Original Article

The Expression Level of Vascular Endothelial Growth Factor Receptor-2, Vascular Endothelial Growth Factor Receptor-3, and Insulin-Like Growth Factor II mRNA Binding Protein 3 in Renal Cell Carcinoma: Can these Markers Indicate Poor Prognosis in Immunohistochemical Examination?

Ömer Eronat, Olcay Kandemir[1], Aykut Onursever[1]

Department of Pathology, Synevo Laboratories, [1]Department of Pathology, Atatürk Training and Research Hospital, Ankara, Turkey

Abstract

Background: The malignant tumors of the kidney are the most aggressive among urologic cancers. To treat patients with renal cell carcinoma (RCC), it is important to understand the disease's prognostic factors. Angiogenesis is an essential process, responsible for the growing, and spreading of neoplastic tissues. Vascular endothelial growth factor (VEGF), the most potent angiogenetic factor known, and its receptor VEGF (VEGFR) play an important role in angiogenesis. Insulin-like growth factor-II mRNA binding protein 3 (IMP-3) is found in some malignant tumors and contributes to cell growth and cell migration during the early stages of embryogenesis. **Material and Methods:** The following study retrospectively evaluated 48 radical, 29 simple, and 23 partial nephrectomy specimens with RCC. Pathologic prognostic parameters, including tumor size, tumor stage, Fuhrman nuclear grade, distant metastasis status, and lymph node involvement status were compared with the immunohistochemical expression levels of VEGFR-2, VEGFR-3, and IMP-3. **Results:** Except the relation between VEGFR-3 and Fuhrman nuclear grade, there was no significant relation with the expressions of VEGFR-2, VEGFR-3, and IMP-3 and the pathologic prognostic parameters such as tumor size, tumor stage, Fuhrman nuclear grade, distant metastasis status, and lymph node involvement status. All three markers showed significant expression in almost all chromophobe and papillary histologic subtypes. The expression rates for chromophobe, papillary type 1, and type 2 RCC were 100%, 90%, and 100% for VEGFR-2, respectively, and 87.5%, 90%, and 100% for VEGFR-3, respectively. The expression rates of IMP-3 were 50% for papillary type 1, 83.3% for papillary type 2, and 100% for chromophobe RCC. **Conclusion:** Although the limited number of cases, current data gathered from our study shows that these markers have no relation with pathologic prognostic parameters and would not provide additional information in the immunohistochemical examination. Anyway, their tendency of expression in chromophobe and papillary type RCC is remarkable which should be evaluated with a larger number of cases.

Keywords: *Insulin-like growth factor-II mRNA binding protein 3, prognosis, renal cell carcinoma, vascular endothelial growth factor receptor-2, vascular endothelial growth factor receptor-3*

Introduction

Malignant tumors of the kidney make up 3% of all fatal malignancies.[1] The American Cancer Society's 2013 estimates for kidney cancer in the United States are as follows: about 65,150 new cases of kidney cancer (40,430 in men and 24,720 in women) and 13,680 deaths (8780 men and 4900 women).[2] The most diagnosed type of cancer among kidney malignancies is renal cell carcinoma (RCC), which makes up 85% of all kidney malignancies.[3,4] The malignant tumors of the kidney are the most aggressive among urologic cancers.[5-7] RCC has a number of different histological subtypes, including clear cell, papillary, chromophobe, multilocular cystic clear cell, and collecting duct types.[8] Papillary and chromophobe RCC have better prognoses compared to clear cell RCC, the most common histological subtype of RCC.[9-12] Some biological features, such as large tumor size, advanced tumor stage, and high-grade nuclear features according to Fuhrman nuclear grading system, indicate a poor prognosis.[11,13,14]

There are several recent studies in the literature that investigated the expression levels of some potential predictive and prognostic markers, such as carbonic

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Address for correspondence:
Dr. Ömer Eronat, Ergin Sokak 45/6 Cömert Apt, Tandogan, Ankara 06580 Turkey.
E-mail: eronatomer@gmail.com

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anhydrase 9, vascular endothelial growth factor (VEGF) and its receptor VEGF (VEGFR) family, and insulin-like growth factor II mRNA binding protein 3 (IMP-3).

