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Expression of growth factors and their receptors in the primary renal cell carcinoma: new data and review

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Introduction The aim of our study was to investigate expression levels and the prognostic value of multiple growth factors and their receptors in the primary tumor cells of renal cell carcinoma (RCC).
Material and methods Expression of vascular endothelial growth factor (VEGF)A, fibroblast growth factor (FGF)2, vascular endothelial growth factor receptor (VEGFR)1, VEGFR2, FGFR1, FGFR2, platelet-derived growth factor receptor (PDGFR)α, and PDGFRβ was investigated in 65 primary RCC specimens by immuhistochemical staining using the appropriate antibodies. Expression levels were evaluated by the semi-quantitative method. A search for correlations of expression levels of investigated growth factors and receptors with RCC features and patients outcomes was performed.
Results Expression of all growth factors and their receptors was detected both on the surface and in the cytoplasm of the primary tumor cells in RCC patients. The expression of all analyzed factors was interconnected. FGFR2 expression correlated with the largest number of other growth factors and receptors. A strong correlation was revealed between high expression of the studied markers, high Fuhrman grade, and advanced RCC stages. In a univariate analysis overexpression of VEGFR2 ($p < 0.0001$) and FGFR2 ($p = 0.014$) had negative influence on cancer-specific survival.
Conclusions Expression of growth factors and tyrosine kinase receptors in the primary tumor cells is strongly interconnected and associated with unfavorable features of RCC.

Key Words: renal cell carcinoma ‹› primary tumor ‹› vascular endothelial growth factor ‹› fibroblast growth factor ‹› platelet-derived growth factor

INTRODUCTION

Mitogenesis, angiogenesis, and lymphangiogenesis underlying renal cell carcinoma (RCC) progression are associated with a high level of hypoxia-induced factor (HIF) and growth factors in the tumor microenvironment. The role of the HIF family has been implicated to be a critical step in clear cell kidney tumorogenesis. Vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and their receptors such as VEGFR, FGFR and platelet-derived growth factor receptors (PDGFR) are considered to be involved in this dynamic process [1–4]. However, there are few studies describing the expression and correlation of multiple growth factors and their receptors in primary kidney tumors. Here, we report the results of investigation of HIF-dependent growth factors and receptor tyrosine kinases (RTKs) expression in the primary RCC.

MATERIAL AND METHODS

Patients

Between January 2014 and August 2016, formalin-fixed paraffin-embedded (FFPE) tissue samples

from 65 RCC patients were collected prospectively. All samples were prepared by the same standard technique. Eligible patients were 18 years of age or older. Any treatment before the surgery was prohibited. The final approval of the patient's participation in the study was taken after receiving histological confirmation of the RCC. The trial was approved by the N.N. Blokhin Russian Cancer Research Center review board, and complied with the Good Clinical Practice guidelines, the Declaration of Helsinki, and local laws. All patients provided written informed consent before any trial procedure.

Immunohistochemistry

Collected surgical specimens of primary renal tumor were studied prospectively. A routine morphological study was performed in all cases. Consecutive sections were used to reduce the variability between assays due to tumor heterogeneity. Two trained pathologists and molecular biologist independently evaluated the morphology and the expression. Nikon Eclipse 80i microscope with DS-Fi1 camera at × 10–40 magnification and Nikon Elements software v.3.0 were engaged in the study.
Expression of growth factors (VEGFA and FGF2) and RTKs (VEGFR1, VEGFR2, FGFR1, FGFR2, PDGFRα, and PDGFRβ) was investigated in RCC tissue by immunohistochemistry using the appropriate Abcam / Santa Cruz Biotech antibodies and REAL™ EnVision™ Detection System, Peroxidase/DAB+ Rabbit/Mouse (Dako). Expression levels were evaluated by the semi-quantitative method for determining the staining intensity (0, 1+, 2+ and 3+) and by calculating the relative number of stained cells, expressed as a percentage (0–100%). The value of the expression level on the immunohistochemical scale (H-score – HS) was calculated by multiplying the percentage of stained cells by an indicator of staining intensity [5].

