Int J Clin Exp Pathol 2014;7(11):7681-7689
www.ijcep.com /ISSN:1936-2625/IJCEP0002298

Original Article

VEGF/VEGFR2 and PDGF-B/PDGFR-β expression in non-metastatic renal cell carcinoma: a retrospective study in 1,091 consecutive patients

Sang Hoon Song[1], In Gab Jeong[1], Dalsan You[1], Jun Hyuk Hong[1], Bumsik Hong[1], Cheryn Song[1], Woon Yong Jung[2], Young Mee Cho[2], Hanjong Ahn[1], Choung-Soo Kim[1]

[1]Department of Urology, [2]Department of Pathology, Asan Medical Center, University of Ulsan College of Medicine, Pungnap-Dong 388-1, Songpa-Gu, Seoul, Korea

Received September 3, 2014; Accepted October 17, 2014; Epub October 15, 2014; Published November 1, 2014

Abstract: Purpose: We aimed to investigate the correlations between the expression of VEGF, PDGF-B, and their receptors (VEGFR2 and PDGFR-β) with pathologic stage or cell type in non-metastatic renal cell carcinoma. Materials and methods: VEGF, VEGFR2, PDGF-B, and PDGFR-β protein expression were evaluated immunohistochemically in prospectively collected 1,423 tumour samples obtained during radical or partial nephrectomy at a tertiary referral center. Intensity of expression was quantified on a scale of 0 to 3, and was compared among renal cell carcinoma cell types. Results: The study cohort consisted of 1,091 patients, of mean age 54 years, including 968 (88.7%) with clear cell, 82 (7.5%) with papillary, 31 (2.8%) with chromophobe, 4 (0.4%) with unclassified, and 6 (0.5%) with other types of renal cell carcinoma. VEGF expression increased with higher T and N stage and Fuhrman nuclear grade. PDGFR-β expression was highest in clear cell renal cell carcinoma, whereas VEGF and PDGF-B expression were highest in papillary renal cell carcinoma. After adjusting for T stage and Fuhrman nuclear grade using multivariate logistic regression analysis, VEGF (OR = 3.57, $P < 0.001$), VEGFR2 (OR = 1.82, $P = 0.017$), and PDGF-B (OR = 2.46, $P = 0.019$) expression were significantly greater in papillary than in clear cell type. Conclusions: Our results indicate that the cytoplasmic expression of VEGF, VEGFR2, PDGF-B, and PDGFR-β in RCC tumour cells is different in various pathologic stage and cell type. Notably, VEGF and PDGF-B expression are higher in papillary than in clear cell renal cell carcinoma. Further studies using quantitative measurement of proangiogenic factors in tumour cell are needed.

Keywords: Carcinoma, renal cell, vascular endothelial growth factor A, vascular endothelial growth factor receptor-2

Introduction

The American Cancer Society has estimated that, during 2012, 64,770 patients were newly diagnosed with kidney cancer, constituting 3.9% of all cancer patients, and that over 13,000 patients died of this disease in the U.S. [1]. In Korea, 3,435 individuals were newly diagnosed with kidney cancer (1.7%) in 2009. RCC is the most common type of kidney cancer in adults. RCC is heterogeneous, with each histological cell type having different genetics, biology, and clinical behaviour [2]. Based on these characteristics, RCC has been divided into several subtypes, including clear cell RCC, papillary RCC, chromophobe RCC, collecting duct RCC, and MITF/TFE family translocation carcinoma [3]. Until recently, RCC was the most lethal urologic cancer, with approximately 40% of patients ultimately dying of disease progression [4]. Thus, it is of great concern to elucidate the molecular biology of each RCC subtype.

Hypoxia, resulting from tumour growth beyond a critical size, has been found to regulate a complex cascade of genes, including the proangiogenic growth factors VEGF and PDGF [5]. VEGF and its receptors play a pivotal role in physiologic and pathologic angiogenesis, which is essential for tumour progression and the development of metastasis [6]. PDGF is a growth factor extensively involved in multidimensional cellular dynamics and in tumorigenesis [7]. Molecules directed against VEGF/

Table 1. Patients' demographic and tumour-specific characteristics

	N (%)
Age (years: mean ± SD) (%)	54.81 ± 0.36
< 40	129 (11.8)
40-49	229 (21.0)
50-59	334 (30.6)
60-69	259 (23.7)
≥ 70	140 (12.8)
Sex	
Male	781 (71.6)
Female	310 (28.4)
BMI, kg/m^2 (514 patients)	
Underweight (< 18.5)	10 (1.9)
Acceptable (18.5 < 23)	137 (26.7)
Increased risk (23 < 27.5)	273 (53.1)
High risk (≥ 27.5)	94 (18.3)
Operative method (n = 466)	
Open	216 (46.3)
Pure laparoscopic	94 (20.1)
HALS	59 (12.6)
Robot-assisted laparoscopic	97 (20.8)
Radical	233 (48.9)
Partial	243 (51.0)
Tumour size (cm: mean ± SD)	4.37 ± 2.94
T stage (pT)	
T1a	621 (57.4)
T1b	250 (23.1)
T2a	57 (5.3)
T2b	59 (5.5)
T3a	88 (8.1)
T3b	4 (0.4)
T3c	0 (0.0)
T4	2 (0.2)
N stage (pN)	
N0	198 (18.4)
N1	14 (1.30)
Nx	879 (80.2)
Cell type	
Clear cell	968 (88.7)
Papillary, type 1	60 (5.5)
Papillary, type 2	22 (2.0)
Chromophobe	31 (2.8)
Unclassified	4 (0.4)
Multilocular cystic	1 (0.1)
Xp11 translocation	3 (0.3)
Others	2 (0.1)