Angiogenesis is an essential process for normal tissue growth and development. Tumor tissues are also dependent on angiogenesis.[15] Among many angiogenetic factors, VEGF is the most potent factor known and plays an important role during this process.[16,17] It is believed that the elevated expression level of VEGF and VEGFR increases the microvessel density inside the tumor so that it can grow and metastasize.[18-21] VEGFR-1 and VEGFR-2, located primarily on endothelial cells, is responsible for angiogenesis, whereas VEGFR-3, which can be detected on lymphatic vessel-derived endothelial cells, is responsible for lymphangiogenesis.[22]

IMP-3, also known as K homology domain that contains protein overexpressed in cancer (KOC), is a protein that plays an important role in cell growth and migration during the early stages of embryogenesis.[23] IMP-3 is expressed in developing epithelial, muscle, and placenta tissues, whereas in adult tissues, the expression level is barely detectable.[23,24] IMP-3, an oncofetal protein reexpressed in many organ malignancies such as pancreas, kidney, lung, breast, esophagus, cervix, and endometrium, plays a critical role in malignant transformation and tumor progression.[24-36] IMP3 has also been recognized as an indicator for metastasis and a predictor of poor prognosis for many types of cancer.[30-32]

In this study, we analyzed and compared different histomorphological parameters with the expression levels of VEGFR-2, VEGFR-3, and IMP-3 in RCC, to determine their prognostic significance. Given that both group of markers were found as useful in the different types of malignant tumor in many studies,[28-30,32,37] our goal was to investigate whether these markers can provide additional information about the prognosis for RCC in routine practice.

Materials and Methods

Patient selection

In our study, we retrospectively reviewed 100 nephrectomy specimens, diagnosed with RCC in the Clinic of Pathology in Ankara Atatürk Training and Research Hospital between 2008 and 2013. The cases were composed of 48 radical, 29 simple, and 23 partial nephrectomy specimens. All slides were reevaluated retrospectively without knowledge of their pathologic outcome.

Clinicopathologic features

We rerecorded tumor size, histologic subtype according to the WHO 2004,[12] nuclear grade according to the Fuhrman nuclear grading system,[38] pathologic tumor stage according to tumor node metastasis (TNM) classification,[39] regional lymph node involvement, and distant metastasis. We then compared these features to the expression levels of VEGRF-2, VEGFR-3, and IMP-3. Furthermore, tumors were grouped into four categories according to tumor size: less than 4, 4.1–7, 7.1–10, and >10 cm to evaluate the relation with other parameters. VEGFR-2, VEGFR-3, and IMP-3 expressions had been examined by immunohistochemical methods. The information about demographic data, such as age, gender, and stage of the diseases, obtained by the search of the patient files retrospectively.

Tissue preparation and immunohistochemical staining

Resection materials obtained after nephrectomy surgery were placed in 10% formaldehyde immediately after the process and fixed for 24 h. After fixation, pathologically sampled tumor tissues were buried into paraffin after routine tissue follow-up. Immunohistochemical staining was applied on cross-sections containing nominal tumor samples that were evaluated with H and E staining. Cross-sections of 5 μm thickness prepared for immunohistochemical staining. After deparaffinization and rehydration, endogenous peroxidase activity was inactivated with 3% hydrogen peroxide for 15 min, and the samples were processed in a microwave oven in EDTA buffer at pH 9.0 to unmask epitopes. After the antigen retrieval process, sections were incubated with diluted primary antibodies for 2 h, followed by washing with phosphate buffered saline (PBS). According to the manufacturer's instructions, the antibodies were diluted and performed as follows: VEGFR-2 (Abcam, Cambridge-UK (ab39256): polyclonal IgG, 200 μg/ml) 1:200 in PBS for 60 min; VEGFR-3 (Novocastra, Newcastle-UK, Clone: KLT9, IgG2b kappa, 200 μg/ml) 1:50 in PBS for 60 min; and IMP-3 (DAKO, California-USA, Clone: 69.1 Monoclonal Mouse Anti-Human IMP3 IgG2a, kappa, 200 μg/ml) 1:100 in PBS for 60 min. After primary antibody incubation, the sections were incubated in biotinylated goat anti-polyvalent solution for 20 min, washed in PBS, and submerged for another 20 min in streptavidin peroxidase solution. To identify the immunoreaction, diaminobenzidine chromogen was performed for 3 min, followed by counterstaining with Harris Hematoxylin.