Statistical analysis

Statistical analysis was performed with commercially available software (IBM SPSS Statistics Base v21.0 (SPSS, Inc., Chicago, IL, USA). The significance of differences between the quantitative factors was calculated with t-test for normally distributed values or with non-parametric Mann-Whitney U test. To compare the qualitative parameters, the Fisher's exact test and c2 were used taking into account nonparametric data and the Poisson distribution. Differences were recognized as significant at $p < 0.05$. To assess the relationship between factors, the Pearson correlation coefficient (r) was calculated and its significance was evaluated. To evaluate predictive efficacy of analyzed factors, receiver operating characteristic curves (ROC) were constructed, and threshold values were selected according to the coordinates of the ROC-curves. Overall survival (OS) was defined as the time from the date of surgery until the last known date alive. Cancer-specific survival (CSS) was defined as the time from the date of surgery until the last known date alive or death from RCC. Recurrence-free survival (RFS) was defined as the time from the date of radical surgery to the date of radiologically confirmed relapse or death from RCC. Progression-free survival (PFS) was defined as the time from the date of cytoreductive surgery to the date of radiologically confirmed progression of the disease or death from RCC. CSS, RFS, and PFS were analyzed with the Kaplan-Meier method, the Mantel-Haenszel log-rank test, and Cox regression model.

RESULTS

Patients

Sixty-five patients with stage pT1a-T4 N0 or N1, M0 or M1 RCC undergone nephrectomy, were included in the study (Table 1). The median age was 59.0 (33–79) years. A male-to-female ratio was 1.9:1. Most patients were diagnosed with advanced stages of the disease. pT3a-4 stages, lymph node and distant metastases were detected in 53 (81.5%), 12 (18.5%), and 45 (69.2%) patients, respectively. Fifty (76.9%) patients had tumor venous invasion.
All patients underwent nephrectomy with retroperitoneal lymphadenectomy. Thrombectomy was performed in 50 (76.9%) cases, and metastasectomy was done in 28 (43.1%) patients. Complete removal of all tumor sites was achieved in 40 (61.5%) patients. Twenty-five (39.5%) patients underwent cytoreductive surgery. RCC was confirmed histologically in all removed samples of the primary tumor, thrombi, and metastases removed. All patients undergone complete removal of all the tumors were under close follow-up, of the 25 patients undergoing cytoreductive surgery, 22 (88.0%) patients received systemic therapy.

Expression of growth factors and RTKs

Expression was detected both in the cytoplasm and on the membrane of the primary tumor cells of RCC patients. There were no differences in the assessment of expression between pathologists and molecular biologists. The highest proportion of positive tumors was detected for VEGFR2 and the low-

Table 1. *Baseline demographic and clinical characteristics*

Characteristics	Study population, N = 65
Age (years), median (range)	59.0 (33–79)
Gender, N (%)	
Male	43 (66.2)
Female	22 (33.8)
Histology, N (%)	
Clear-cell RCC	59 (90.8)
Non-clear cell RCC	6 (9.2)
RCC with sarcomatoid features	0
Fuhrman grade, N (%)	
G1-2	29 (44.6)
G3-4	36 (65.4)
Size of the primary tumor, diameter, median (range), cm	10 (2.5–26)
The primary tumor side, N (%)	
Unilateral	59 (90.8)
Bilateral	6 (9.2)
TNM stage, N (%)	
pT1-T2	12 (18.5)
pT3-T4	53 (81.5)
pT4	0
N0	53 (81.5)
N1	12 (18.5)
M0	20 (30.8)
M1	45 (69.2)
Tumor venous thrombus, N (%)	50 (76.9)
Perirenal	6 (9.2)
Subhepatic	3 (4.6)
Intrahepatic	20 (30.8)
Supradiaphragmatic	21 (32.3)
Tumor invasion of the venous wall	4 (6.2)
Tumor invasion of paranephric fat, N (%)	29 (44.6)
Metastatic sites, N (%)	
1	22 (33.8)
≥2	23 (35.4)
Sites of metastases, N (%)	
Adrenal gland	28 (43.1)
Lungs	22 (33.8)
Bones	5 (7.7)
Liver	2 (3.1)
Surgery, N (%)	
Nephrectomy, retroperitoneal lymphadenectomy	65 (100)
Thrombectomy	50 (76.9)
Metastasectomy, N (%)	28 (43.1)
Adrenalectomy	24 (36.9)
Contralateral partial nephrectomy	1 (1.5)
Bone metastasectomy	1 (1.5)
Pulmonary resection	1 (1.5)
Liver resection	1 (1.5)
Complete removal of all tumor sites, N (%)	40 (61.5)
Cytoreductive nephrectomy, N (%)	25 (39.5)
Systemic therapy following cytoreductive nephrectomy, N (%)*	22 (88.0)
immunotherapy	3 (5)
targeted therapy	19 (29)

RCC – renal cell carcinoma; N – number; TNM – The UICC TNM Classification
*from 25 patients undergone cytoreductive nephrectomy