Abbreviations: HALS = hand assisted laparoscopic surgery; SD = standard deviation.

PDGF protein and VEGF/PDGF receptor signaling are available for RCC treatment. To date, seven agents have been approved by the U.S. FDA for the treatment of advanced RCC, including the small molecule tyrosine kinase inhibitors sunitinib, sorafenib, pazopanib, temsirolimus, everolimus, and axitinib, and the monoclonal antibody bevacizumab, in combination with interferon-α [8, 9].

VEGF and its receptors have been associated with RCC tumour stage and survival, with different RCC types having different expression patterns of VEGF and VEGF receptor mRNAs [10]. It is unclear, however, whether the expression of these tumour molecules differ according to RCC cell types in patients with non-metastatic disease. We have therefore evaluated the correlations between the expression of VEGF, PDGF-B, and their receptors (VEGFR2 and PDGFR-β) with pathologic stage or cellular classification in patients with non-metastatic RCC.

Materials and methods

Patients and tumours

Tumour samples were collected from surgical specimens of patients, who underwent radical or partial nephrectomy for RCC between January, 2008, and March, 2012. All patients underwent a physical examination, chest radiography, and CT, with tumours staged according to the 2010 TNM classification system and histopathologically graded according to Skinner et al., based on worst grade. RCC type was classified according to the 2004 WHO classification [11]. The report was prepared following the guidelines of the Institutional Review Board of Asan Medical Center (IRB No. S2013-1479-0001).

Immunohistochemistry

IHC of the tumour tissue samples was performed by using the autoimmunostainer Ventana XT (Roche, CA, USA) with Optiview Dab Detection Kit (Roche, CA, USA) according to the manufacturer's instructions and using the reagents supplied with the kit. Formalin-fixed, paraffin-embedded sections of 4 μm were mounted on silanized charged slides and allowed to dry for 10 min at room temperature, followed by 20 min in an incubator at 65 C. Then, sections were performed by Heat Induced Epitope Retrieval (Cell Conditionning 1) for 24 min and incubated for 16 min with antibodies to VEGF (anti-Mouse, dilution 1:500; Pharmingen, NJ, USA), VEGFR2 (anti-Rabbit, dilution