Evaluation of immunohistochemical staining

The expression levels of all markers were divided into subcategories. Staining was scored as follows: 0 indicated no staining; 1+ indicated <10%; 2+ indicated 10%–75%; and 3+ indicated >75%, according to the percentages of the positive cytoplasmic staining areas of tumor cells in contrast to the whole area. Score 0 was accepted as negative, and score 1, 2, and 3 as positive. For each marker, cytoplasmic reactions in the tumor cells were evaluated as positive staining. There were no accompanying membranous or nuclear staining. Appropriate positive controls were used as follows: vascular structures of placental tissue for VEGFR-2 and VEGFR-3, and tonsil for IMP-3 as a positive control. Beside positive controls, we observed cytoplasmic reaction with all three markers in renal tubulus

epithelium [Figures 1 and 2]. We detected no staining in glomerulus epithelium, vascular structures, and perirenal fat tissue or other nonneoplastic tissues.

Statistical methods

Relations of expression levels of VEGFR-2, VEGFR-3, and IMP-3 with clinicopathologic features were analyzed using Fisher's exact probability and Chi-square tests. A value of $P < 0.05$ was considered as statistically significant. Statistical analyses were performed using the SPSS software version 16.0 (IBM, Chicago, Illinois, US).

Results

A total of 100 RCC patients, 29 (29%) of whom were female and 71 (71%) of whom were male, were included in the study. Male/female ratio was 244:1. Mean age of female patients was 60.7 (range 46–78), and mean age of male patients was 57.5 (range 30–81). Clear cell RCC was determined in 74 (74%) of the patients, papillary type 1 RCC was determined in 10 patients (10%), papillary type 2 RCC [Figure 3] was determined in 6 patients (6%), chromophobe RCC was determined in 8 patients (8%), and multilocular cystic RCC was determined in 2 patients (2%). The median diameter of tumors was 6.02 cm (range 0.8–17.5 cm). The number of cases with tumor diameter <4 cm, between 4 and 7 cm, between 7 and 10 cm, and over 10 cm were 40 (40%), 34 (34%), 16 (16%), and 10 (10%), respectively. According to the Fuhrman nuclear grade, 7 patients (7%) had grade 1, 26 patients (26%) had grade 2, 51 patients (51%) had grade 3, and 16 patients (16%) had grade 4 tumor. T1a disease was detected in 37 patients (37%), T1b disease was detected in 33 patients (33%), T2, T3a, T3b, and T4 disease was stated in 17 patients (17%), 9 patients (9%), 2 patients (2%), and 2 patients (2%), respectively. Regional lymph node involvement was positive in 4 patients (4%). Distant metastasis was positive in 5 patients (5%). Table 1 shows the results of clinicopathologic features.

Each marker indicated a tendency to be expressed in both papillary and chromophobe histologic subtypes [Figures 4-7]. The expression rate in clear cell RCC was 66.2% for VEGFR-2, 68.9% for VEGFR-3, and 36.5% for IMP-3. The expression rates for chromophobe, papillary type 1, and type 2 RCC were 100%, 90%, and 100% for VEGFR-2, respectively, and 87.5%, 90% and 100% for VEGFR-3, respectively. The expression rates of IMP-3 were 50% for papillary type 1, 83.3% for papillary type 2, and 100% for chromophobe RCC. Of the two multilocular cystic types, clear cell RCC cases, we identified immunoreactivity in one case with VEGFR-2 (50%) and IMP-3 (50%), and immunoreactivity in both cases with VEGFR-3 (100%) [Table 2].

The relation of tumor size with VEGFR-2, VEGFR-3, and IMP-3 expressions had no statistical significance ($P = 0.249$, 0.554, 0.890, respectively) [Table 3].

