0.22
	<25	11 (37%)	6.9± 4.01	
Hypertensive	Yes	17 (57%)	6.23± 3.56	0.44
	No	13 (43%)	5.15± 3.93	

M = male, F = female, Age expressed in years; Body mass index expressed in kg/m^2; Statistical analysis done using independent Student's t test to compare the VEGF Q scores[12] and patient details; p value < 0.05 was considered as significant

Table 2: Comparison of VEGF Q scores with tumor characteristics

Parameter		Subjects n (%)	Q score	p value
Type of RCC	Clear cell	23 (77%)	6.22± 3.908	0.74
	Papillary	3 (10%)	4.00± 2.0	
	Chromophobe	3 (10%)	6.00± 2.0	
	Collecting duct	1 (3%)	4.00	
Grade	I	4 (13%)	6	0.19
	II	23 (77%)	5.91± 3.716	
	III	2 (7%)	2.5± 2.121	
	IV	1 (3%)	12	
Stage	I	13 (43%)	5.46± 3.643	0.58
	II	11 (37%)	6.18± 3.125	
	III	4 (13%)	5.00± 4.76	
	IV	2 (7%)	9.00± 4.243	

Statistical analysis was done using one-way ANOVA to compare the VEGF Q score[12] and tumor characteristics; p value < 0.05 was considered as significant

Discussion

In a study conducted by Protzel *et al.* in 2012, RCC was found to be common in elderly males.[13] Our study also shows similar findings since most of the renal cell carcinoma patients were males. According to Yu *et al.,* the major risk factors like smoking, obesity, and hypertension were found to be associated with renal cell carcinoma.[4] In our study, more than 50% of the subjects were obese, smokers and hypertensive. Most of the renal cell carcinoma cases were clear cell type (77%), grade II (77%), stage I (43%) and stage II (37%).

All the immunostained tumor samples strongly (90%) expressed VEGF whereas the adjacent normal tissue expressed weak staining. CcRCC showed strong and diffuse staining while papillary, chromophobe RCC and collecting duct carcinoma expressed weak and moderate staining. Djordjevic *et al.,* in 2007 proved that the overexpression of VEGF predicted worse histologic prognostic parameter in clear cell type RCC.[8] But a study done by Jacobsen *et al*. found that there was no difference in VEGF expression among the types of RCC.[14]

Both tumor tissue and the adjacent normal tissue expressed immunoreactive VEGF. Few studies have explained the reason behind this as the presence of different isoforms of VEGF expressed in different areas.[14] The differences in the expression of isoforms are clearly studied only by doing Western blot. Normal tissue samples have been found to express VEGF189 and VEGF165 isoforms, but not VEGF121; whereas in RCC samples, VEGF189 had the highest expression, VEGF165 had intermediate expression and VEGF121 was rarely detected. [14] VEGF121 expression was considered positive when a band was visually detected by doing the Western blot.[15] In our study, Western blot was not done to confirm the isoforms, which was a limitation. Hence the VEGF was noticed in both tumor and adjacent normal tissue.

In our study, Q scoring was done to find the strength of the VEGF antigen present in the tumor tissue. The VEGF Q score was compared with the various parameters like risk factors and tumor characteristics. But no significant difference was found, which could be due to the small sample size. Studies have also been conducted to assess the postoperative recurrence of RCC using the serum levels and the immunohistochemical (IHC) expression of VEGF.[16]

In our study, VEGF was found to play a significant role in clear cell renal cell carcinoma. This may prove to be a potential diagnostic and prognostic marker. However a large scale study is needed to show the actual role of the

markers in RCC. In future, more information about the presence of the inflammatory mediators can provide additional insights in the diagnosis and management of renal cell carcinoma. The knowledge of the risk factor association can promote preventive measures to reduce morbidity and mortality due to renal cell carcinoma

Conclusion

Our study revealed that vascular endothelial growth factor (VEGF) was strongly expressed in the renal cell carcinoma (RCC) tissue especially in clear cell renal cell carcinoma showing its involvement in tumorigenesis. VEGF markers may prove useful as potential diagnostic markers in assessing the prognosis and designing therapy in these tumors.

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Conflicts of interest: Nil

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