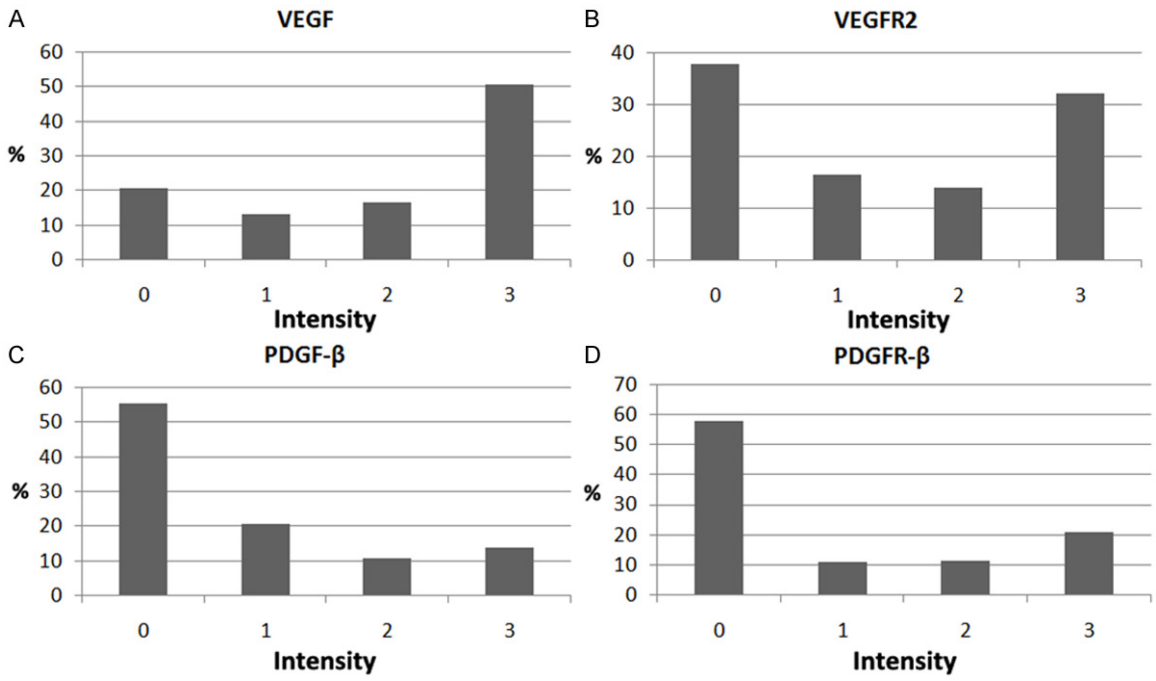

Figure 1. Distribution of staining intensity in each staining of (A) VEGF, (B) VEGFR2, (C) PDGF-B, and (D) PDGFR-β.

1:100; Cell Signaling Technology, MA, USA), PDGF-B (anti-Rabbit, dilution 1:400; Bioworld, MN, USA), and PDGFR-β (anti-Rabbit, dilution 1:200; Eptomics, CA, USA) in the autoimmunostainer. All sections were counterstained with haematoxylin. Tumour sections with documented positivity were used as positive controls. Negative controls included omitting the primary antibody and replacing the primary antibody with an irrelevant antibody.

Assessment of expression

IHC results were independently evaluated by two specialised pathologists, blinded to each patient's clinical data. A semiquantitative scoring system was used, based on staining intensity (0, negative; 1, weak; 2, intermediate; 3, strong), which was corresponded to the percentage of positive stained cells (0, 0%; 1, < 25% positive; 2, 26-50% positive; 3, ≥ 50% positive). A score ≥ 1 represented a positive immunohistochemical identification of a marker.

Statistical analysis

Correlations between protein expression and oncologic parameters, especially the pathologic classification according to RCC cell, were analysed statistically using the Mann-Whitney U, Kruskal-Wallis, and Spearman rank correlation tests. Logistic regression was used for multivariate analysis. All statistical tests were two-sided, with the level of significance set at 0.05.

Results

A total of 1,423 patients diagnosed with RCC underwent radical or partial nephrectomy during the study period. After excluding 292 patients with pathologically proven tumours other than RCC or 50 with metastasis, 1,091 patients of mean age 54.8 years were involved in the analysis. Patients' demographic and tumour-specific characteristics are listed in **Table 1**. Of these samples, 79.6%, 62.4%, 45.0%, and 42.4% were positive for VEGF, VEGFR2, PDGF-B, and PDGFR-β, respectively (**Figure 1**).

Cell type and immunohistochemical staining intensity

Of the 1,091 patients, 962 (88.8%) had clear cell, 60 (5.5%) had papillary type 1, 20 (2.0%) had papillary type 2, 31 (2.9%) had chromophobe, 4 (0.3%) had unclassified, and 6 (0.5%) had other types of RCC. The representative IHC results of VEGF and VEGFR2 in clear cell, papillary, and chromophobe RCC are shown in **Figure**