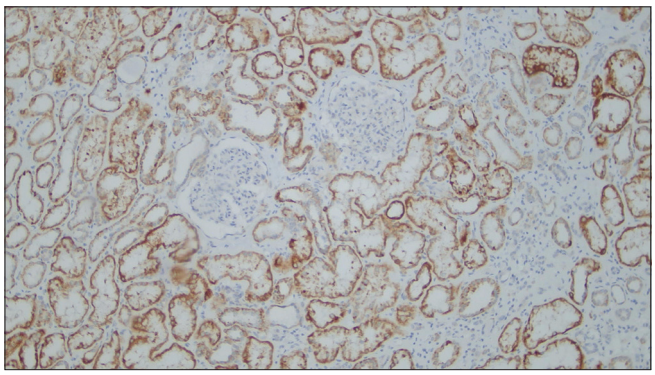

Figure 1: IMP-3 expression in renal tubulus epithelium (X100)

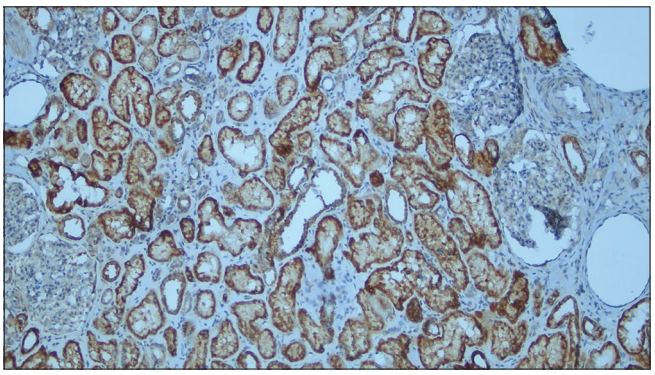

Figure 2: VEGFR-2 expression in renal tubulus epithelium (X100)

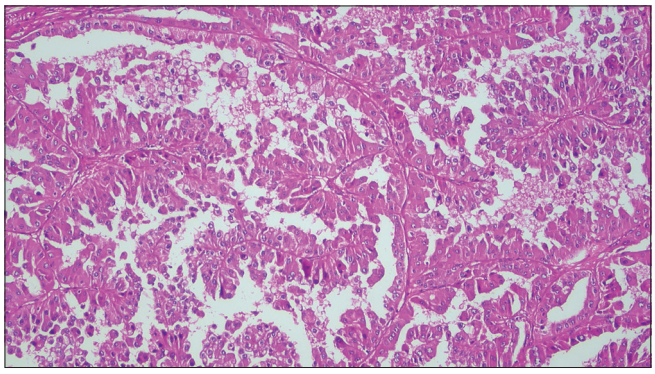

Figure 3: Papillary type 2 RCC (H&E X100)

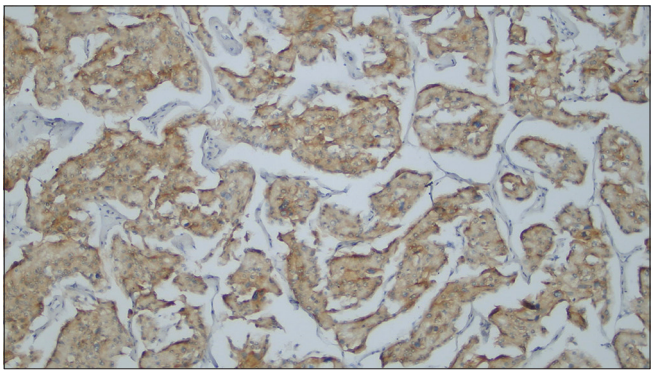

Figure 4: VEGFR-2 expression in chromophobe RCC (X100)

Table 1: Results of clinicopathologic features

Features	Results
Sex	
Women	29 (29%)
Men	71 (71%)
Men/Female Ratio	2,44:1
Age, median (range)	
Women	60,7 (46-78)
Men	57,5 (30-81)
Histologic subtype	
Clear Cell	74 (74%)
Papillary Type 1	10 (10%)
Papillary Type 2	6 (6%)
Chromophobe	8 (8%)
Multilocular Cystic	2 (2%)
Tumor size (cm)	
Median (range)	6,02 (0,8-17,5)
<4 cm	40 (40%)
4-6,9 cm	34 (34%)
7-10 cm	16 (16%)
<10 cm	10 (10%)
Fuhrman nuclear grade	
Grade 1	7 (7%)
Grade 2	26 (26%)
Grade 3	51 (51%)
Grade 4	16 (16%)
TNM classification	
pT1a	37 (37%)
pT1b	33 (33%)
pT2	17 (17%)
pT3a	9 (9%)
pT3b	2 (2%)
pT3c	0
pT4	2 (2%)
Distant metastasis	
M0	95 (95%)
M1	5 (5%)
Regional lymph node involvement	
Nx	96 (96%)
N0 and N1	4 (4%)

There were no relation between tumor stage with VEGFR-2, VEGFR-3, and IMP-3 expressions (P = 0.345, 0.847, 0.796, respectively) [Table 4].