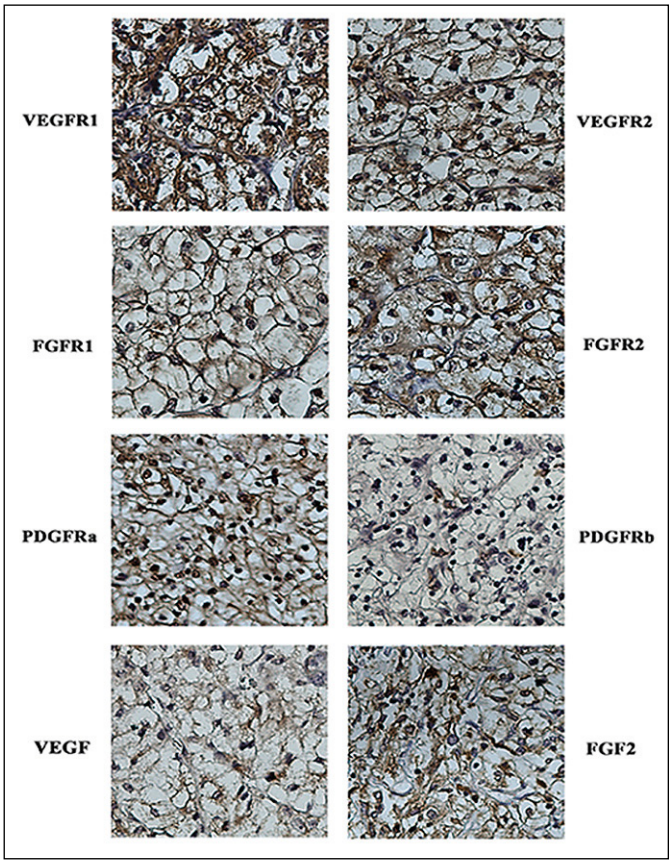


Figure 1. *Expression of growth factors and receptor tyrosine kinases in primary tumor cells in patients with kidney cancer (scale bar 50 mkm).*

VEGFR – vascular endothelial growth factor receptor; FGFR – fibroblast growth factor receptor, PDGFRa – platelet-derived growth factor receptor a; PDGFRb – platelet-derived growth factor receptor b, VEGF – vascular endothelial growth factor; FGF – fibroblast growth factor

est proportion was detected for FGFR1. Percentage of tumors with any grade expression was 55.4% (VEGFA), 55.4% (VEGFR1), 75.4% (VEGFR2), 32.3% (FGFR1), 66.2% (FGFR2), 58.5% (PDGFRα), and 44.6% (PDGFRβ) in the study. Mean expression level (± SD) was 32.4 ±5.5 HS (VEGFA), 97.2 ±10.2 HS (VEGFR1), 39.2 ±6.5 HS (VEGFR2), 7.5 ±2.2 HS (FGFR1), 46.6 ±6.3 HS (FGFR2), 62.9 ±8.4 HS (PDGFRα), and 26.6 ±5.3 HS (PDGFRβ) in primary tumors. Figure 1 demonstrates expression of growth factors and receptor tyrosine kinases in primary tumor cells in patients with kidney cancer.

Correlation analysis revealed a strong relationship between expression levels of growth factors VEGFA and FGF2 (Table 2). A high correlation was noted between the expression level of VEGFA and such RTKs as FGFR1 and FGFR2. FGF2 expression level correlated with the expression of RTKs VEGFR1, VEGFR2, FGFR2, and PDGFRα.

Table 2. *Correlations between the growth factors and tyrosine kinase receptors expression in primary tumor cells in renal cell carcinoma patients*

Expression levels	Pearson correlation (r), 2-tailed significance	Expression levels							
		VEGFA	FGF2	VEGFR1	VEGFR2	PDGFRα	PDGFRβ	FGFR1	FGFR2
VEGFA	r	–	.350**	.199	-.170	.013	.064	.257*	.287*
	Sig.		.004	.113	.175	.918	.611	.039	.021
FGF2	r	.350**	–	.420**	.296*	.246*	.099	.035	.390**
	Sig.	.004		.001	.017	.048	.433	.784	.001
VEGFR1	r	.199	.420**	–	.711**	.618**	.465**	.185	.347**
	Sig.	.113	.001		.000	.000	.000	.140	.005
VEGFR2	r	-.170	.296*	.711**	–	.484**	.381**	-.044	.193
	Sig.	.175	.017	.000		.000	.002	.731	.124
PDGFRα	r	.013	.246*	,618**	.484**	–	.521**	.187	.338**
	Sig.	.918	.048	.000	.000		.000	.135	.006
PDGFRβ	r	.064	.099	.465**	.381**	.521**	–	.391**	.583**
	Sig.	.611	.433	.000	.002	.000		.001	.000
FGFR1	r	.257*	.035	.185	-.044	.187	.391**	–	.442**
	Sig.	.039	.784	.140	.731	.135	.001		.000
FGFR2	r	.287*	.390**	.347**	.193	.338**	.583**	.442**	–
	Sig.	.021	.001	.005	.124	.006	.000	.000	

r – correlation coefficient; Sig. – significance (P-value); VEGF – vascular endothelial growth factor; FGF –fibroblast growth factor, FGFR – fibroblast growth factor receptor; VEGFR – vascular endothelial growth factor receptor; PDGFR – platelet-derived growth factor receptor
*correlation is significant at the P <0.05 (two-sided)
**correlation is significant at the P ≤0.01 level (two-sided)