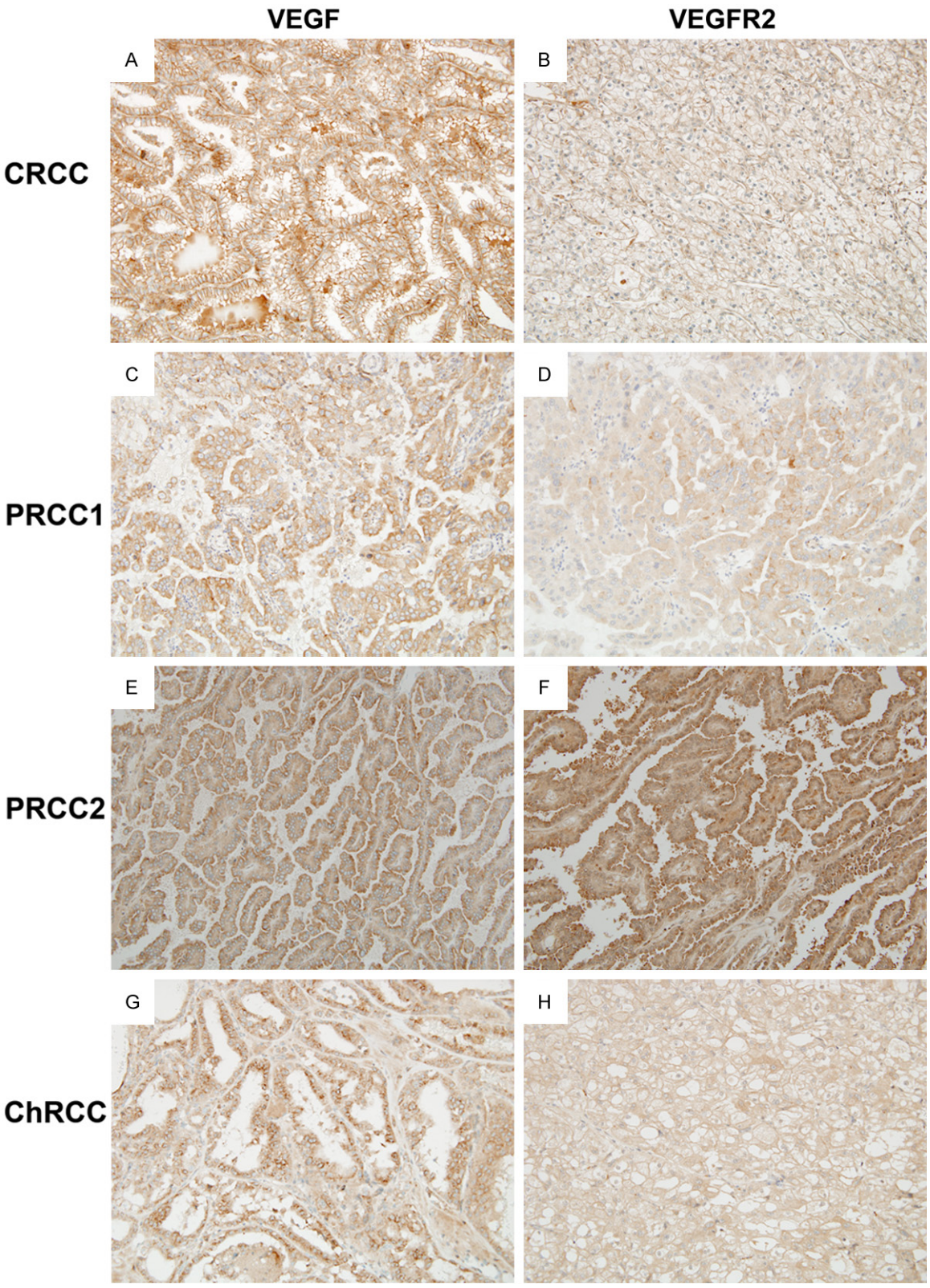

Figure 2. Representative immunohistochemical localization of VEGF, and VEGFR2 in clear cell, papillary, and chromophobe renal cell carcinoma. Expression of VEGF is localized to cellular membrane and cytoplasm of tumour cells and vascular smooth muscle cells (A, C, E, G) (× 200). Expression of VEGFR2 is more prominent in cellular membrane than in cytoplasm of tumour cells (B, D, F, H) (× 200). VEGF = vascular endothelial growth factor type

2, VEGFR2 = vascular endothelial growth factor receptor type 2, CRCC = clear cell renal cell carcinoma, PRCC1 = papillary renal cell carcinoma type 1, PRCC2 = papillary renal cell carcinoma type 2, ChRCC = chromophobe renal cell carcinoma.