The P values indicating that the relation of distant metastasis status with VEGFR-2, VEGFR-3, and IMP-3 expressions was 0.324, 0.328, and 0.820, respectively.

There were 4 cases with lymph node dissection, in which all were involved with tumor. In all of these cases, we identified immunoreactivity only with VEGFR-3. We categorized all other cases as Nx and due to these lymph nodes with unknown status statistical analyses between lymph node involvement and expression of VEGFR-2, VEGFR-3, and IMP-3 could not be evaluated.

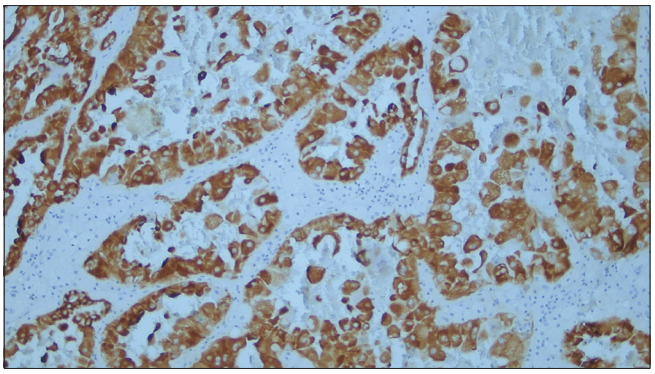

Figure 5: VEGFR-2 expression in papillary type 2 RCC (X100)

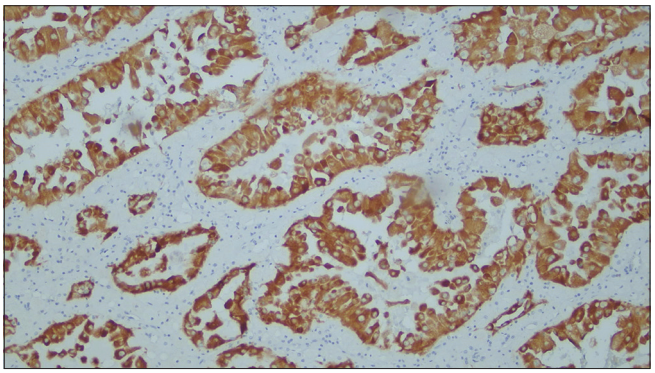

Figure 6: VEGFR-3 expression in papillary type 2 RCC (X100)

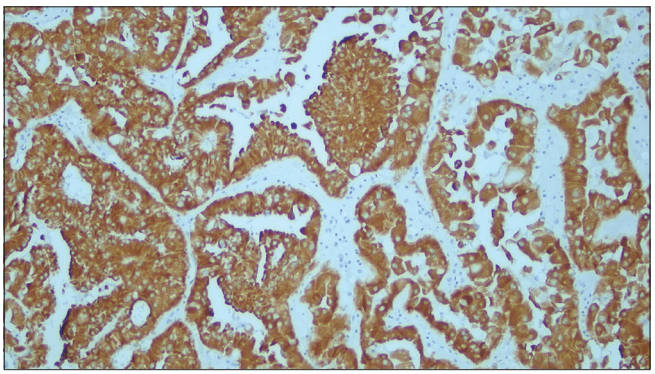

Figure 7: IMP-3 expression in papillary type 2 RCC (X100)

The relationship between the nuclear grade of RCC according to the Fuhrman and the expression levels of VEGFR-2 and IMP-3 had no statistical significance (P > 0.265, P > 0.564, respectively). In contrast, the comparison of the Fuhrman nuclear grade and the expression level of VEGFR-3 was statistically significant (P < 0.018). The results are summarized in Table 5.

Discussion

Malignant tumors of the kidney are the most aggressive neoplasms among urologic cancers. In 25%–30% of patients, the disease will spread from the initial time of diagnosis, and 40% of patients will die due to RCC.