Other strong correlations have been identified for pairs of VEGFR1/VEGFR2, VEGFR1/FGFR2, VEGFR1/PDGFRα, VEGFR1/PDGFRβ, VEGFR2/PDGFRα, VEGFR2/PDGFRβ, PDGFRα/PDGFRβ, PDGFRα/FGFR2, PDGFRβ/FGFR1, FGFR1/FGFR2 (p <0.05 for all). Modified Venn diagram demonstrates all possible relations between growth factors and RTKs (Figure 2).

We studied correlations of growth factors (VEGFA and FGF2) and RTKs (VEGFR1, VEGFR2; PDGFRα, PDGFRβ, FGFR1, and FGFR2) expressions with RCC tumor characteristics (laterality and size of the primary tumor, RCC histological subtype, Fuhrman grade, pT category, paranephric fat tumor invasion, tumor venous thrombosis, thrombus level, tumor invasion into the venous wall, pN and M categories, number and sites of metastases). There were no significant relationships between RCC histological subtype and the expression levels of growth factors and RTKs (p >0.05 for all growth factors and RTKs). A strong correlation of some analyzed markers overexpression with unfavorable tumor characteristics and advanced RCC was noted. Fuhrman grade had a high relationship with FGF2, VEGFR1, VEGFR2, PDGFRα, and PDGFRβ expression levels (p <0.05 for all). pT category significantly increased along with rising of VEGFR1 and VEGFR2 expression levels (p <0.05 for all). Paranephric fat invasion had

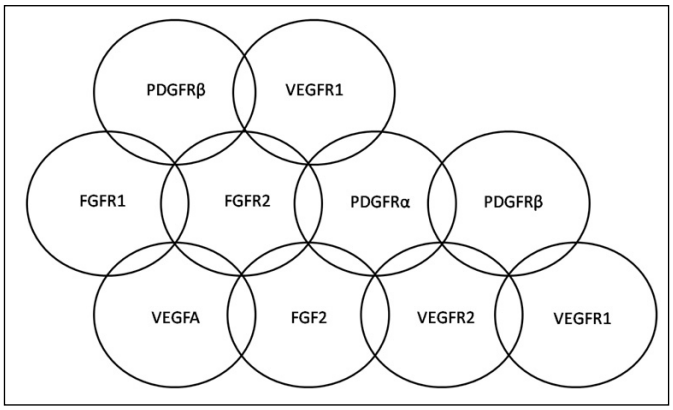


Figure 2. *Modified Venn diagram shows all possible relations between growth factors and RTKs. Circles that overlapped demonstrate the significant correlation between the factors, while circles that do not overlap do not share those trades.*
VEGF – vascular endothelial growth factor; FGF – fibroblast growth factor; FGFR – fibroblast growth factor receptor; VEGFR – vascular endothelial growth factor receptor; PDGFR – platelet-derived growth factor receptor

a strong relationship with overexpression of VEGFA, FGF2, VEGFR1, and FGFR2 ($p < 0.05$ for all). Tumor venous invasion correlated with increased expression levels of VEGFR1 and VEGFR2 ($p < 0.05$ for all). Tumor thrombus levels correlated with PDGFRβ, and venous wall tumor invasion correlated with FGFR2 ($p < 0.05$ for all). An appearance of regional and distant metastases correlated with PDGFRα and PDGFRβ expression, respectively ($p < 0.05$ for all). A strong relationship between the number of metastases and VEGFR2 expression level was obtained ($p = 0.009$). Adrenal metastases development correlated with overexpression of VEGFA, VEGFR2, FGFR1, and PDGFRβ ($p < 0.05$ for all). Liver metastases appearance correlated with overexpression of FGF2, VEGFR1, and FGFR2 ($p < 0.05$ for all).