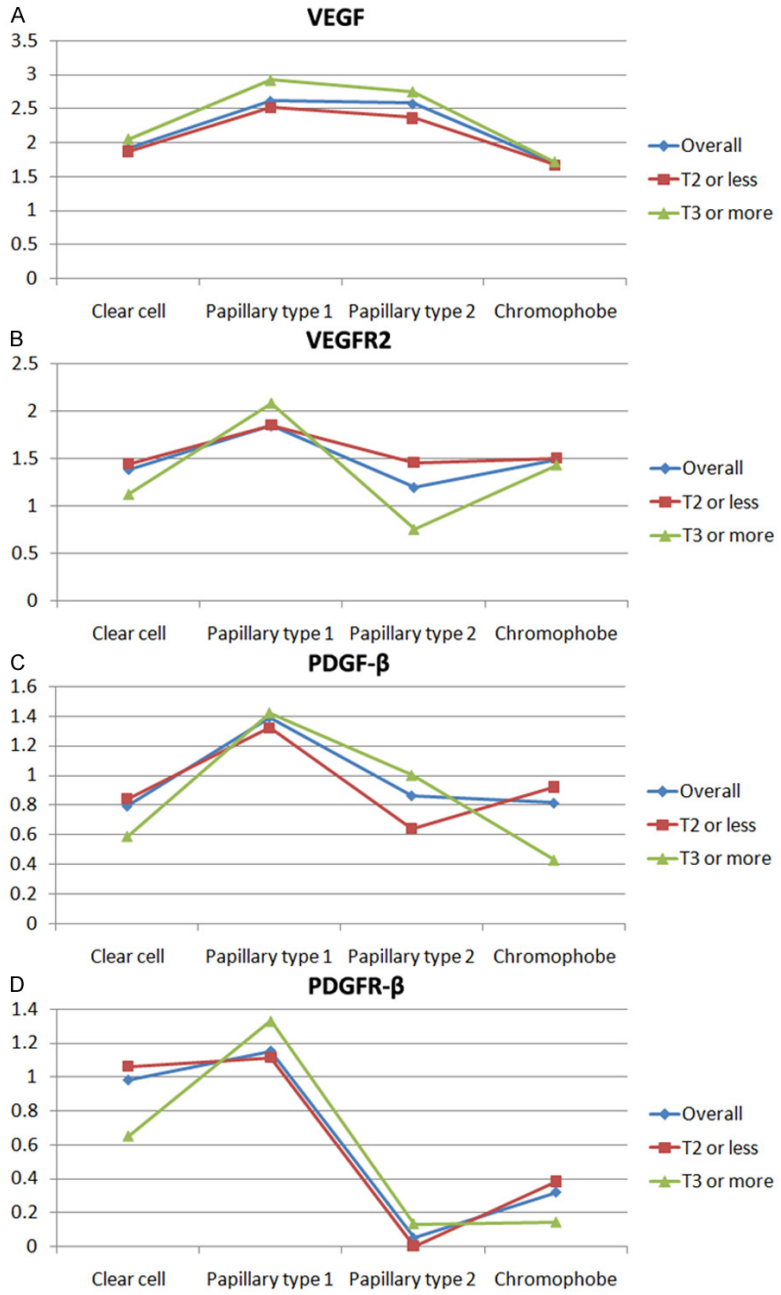

Figure 3. Broken line graph shows that the intensity of immunohistochemical staining of VEGF, VEGFR2, PDGF-B, PDGFR-β are different according to the cell type of renal cell carcinoma: A. Staining intensity for VEGF was lower in chromophobe than in papillary types 1 and 2 but was similar to that in clear cell RCC; B. VEGFR2; C. PDGF-B; D. PDGFR-β staining was higher in clear cell and papillary type 1 than in papillary type 2 or chromophobe RCC.

2. The mean staining intensities of VEGF, VEGFR2, PDGF-B, and PDGFR-β in clear cell, papillary types 1 and 2, and chromophobe RCC are shown in **Figure 3**. The staining intensities of VEGF ($P < 0.001$), VEGFR2 ($P = 0.010$), and PDGF-B ($P < 0.001$) were each significantly greater in papillary type 1 than in clear cell RCC (**Table 2**), although their mean tumour sizes were similar (4.27 cm vs. 4.90 cm, $P = 0.280$). Although the group with papillary type 2 RCC included a higher percentage of patients with T3 or higher stage, the differences in distribution of stage by cell types were not statistically significant ($P = 0.053$, Fisher's exact test). In patients with higher pathologic stage (pT3 or more), the differences in staining intensity between clear cell and papillary type 1 were more obvious (**Figure 3**). Staining intensity for VEGF was lower in chromophobe than in papillary types 1 ($P = 0.002$) and 2 ($P = 0.041$) but was similar to that in clear cell ($P = 0.697$) RCC. PDGFR-β staining was higher in clear cell and papillary type 1 than in papillary type 2 or chromophobe RCC.

Fuhrman's nuclear grade and immunohistochemical staining intensity

Fuhrman's nuclear grade was associated with VEGF, VEGFR2, and PDGFR-β expression in clear cell RCC (**Figure 4**). VEGF expression was higher in clear cell RCCs of Fuhrman's grade 4 than in grades 1 or 2, and VEGFR-2 expression was higher in grades 3 ($P < 0.001$) and 4 ($P = 0.040$) than in grade 2. Although PDGF-B expression did not differ significantly by nuclear grade, PDGFR-β expression was significantly

Table 2. Expression of VEGF2, VEGFR2, PDGF-B, and PDGFR-β in different cellular subtypes of RCC

	VEGF2		*P* value	VEGFR2		*P* value	PDGF-B		*P* value	PDGFR-β		*P* value
	Weak	Strong		Weak	Strong		Weak	Strong		Weak	Strong	
Cell type, n (%)			< 0.001			0.166			< 0.001			0.002
Clear cell	340 (35.3)	622 (64.7)		532 (55.3)	430 (44.7)		747 (77.7)	215 (22.3)		646 (67.2)	316 (32.8)	
Papillary	9 (11.3)	71 (88.8)		34 (42.5)	46 (54.5)		44 (55.0)	36 (45.0)		58 (72.5)	22 (27.5)	
Chromophobe	14 (45.2)	17 (54.8)		16 (51.6)	15 (48.4)		22 (71.0)	9 (29.0)		28 (90.3)	3 (9.7)	
Others	1 (10.0)	9 (90.0)		5 (50.0)	5 (50.0)		8 (80.0)	2 (20.0)		10 (100.0)	0 (0.0)	

Abbreviations: VEGF2 = vascular endothelial growth factor type 2; VEGFR2 = vascular endothelial growth factor receptor type.