Table 2: The expression levels of VEGFR-2, VEGFR-3, and IMP-3 in histological subtypes

Marker	Staining	Histologic Subtype					*P*
		Clear cell	Chromophobe	Papillary type 1	Papillary type 2	Multilocular cystic	
VEGFR2	0	25	0	1	0	1	*P*<0,001
	1+	20	0	1	0	0	
	2+	19	2	3	2	0	
	3+	10	6	5	4	1	
VEGFR3	0	23	0	1	0	0	*P*<0,001
	1+	30	1	2	1	0	
	2+	18	2	3	1	1	
	3+	3	5	4	4	1	
IMP3	0	47	0	5	1	1	*P*<0,001
	1+	16	1	2	0	0	
	2+	9	1	0	0	1	
	3+	2	6	3	5	0	

Table 3: Comparison of tumor size and the expression levels of VEGFR-2, VEGFR-3, and IMP-3

Marker	Staining	Tumor Size				*P*
		<4 cm	4,1-7 cm	7, 1-10 cm	>10 cm	
VEGFR2	0	8	9	7	3	*P*>0,249
	1+	5	9	5	2	
	2+	14	9	2	1	
	3+	13	7	2	4	
VEGFR3	0	10	6	6	2	*P*>0,554
	1+	11	13	6	4	
	2+	9	9	4	3	
	3+	10	6	0	1	
IMP3	0	24	17	8	5	*P*>0,890
	1+	6	9	3	1	
	2+	3	4	2	2	
	3+	7	4	3	2	

Table 4: Comparison of tumor stage and the expression levels of VEGFR-2, VEGFR-3, and IMP-3

Marker	Staining	Tumor Stage (TNM)						*P*
		pT1a	pT1b	pT2	pT3a	pT3b	pT4	
VEGFR2	0	8	8	5	2	2	2	*P*>0,345
	1+	4	9	4	4	0	0	
	2+	13	9	3	1	0	0	
	3+	12	7	5	2	0	0	
VEGFR3	0	10	6	4	3	0	1	*P*>0,847
	1+	10	12	6	3	2	1	
	2+	9	9	6	1	0	0	
	3+	8	8	1	2	0	0	
IMP3	0	23	16	7	4	2	2	*P*>0,796
	1+	5	9	2	3	0	0	
	2+	3	4	3	1	0	0	
	3+	6	4	5	1	0	0	

Patients with localized RCC in the kidneys has a recurrence or metastases rate of approximately 30%–50%.[40-43] To treat cancer with such a high mortality rate, it is essential to understand its underlying pathophysiologic processes.

There are several studies about prognostication of RCC that attempt to provide additional information, such as defining the tendency for distant metastasis and lymph node involvement.[37] Some of these studies investigate the expression of different biological markers, specifically, whether or not they can affect treatment of the disease.[44-46]

Angiogenesis, which is modulated by a series of angiogenetic factors, is an essential process for every solid tumor to grow and metastasize.[15] Among these angiogenetic factors, VEGF stimulates with the aid of a paracrine mechanism, endothelial cell hyperpermeability, and proliferation so the tumor can progress.[16,17] In this respect, VEGF expression in cancers such as kidney, breast, stomach, colon, and its appreciable relation with the microvessel density inside the tumor increases the possibility that VEGF may have prognostic value for these kinds of tumors.[18-21] Well-known receptors in the VEGFR family are as follows: VEGFR-1 (Flt-1), VEGFR-2 (KDR/Flt-2), and VEGFR-3 (Flt-4). VEGFR-1 and VEGFR-2 are located primarily on endothelial cells, whereas VEGFR-3 can be detected on lymphatic vessel-derived endothelial cells and is responsible for lymphangiogenesis.[22] VEGFR-B is the ligand for VEGFR-1, but it can also react with VEGFR-2 and VEGFR-3. VEGF-C, in contrast with VEGF-B, is the ligand for VEGFR-3, but it can also react with VEGFR-2, and thus, it can stimulate both angiogenesis and lymphangiogenesis.[47-49]

RCC is a tumor characterized by hypervascularity. The VEGF gene is overexpressed in RCC,[50,51] and Sasaki claimed that the overexpression level of the VEGF gene correlates with the microvessel density inside the tumor.[20,52] All current observations suggest that VEGF plays an important role in RCC.