Survival analysis

Median follow-up was 19.9 months. RCC recurrences developed in 17 (42.5%) of 40 patients following radical surgery. In all cases an appearance of distant metastases was recorded. Forty-two (64.6%) of 65 patients were still alive: 24 (36.9%) with no disease progression, 18 (27.7%) with metastases. Twenty-three (35.4%) patients died including 22 (33.8%) patients due to RCC progression, and 1 (1.5%) due to complications of surgical treatment. Median OS and CSS were 43.8 (95% confidence interval (CI), 28.7–58.9) months and 52.1 (95% CI, 36.4–67–9) months, respectively. Median RFS of 40 patients undergone radical surgery was 79.2 (95% CI, 8.1–150.5) months. Median PFS of 25 patients following cytoreductive nephrectomy was 7.4 (95% CI, 2.6–12.2) months.

Prognostic value of RCC characteristics (RCC subtype, Fuhrman grade, laterality of kidney tumor, pT category, paranephric fat tumor invasion, tumor venous thrombus, pN and M stages, number of metastases, and incomplete removal of all the tumors) was assessed for CSS, RFS, and PFS. Univariate analysis revealed a negative predictive value of Fuhrman grade G3–4, unilateral kidney tumor, pT3–T4 categories, tumor venous thrombus, multiple metastases and cytoreductive surgery for CSS ($p < 0.05$ for all). Univariate analysis detected a tendency toward RFS decrease in patients with tumor venous thrombus ($p = 0.087$) and a tendency to PFS worsening in patients with Fuhrman grade G3-4 following cytoreductive surgery ($p = 0.073$); Table 3. No independent risk factors for RFS and PFS were identified in multivariate analysis.

We analyzed value of the growth factors and RTKs expression levels for prediction of RCC recurrence following radical surgery, RCC progression after cytoreductive nephrectomy, and also for death from RCC by ROC-curves (Figure 3). VEGFA expression level had a tendency to influence on the rate of RCC progression following cytoreductive nephrectomy ($p = 0.082$). Expression levels of VEGFR2 ($p = 0.089$) and FGFR2 ($p = 0.092$) had a tendency towards a significant effect on the rate of death from RCC. FGF2 and other TKRs expression levels did not affected RCC outcomes significantly (all $p < 0.05$).

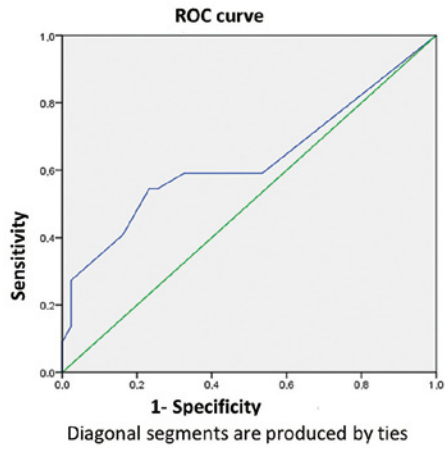
A detected threshold for VEGFA expression level was of 80 HS. There was a strong tendency for PFS worsening in patients with expression of VEGFA ≥80 comparing with those with VEGFA <80 HS (median PFS 6.7 vs. 48.8 months, respectively, $p = 0.054$).

Table 3. *Survival risk factors of cancer-specific survival of renal cell carcinoma (RCC) patients in univariate analysis*

Clinical and pathological risk factors	Median survival, months	P
Cancer-specific survival (N = 65)		
Fuhrman grade G		
G1-2	79.3	0.002
G3-4	33.8	
Tumor laterality		
Unilateral	43.7	0.043
Bilateral	Not reached	
pT category		
pT1-T2	Not reached	0.018
pT3-T4	43.8	
Tumor venous thrombosis		
No	79.3	0.008
Yes	33.8	
Metastases number		
1	79.3	0.020
>1	42.7	
Surgery		
Radical	79.3	0.010
Cytoreductive	33.8	
VEGFR2 in the primary RCC tumor		
<100 HS	59.3	<0.0001
≥100 HS	6.2	
FGFR2 in the primary RCC tumor		
<80 HS	52.1	0.014
≥80 HS	15.7	
Recurrence-free survival (N = 40)		
Tumor venous thrombosis		
No	79.3	0.087
Yes	43.4	
Progression-free survival (N = 25)		
Fuhrman grade G		
G1-2	14.6	0.073
G3-4	6.3	
VEGFA in the primary RCC tumor		
<80 HS	48.8	0.054
≥80 HS	6.7	

N – number; HS – H-score; VEGFR – vascular endothelial growth factor receptor; VEGFA – vascular endothelial growth factor A; FGFR2 – fibroblast growth factor receptor 2