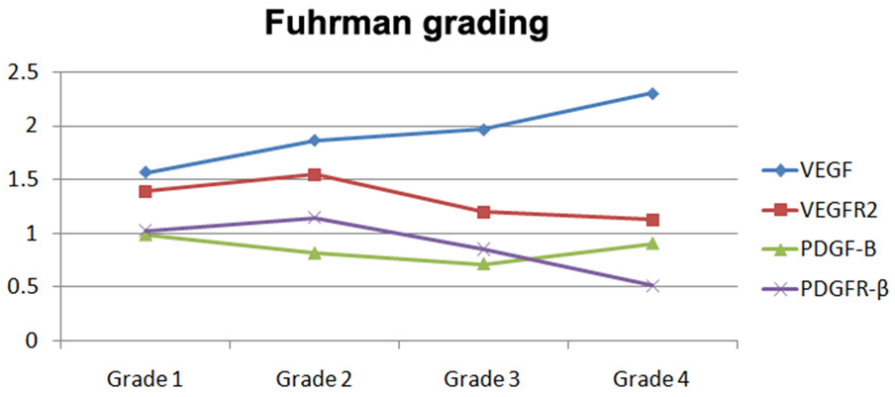

Figure 4. Fuhrman's nuclear grading and staining intensity in each staining of VEGF, VEGFR2, PDGF-B, PDGFR-β in clear cell renal cell carcinoma. VEGF expression was higher in clear cell RCCs of Fuhrman's grade 4 than in grades 1 or 2, and VEGFR-2 expression was higher in grades 3 and 4 than in grade 2. Although PDGF-B expression did not differ significantly by nuclear grade, PDGFR-β expression was significantly lower in grades 3 and 4 than in grade 2.

lower in grades 3 ($P = 0.003$) and 4 ($P < 0.001$) than in grade 2. Expression was not associated with nuclear grade in papillary RCC types 1 and 2, except that only VEGFR2 staining was higher in grade 4 than in grade 2 papillary RCC type 2 ($P = 0.030$).

Immunohistochemical staining intensity after adjusting for confounders

Only VEGF expression was significantly higher as T or N stage increased. After adjusting the T stage and Fuhrman nuclear grade using multivariate logistic regression analysis, papillary RCC showed significantly stronger expression of VEGF (OR = 3.57, 95% CI 1.74-7.32, $P < 0.001$), VEGFR2 (OR = 1.82, 95% CI 1.11-2.99, $P = 0.017$), and PDGF-β (OR = 2.46, 95% CI 1.49-4.06, $P = 0.019$) compared to clear cell RCC.

Discussion

Among the five major subtypes of RCC, clear cell, papillary types 1 and 2, chromophobe, collecting duct, and unclassified, clear cell is the most frequent [2]. Clear cell RCCs are highly vascular tumours, with high expression of VEGF, VEGFR, and PDGFR [12]. Although VEGF and PDGF are proangiogenic growth factors believed to play critical roles in the development and progression of clear cell RCCs [13], less is known about the expression of proangiogenic factors in other RCC cell types. Moreover, few studies have assayed VEGF staining by IHC and correlated the results with clinicopathological findings and different RCC subtypes [14]. These results showed that VEGF/VEGFR2 and PDGF-B/PDGFR-β expression are significantly associated with pathological stage or histopathological tumour cell type in patients with non-metastatic RCC. Notably, this study involved the largest number of prospectively collected pathologic specimens to date to assess differences in VEGF/VEGFR2 and PDGF-B/PDGFR-β staining by IHC in RCCs other than clear cell and papillary in a single centre.

We found that the levels of expression of VEGF, VEGFR2, and PDGF-β protein were lower in clear cell than in papillary RCC. Previous studies comparing VEGF expression according to RCC cell type have yielded conflicting results. For example, the expression of VEGF and VEGFR1 mRNA was reported lower in papillary than in clear cell RCC [15], whereas a subsequent study from the same group showed higher VEGF mRNA levels in clear cell than in papil-

lary RCC, with only marginal significance (P = 0.050). Moreover, the sample size was relatively small, and protein expression was not assessed. Other studies reported no apparent correlation between VEGF expression pattern and histological grade or type [14, 16]. Our findings are in good agreement with those of an earlier study, which reported that VEGF protein expression, as assessed by IHC, was significantly lower in patients with clear cell than papillary RCC (P < 0.010), but did not differ between clear cell and other RCC subtypes [17]. Interestingly, 77.4-95.5% of our samples were positive for VEGF, compared with 12.5-28.6% of samples in the previous study.

VEGF is a proangiogenic factor associated with the cascade of hypoxia inducible tumour growth [5]. Generally, RCCs are characterised as having abundant blood vessels, with extensive haemorrhage, necrosis, and tumour thrombi associated with angiogenic activity [18]. VEGF is a potent promoter of tumour angiogenesis, with VEGF mRNA and protein levels higher in RCC than in normal kidney cortex [16, 19]. We expected that VEGF expression would be elevated in clear cell RCCs with VHL mutations, despite normal oxygenation conditions. VHL mutations are rarely observed in papillary and chromophobe RCCs. However, we found VEGF and PDGF-B expression were higher in papillary than in other RCC subtypes, whereas tumour size did not differ in clear cell and papillary RCCs. This result indicates that VEGF expression could be promoted without relating to hypoxia or VHL mutated pathways.