The VEGF signaling pathway is a target in anticancer therapy, used in advanced and/or metastasized RCC for multitargeted receptor tyrosine kinase inhibitor drugs, such as sunitinib[44] and sorafenib.[45] Recently, Escudier *et al*. in their study found that the clinical use

Table 5: Comparison of Fuhrman nuclear grade and the expression levels of VEGFR-2, VEGFR-3, and IMP-3

Marker	Staining	Fuhrman nuclear grade				*P*
		Grade 1	Grade 2	Grade 3	Grade 4	
VEGFR2	0	4	5	10	8	*P*>0,265
	1+	0	6	12	3	
	2+	1	8	14	3	
	3+	2	7	15	2	
VEGFR3	0	4	3	10	7	*P*<0,018
	1+	0	11	16	7	
	2+	2	8	13	2	
	3+	1	4	12	0	
IMP3	0	6	14	23	11	*P*>0,564
	1+	0	5	11	3	
	2+	1	3	6	1	
	3+	0	4	11	1	

of bevacizumab, an anti-VEGF monoclone antibody, has benefits in RCC treatment.[46] All current data indicate that the selection of the angiogenesis process, triggered by the tumor itself as a target, might be a strategic treatment option for RCC.[53]

In our study, we compared the expression level of VEGFR-2, VEGFR-3, and IMP-3 in RCC, and their relationship with the following well-known prognostic factors: tumor size, Fuhrman nuclear grade, pathologic tumor stage, distant metastasis, and lymph node involvement. We also analyzed these markers in different histological RCC subtypes. To categorize the percentage of immune reaction we divided positive staining into subgroups. There are similar approaches to categorize immunohistochemical staining based on the percentage of tumor cells with a positive reaction.[54]

The comparisons of tumor size, pathologic tumor stage, Fuhrman nuclear grade, and distant metastasis to the expression levels of VEGFR-2 and VEGFR-3 had no significant relation. The only significant association, among all the prognostic factors, was between Fuhrman nuclear grade and the expression level of VEGFR-3. We observed almost equal expression levels in our cases with different tumor size, stage, and Fuhrman grade. We also observed strong immunoreaction with both markers in normal renal tubulus epithelium adjacent to the tumor [Figures 1 and 2]. The outcome of this study indicates that VEGFR-2 and VEGFR-3 are not appropriate for routine use as markers to predict poor prognosis.

Studies in the literature have produced similar results. Bierer *et al*. worked on 166 clear cell and papillary RCC cases, examining the expression level of lymphangiogenetic factors, including VEGF-C, VEGF-D, and VEGFR-3. The only significant association they found were the increased expressions of VEGF-C and VEGF-D in papillary RCC. VEGFR-3 showed similar expression levels in both histologic subtypes. None of these markers had any significant association with TNM, progression-free survival, and overall survival.[53]

Zhonghua Zhong Liu Za Zhi *et al*. investigated the expression levels of VEGFR-1, VEGFR-2, VEGFR-3 and their prognostic significance in papillary RCC cases. The expression rates of these angiogenetic factors were as follows: 82.93% for VEGFR-1, 63.41% for VEGFR-2, and 34.15% for VEGFR-3. VEGFR-1 was not correlated with any clinicopathologic parameter. Increased VEGFR-2 expression showed significant association with tumor size, histological grade, and distant metastasis. VEGFR-3 was correlated with histological grade, lymph node involvement, and distant metastasis, but not correlated with gender, age, location, tumor size, and TNM staging. According to this study, VEGFR-2 and VEGFR-3 can serve as markers for the prognosis of papillary RCC. In other words, VEGFR-3 is a predictor of lymph node metastasis, and increased VEGFR-2 expression could be used to predict a potential blood dissemination.[37]

In this study, we observed a high rate of VEGFR-2 and VEGFR-3 immunoreaction in papillary and chromophobe RCC. Almost all cases of the two histological subtypes were stained with both markers [Table 2], but further studies with a larger number of cases are necessary to determine if these two markers are specific for both histological subtypes. The aim of this study was to determine whether or not these markers are predictors of poor prognosis. Except the relation between the Fuhrman nuclear grade and VEGFR-3 [Table 5], we did not observe any association between well-known histopathological parameters related to poor prognosis and the angiogenic receptor markers. We detected immunoreaction in histological subtypes that indicate a better outcome.[9-12] All four cases with lymph node involvement were stained with VEGFR-3. This could predict lymph node metastasis, but due to the limited number of cases with lymph node involvement in this study, further investigations are necessary. The results of VEGFR-2 and VEGFR-3 indicate that these two

markers are not appropriate to use as predictors of poor prognosis. A study with more chromophobe and papillary RCC cases, which could show these two markers to be specific for both histological subtypes, would be beneficial in cases with unknown primary origins, in addition to an immunohistochemical panel.