A



Area under the curve

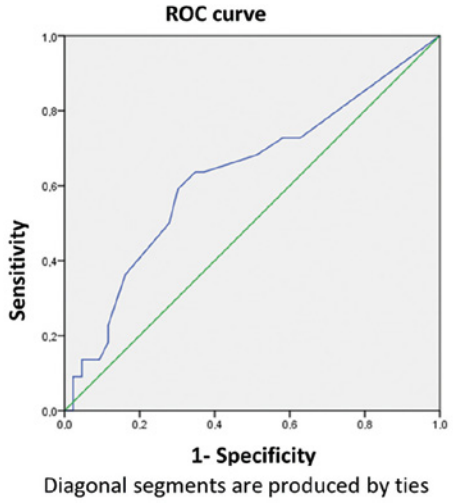
Area	Std. error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Int.	
			Lower board	Lower board
0.629	0.081	0.089	0.471	0.788

The test result variable: higher vascular endothelial growth factor receptor (VEGFR)2 has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased

[a] Under the nonparametric assumption

[b] Null hypothesis: true area = 0

B



Area under the curve

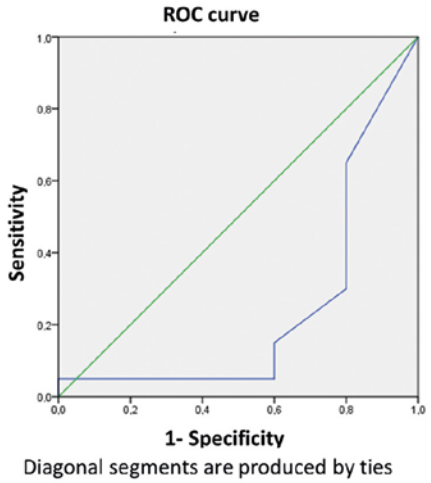
Area	Std. error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Int.	
			Lower board	Lower board
0.628	0.076	0.092	0.479	0.778

The test result variable: higher FGFR2 has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.

[a] Under the nonparametric assumption.

[b] Null hypothesis: true area = 0.5

C



Area under the curve

Area	Std. error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Int.	
			Lower board	Lower board
0.294	0.115	0.081	0.069	0.520

The test result variable: lower vascular endothelial growth factor (VEGF) has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.

[a] Under the nonparametric assumption

[b] Null hypothesis: true area = 0.5

Figure 3. *Value of the growth factors and receptor tyrosine kinases (RTKs) expression levels for prediction of renal cell carcinoma (RCC) recurrence following radical surgery, RCC progression after cytoreductive nephrectomy, and also for death from RCC by ROC-curves.* **A.** *Testing variable: higher vascular endothelial growth factor receptor (VEGFR)2 = higher risk of renal cell carcinoma (RCC) – associated death.* **B.** *Testing variable: higher fibroblast growth factor receptor (FGFR)2 = higher risk of RCC-associated death.* **C.** *Testing variable: lower vascular endothelial growth factor receptor (VEGFR) = higher risk of renal cell carcinoma (RCC) progression after cytoreductive nephrectomy.*

A detected threshold for VEGFR2 expression level was of 100 HS. A significant decrease of median CSS from 59.3 to 6.2 months was noted in patients with expression of VEGFR2 ≥100 HS compared with those with VEGFR2 expression <100 HS (p <0.0001). A detected threshold for FGFR2 expression level was of 80 HS. Expression of FGFR2 ≥80 HS was associated with significant worsening of CSS comparing with lower FGFR2 production (15.7 vs. 52.1 months, respectively; p = 0.014).

Fuhrman grade, kidney tumor laterality, tumor venous thrombus, not complete removal of all tumor sites as well as VEGFR2 and FGFR2 expression levels were included into multivariate analysis of CSS. Fuhrman grade G3–4 (hazard ratio (HR), 1.7 (95% CI, 1.0–3.1); p = 0.072), tumor thrombosis (HR 6.2 (95% CI, 0.8–49.1); p = 0.082), and expression of VEGFR2 ≥100 HS (HR 2.4 (95% CI, 0.96.5); p = 0.081) had a non-significant impact on CSS.

DISCUSSION

RCC is characterized by overexpression of HIF and activation of the underlying signaling pathways involved in mitogenesis and angiogenesis [6]. Our study was designed to evaluate the expression of growth factors and RTKs that are directly regulated by HIF at one and the same level of signal transmission, and possibly interrelated with each other. We selected the molecules with the highest proven proangiogenic and proliferative activity such as VEGFA, FGF2, VEGFR1, VEGFR2, FGFR1, FGFR2, PDGFRα, and PDGFRβ.

In our study, we collected paired surgical RCC specimens and performed an immunohistochemical semi-quantitative assessment of protein expression in tumor cells, including quantification of both the number of stained cells and the degree of staining. This methodology is well reproducible and has been proven in early studies [5]. It is currently widely used by other researchers [7, 8].