We assayed only one of the three structurally related VEGF receptors, VEGFR2. Although VEGFR1 plays a role in tumour progression and binds VEGFA with at least 10-fold higher-affinity than does VEGFR2, VEGFR1 is expressed at low levels in RCC [20]. The expression of VEGFR3 in tumour cells is unclear [21], although this receptor and its ligands are involved in tumour lymphangiogenesis rather than angiogenesis [22]. VEGFR2 is the major mediator of VEGFA-driven responses in endothelial cells and a pivotal signal transducer during both physiologic and pathologic angiogenesis [23]. Moreover, VEGFR2 expression is significantly upregulated in the vascular endothelium of most common human solid tumours [20].

The PDGF family consists of four different isoforms: PDGF-A, -B, -C, and -D. PDGF receptors are dimers consisting of two different receptor chains (PDGFR-α and -β) [24]. PDGF-A and -B and PDGFR-α and -β are expressed in postnatal human and rodent kidneys [25], and PDGFR-β, but not PDGFR-α, has been reported to be expressed in epithelial cells of human glomeruli [26]. PDGFR-β is expressed in glomerular parietal epithelial cells, vascular smooth muscle cells, and the interstitium of healthy human kidneys [26, 27]. In experimental renal disease models, PDGF-B is mainly overexpressed in the glomeruli, arterial, and tubular cell [28]. Therefore, staining for only PDGF-B and PDGFR-β, not for the other isoforms, may be sufficient to assess expression of this protein family in renal epithelial tumours.

PDGFR-β is one of the molecular markers, along with alpha smooth muscle actin, non-muscle myosin, tropomyosin, desmin, nestin, and aminopeptidase A, frequently used to identify pericytes [29], which reinforce vascular structure and regulate microvascular blood flow. Mature endothelial cells secrete PDGF-B, which binds to and activates of PDGFR-β receptors expressed on the surface of pericyte progenitors, promoting their proliferation and migration. This mechanism leads to pericyte coverage of early endothelial tubes. The synergistic effects of anti-endothelial and anti-pericytic molecules may make pericytes targets in anti-cancer treatment. We found that PDGF-B staining was stronger in papillary type 1 than in clear cell or chromophobe RCC, whereas PDGFR-β expression was higher in clear cell and papillary type 1 than in papillary type 2 and chromophobe RCC, but did not different in clear cell and papillary type 1. These findings suggest that pericyte proliferation may be greater in papillary type 1, especially since vascularity is lower in papillary than in clear cell RCC.

This study had several limitations. Although we analysed protein expression by IHC, we did not utilise quantitative methods, such as quantitative RT-PCR or ELISA of cytosolic extracts. However, the expression of individual isoforms of a protein may not correlate with mRNA expression, because mRNA and protein expression may be regulated by different control mechanisms [30]. For example, a significant correlation between $VEGF_{165}$ mRNA and protein was observed in only 17% of lung adenocarcinomas [30]. Second, the follow-up period in our patients was insufficient for analysis of surviv-

al. We included samples from patients, who underwent radical or partial nephrectomy between January, 2008, and March, 2012. Thus, the median follow-up duration was 2.9 years and the maximum was only 5 years. Moreover, 80.5% of these patients had early stage disease (pT1). Longer term follow-up of these patients may yield correlations between the expression of VEGF, VEGFR2, PDGF-B, and PDGFR-β and patient survival. Despite these limitations, we think this study is valuable because it is the largest prospective study to date to analyse VEGF/VEGFR2 and PDGF-B/PDGFR-β expression by IHC in RCCs other than clear cell and papillary types.

Conclusions

IHC showed that the cytoplasmic expression of VEGF, VEGFR, PDGF-B, and PDGFR-β in tumour cells was dependent on the pathologic stage and cellular type of RCC. VEGF and PDGF-B expression were higher in papillary than in other RCC cell types. These findings should be considered when designing new treatment strategies targeting angiogenesis in patients with papillary RCC.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Choung-Soo Kim, Department of Urology, Asan Medical Center, University of Ulsan College of Medicine, Pungnap-Dong 388-1, Songpa-Gu, Seoul, Korea. Tel: +82-2-3010-3734; Fax: +82-2-477-8928; E-mail: cskim@amc.seoul.kr

References

[1] Siegel R, Naishadham D and Jemal A. Cancer statistics, 2012. CA Cancer J Clin 2012; 62: 10-29.

[2] Farber LJ, Furge K and Teh BT. Renal cell carcinoma deep sequencing: recent developments. Curr Oncol Rep 2012; 14: 240-248.

[3] Kovacs G, Akhtar M, Beckwith BJ, Bugert P, Cooper CS, Delahunt B, Eble JN, Fleming S, Ljungberg B, Medeiros LJ, Moch H, Reuter VE, Ritz E, Roos G, Schmidt D, Srigley JR, Storkel S, van den Berg E and Zbar B. The Heidelberg classification of renal cell tumours. J Pathol 1997; 183: 131-133.

[4] Janzen NK, Kim HL, Figlin RA and Belldegrun AS. Surveillance after radical or partial nephrectomy for localized renal cell carcinoma and management of recurrent disease. Urol Clin North Am 2003; 30: 843-852.