IMP-3 is composed of IMP-1, 2, and 3.[24] These proteins are responsible for the transcription of insulin-like growth factor 2, cell migration, and growth during the early stages of embryogenesis.[23] The IMP-3 gene is located on chromosome 7p11.2 and shows homology with KOC.[25] IMP-3 is expressed during the early stages of embryogenesis, but it is barely detectable in adult human tissues.[23,24] This protein is expressed in many organ malignancies, such as pancreas, lung, stomach, and colon, but not in normal tissues adjacent to the neoplastic tissue.[24-27] The oncofetal protein IMP-3, which is reexpressed in many organ malignancies, plays a critical role in malignant transformation and tumor progression.[28,29]

Most of the studies in the literature support IMP-3 as a poor prognostic, predictive marker for RCC. Hoffman *et al*. worked on 716 RCC cases, investigating the expression level of IMP-3, and compared these levels with prognostic parameters. According to the results of this study, they came to the conclusion that IMP-3 positive patients with the clinically localized disease are 5 times more likely to develop distant metastasis and that IMP-3 could be used as an independent marker that predicts biological aggressive behavior.[30]

Jiang *et al*. investigated that 501 primary and metastatic RCC cases to determine whether or not IMP-3 expression can predict a probable metastasis, and the prognostic value of IMP-3. They discovered that both the metastasis-free and overall survival were longer in IMP-3 negative RCC patients compared to IMP-3 positive patients. Given that, patients with IMP-3 positive RCC had remarkably lower metastasis-free survival than IMP-3 negative patients, they concluded that IMP-3 is an effective marker to detect tumors with a subsequent tendency of metastasis in patients, which indicates that an early systemic therapy would be effective.[31] In another study by Jiang *et al*., with 334 chromophobe and papillary RCC patients, they investigated the expression of IMP-3 and its significance as a metastasis predictor. IMP-3 positive patients were 10 times more likely to develop metastasis and twice as likely to die compared to patients with IMP-3 negative tumors.[32]

In this study, we investigated angiogenic receptor markers, the expression of IMP-3, and its relation with histological prognostic parameters. Statistically, there was no significant association between IMP-3 expression and any histological parameter that was related to poor prognosis. In addition to VEGFR-2 and VEGFR-3, IMP-3 also had a tendency to be expressed in chromophobe (100%) and papillary type 2 (83.3%) cases. Fifty percent of papillary type 1 cases were stained, as well as 36.5% of clear cell cases. We observed in one of two multilocular cystic, clear cell cases a positive reaction with IMP-3, which is insufficient to interpret the statistical results. The tendency of IMP-3 to be expressed in histological subtypes that behave less aggressively than conventional clear cell RCC, as well as the protein's insignificant association with prognostic parameters, suggest that IMP-3 is not appropriate for routine use to predict poor prognosis. In addition, we observed the strong reaction in renal tubulus epithelium adjacent to neoplastic tissues in all cases [Figures 1 and 2]. As discussed, the literature indicates that IMP-3 is barely detectable in adult tissues.[23,24] These findings lead us to question the oncofetal definition of IMP-3.

As stated in many studies those we have discussed previously, vascular growth factor markers and IMP-3 predicts poor prognosis based on their individual results. Given the small number of cases in our study, this represents a limitation that might be a reason for insignificant statistical results.

Conclusion

According to the results of this study, VEGFR-2, VEGFR-3, and IMP-3 have no correlation with poor prognosis and are not appropriate predictors. Each marker's tendency to be expressed in papillary and chromophobe RCC is remarkable. However, additional studies on both histologic subtypes of these markers are required to determine whether these markers are specific or not.

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Conflicts of interest

There are no conflicts of interest.

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