As was expected, we found expression of all investigated growth factors and RTKs on the surface and in cytoplasm of primary tumor cells. The rate and intensity of growth factors expression were comparable. RCC cells most actively expressed VEGFR1 and FGFR2, modestly expressed VEGFR2 and PDGFRα. Expression of PDGFRβ and FGFR1 was the lowest.

More than half of the tumors were VEGFA, VEGFR1, and VEGFR2 positive. High expression of VEGF family members was described in other publications (VEGFA, 80.6% [9], VEGFR1, 46.8% [10], and VEGFR2, 62.4% [11]). However, it is difficult to compare our data with the results of other researchers due to applying of different antibodies and expression level assessment methods in various studies [12, 13]. We found a high correlation of VEGFA expression with some RCC characteristics, which were proved to be poor prognostic factors in large series, including tumor invasion of paranephric fat and adrenal metastasis [14]. Other authors also noted that the relationship between VEGF expression and tumor features could be precursors of 'aggressive' RCC 'duration'. For example, Minardi et al. revealed a correlation of VEGF expression with RCC stage, Fuhrman grade G3-4, and the prognostic group [7, 8]. In contrast, Tsuchiya et al. did not detect the relationship of VEGF expression with clinical and morphological characteristics of the primary tumor in 23 RCC cases [15].

In our study, we failed to reveal if VEGF expression level influenced survival. Similar results were obtained in other studies [8, 15]. In univariate analysis, Jacobsen et al. found that VEGF expression correlated with survival, however, this prognostic information was lost in multivariate analysis [12]. Differences in overall survival (OS) reached a statistically significant level in the Minardi study [7] showing longer OS in patients with lower VEGF expression.

Otherwise, we found a tendency toward a PFS decrease in patients with VEGFA expression of <80 HS compared to VEGFA ≥80 HS. The inverse correlation between VEGF levels and PFS in our series may be explained by both small cases number and a 'robbery' effect, when tumors with high VEGFR expression bind VEGF before it can be secreted. We consider the absence of a strong correlation between the levels of VEGF, VEGFR1, and VEGFR2 to be an indirect confirmation of this hypothesis. It should be noted that Kluger et al. made similar conclusions [16].

We found a strong relationship of VEGFR1 and VEGFR2 expression with a high Fuhrman grade and advanced RCC (pT3-4 stages, paranephric fat tumor invasion, tumor venous invasion, multiple metastases, and adrenal metastasis). Data on the prognostic value of VEGF receptors obtained by other authors differ significantly. Lkhagvadorj et al. noted that higher expression of VEGFR1 correlated with a low Fuhrman grade and the absence of renal sinus tumor invasion in 126 samples of clear cell RCC [10]. Jacobsen et al. analyzing data of 84 patients showed higher expression of VEGFR2 in the early stages of RCC [12]. Eronat et al. did not obtain a correlation of VEGFR2 with the size, histological subtype, Fuhrman grade, pT stage, regional and distant metastases in 48 RCC patients [17]. In contrast, Kluger et al. showed that VEGFR expression correlated with Fuhrman grade in 334 RCC samples [16].

In our multivariate analysis, VEGFR2 expression ≥100 HS tended to have an independent influence on

CSS of patients along with Fuhrman grade G3-4 and tumor venous thrombosis. Lkhagvadorj et al. noted that the higher expression of VEGFR1 did not affect OS in 126 RCC patients [10]. On the other hand, in a series of 334 samples, high expression of VEGFR in tumor cells was an independent OS risk factor [16]. We detected FGF2 staining in 60% of RCC samples and found higher FGFR2 expression rate (66.2%) comparing with FGFR1 (32.3%). Horstmann et al. recorded FGF2 staining in ≥5% of RCC cells in 37.7% of specimens from the marginal, and in 28.2% of specimens from the central zone of 259 tumors [18]. On other note, Tsimafeyeu et al. described a high expression of FGFR1 in clear-cell RCC (n = 100) [19]. FGFR1 expression was observed in 98% of primary tumors samples and FGFR2 staining was recorded much less frequently, in 4% of cases [19]. In a small series by Lacovelli et al. the expression of FGFR1 and FGFR2 was unexpectedly low: staining of ≥5% of tumor cells occurred only in 16% and 30% of 36 RCC samples, respectively [20]. It is not correct to compare the results of different studies, but RCC cells seem to be characterized with high expression of FGF / FGFR.

Prognostic value of FGF / FGFR in RCC patients has not been studied, but this signal axis activation is believed to have a negative effect on survival. In our series, expression of FGFR2 ≥80 HS adversely influenced CSS, however, in multivariate analysis the prognostic value of this marker was lost. Several studies demonstrated that increased FGF / FGFR expression affected negatively on the prognosis of RCC patients. High expression of FGF2 in the tumor growth margin was an independent risk factor for OS along with high Fuhrman grade and N+ category [18]. Lacovelli et al. revealed the correlation of low FGFR2 expression with PFS increase during targeted antiangiogenic therapy [20]. Ho et al. noted a significant PFS decrease in 40 metastatic RCC patients who had overexpression of FGFR1 and FGFR2 and received sorafenib as the third-line therapy [21]. We found expression of PDGFRα in 58.5% and PDGFRβ in 44.6% of tumors. Sulzbacher et al. performed an immunohistochemical study of 112 surgical RCC samples and revealed PDGFRα expression in 87.5% of cases [22]. Song et al. detected PDGFRβ overexpression in 32.8% of 1,423 RCC specimens [11]. Cumpănas et al. found the expression of PDGFRβ in one third of 50 RCC samples, while the expression level was low in all stained tissue samples [23].

We obtained a strong correlation of PDGFRα and PDGFRβ expression with a high Fuhrman grade and advanced RCC (the extent of tumor thrombus, pN+ and M+ stages, including adrenal metastases). Similarly, Sulzbacher et al. noted, that PDGFRα overexpression (staining >38.8% of 500 cells) correlated with Fuhrman grade G3-4 [22]. Frödinet al. revealed perivascular expression of PDGFRβ to be correlated with high stage and Fuhrman grade G3-4 in 314 RCC specimens [24]. In contrast, Tawfik et al. failed to demonstrate any relationship between PDGFRα expression and characteristics of 62 RCC surgical specimens [25].

We did not reveal the effect of PDGFR expression on the survival of RCC patients. In contrast, Tawfik et al. recognized PDGFRα expression as an independent risk factor for OS in 62 RCC patients [26]. Frödinet al. found that PDGFRβ overexpression was associated with a significant decrease of OS in 314 RCC patients [24]. According to Sulzbacher et al., PDGFRα overexpression correlated with poor RFS in univariate analysis of 112 RCC specimens, but lost its significance in multivariate analysis [22]. Expression of PDGFRα was an independent PFS risk factor in the study by Kusuda Y [26].

The most interesting result obtained in our study was the detection of a strong cross-relationship between the expression of different signal chains. FGFR2 expression correlated with the largest number of factors. There was a strong relationship between VEGFA and FGF2 expression levels. In addition, we demonstrated the correlation of growth factor VEGFA expression with receptors of FGF as well as growth factor FGF2 expression with receptors of VEGF and PDGF. VEGFR / FGFR / PDGFR tyrosine kinase expression levels also strongly correlated with each other, with almost complete cross-correlation between all signal transmission chains.

In conclusion, our study assumes that the relationship between the expression of angiogenic growth factors and RTKs can indicate their coordinated contribution to the complex and multi-stage process of RCC development. This hypothesis is supported by data from previous studies. It was shown that FGF / FGFR pair leads to the tumor cells proliferation, degradation of the intercellular matrix, release of growth factors, and also promotes the reproduction and migration of endothelial cells. VEGF / VEGFR pair plays an important role in neoangiogenesis and angioinvasion at the endothelial cell level; PDGFRs impact on cancer cells stimulation, control of tumor interstitial pressure, and attract pericytes to the forming vessels [27, 28, 29].

Moreover, co-expression of different growth factors and RTKs could be explained as pro-angiogenic cross-signaling in RCC. In 2007, Ball et al. showed that VEGFA stimulates the expression of PDGFRα, PDGFRβ and, in addition, binds to both types of receptors, which makes VEGFA a potential regulator for attracting both endothelial and perivascular cells

[30]. A cross-relationship between the transmission of pro-angiogenic FGF and VEGF signals has been proven in cell lines [28]. In an experimental glioma model, PDGF has been shown to enhance angiogenesis by stimulating VEGF expression in tumor-specific endothelial cells and by attracting pericytes [31]. Similar results were obtained by Tsimafeyeu et al. [32].

We can also suppose that only an individually balanced combination of HIF-dependent growth factors and RTKs can promote tumor activity, thus, no study determined exact factors or even combinations of factors that could be predictors of RCC prognosis. Finally, in our series, VEGFA expression did not correlate with VEGFR, as well as FGF2 expression was not related to FGFR1. We suggest that it may be due to a 'robbery' effect when an excessive amount of RTKs specifically and quickly binds growth factors before staining.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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