[5] Levy AP, Levy NS, Wegner S and Goldberg MA. Transcriptional regulation of the rat vascular endothelial growth factor gene by hypoxia. J Biol Chem 1995; 270: 13333-13340.

[6] Berger DP, Herbstritt L, Dengler WA, Marme D, Mertelsmann R and Fiebig HH. Vascular endothelial growth factor (VEGF) mRNA expression in human tumor models of different histologies. Ann Oncol 1995; 6: 817-825.

[7] Farooqi AA, Waseem S, Riaz AM, Dilawar BA, Mukhtar S, Minhaj S, Waseem MS, Daniel S, Malik BA, Nawaz A and Bhatti S. PDGF: the nuts and bolts of signalling toolbox. Tumour Biol 2011; 32: 1057-1070.

[8] Posadas EM and Figlin RA. Systemic therapy in renal cell carcinoma: advancing paradigms. Oncology (Williston Park) 2012; 26: 290-301.

[9] Yang LP and McKeage K. Axitinib: in advanced, treatment-experienced renal cell carcinoma. Drugs 2012; 72: 2375-2384.

[10] Ljungberg BJ, Jacobsen J, Rudolfsson SH, Lindh G, Grankvist K and Rasmuson T. Different vascular endothelial growth factor (VEGF), VEGF-receptor 1 and -2 mRNA expression profiles between clear cell and papillary renal cell carcinoma. BJU Int 2006; 98: 661-667.

[11] Lopez-Beltran A, Scarpelli M, Montironi R and Kirkali Z. 2004 WHO classification of the renal tumors of the adults. Eur Urol 2006; 49: 798-805.

[12] Mena AC, Pulido EG and Guillen-Ponce C. Understanding the molecular-based mechanism of action of the tyrosine kinase inhibitor: sunitinib. Anticancer Drugs 2010; 21 Suppl 1: S3-11.

[13] Linehan WM. Targeting VEGF receptors in kidney cancer. Lancet Oncol 2007; 8: 956-957.

[14] Jacobsen J, Grankvist K, Rasmuson T, Bergh A, Landberg G and Ljungberg B. Expression of vascular endothelial growth factor protein in human renal cell carcinoma. BJU Int 2004; 93: 297-302.

[15] Ljungberg B, Jacobsen J, Haggstrom-Rudolfsson S, Rasmuson T, Lindh G and Grankvist K. Tumour vascular endothelial growth factor (VEGF) mRNA in relation to serum VEGF protein levels and tumour progression in human renal cell carcinoma. Urol Res 2003; 31: 335-340.

[16] Tomisawa M, Tokunaga T, Oshika Y, Tsuchida T, Fukushima Y, Sato H, Kijima H, Yamazaki H, Ueyama Y, Tamaoki N and Nakamura M. Expression pattern of vascular endothelial growth factor isoform is closely correlated with tumour stage and vascularisation in renal cell carcinoma. Eur J Cancer 1999; 35: 133-137.

[17] Dirim A, Haberal AN, Goren MR, Tekin MI, Peskircioglu L, Demirhan B and Ozkardes H. VEGF,

COX-2, and PCNA expression in renal cell carcinoma subtypes and their prognostic value. Int Urol Nephrol 2008; 40: 861-868.
[18] Ferrara N. Role of vascular endothelial growth factor in the regulation of angiogenesis. Kidney Int 1999; 56: 794-814.
[19] Takahashi A, Sasaki H, Kim SJ, Tobisu K, Kakizoe T, Tsukamoto T, Kumamoto Y, Sugimura T and Terada M. Markedly increased amounts of messenger RNAs for vascular endothelial growth factor and placenta growth factor in renal cell carcinoma associated with angiogenesis. Cancer Res 1994; 54: 4233-4237.
[20] Rapisarda A and Melillo G. Role of the VEGF/VEGFR axis in cancer biology and therapy. Adv Cancer Res 2012; 114: 237-267.
[21] Petrova TV, Bono P, Holnthoner W, Chesnes J, Pytowski B, Sihto H, Laakkonen P, Heikkila P, Joensuu H and Alitalo K. VEGFR-3 expression is restricted to blood and lymphatic vessels in solid tumors. Cancer Cell 2008; 13: 554-556.
[22] Shibuya M and Claesson-Welsh L. Signal transduction by VEGF receptors in regulation of angiogenesis and lymphangiogenesis. Exp Cell Res 2006; 312: 549-560.
[23] Hicklin DJ and Ellis LM. Role of the vascular endothelial growth factor pathway in tumor growth and angiogenesis. J Clin Oncol 2005; 23: 1011-1027.
[24] van Roeyen CR, Ostendorf T and Floege J. The platelet-derived growth factor system in renal disease: an emerging role of endogenous inhibitors. Eur J Cell Biol 2012; 91: 542-551.
[25] Floege J, Eitner F and Alpers CE. A new look at platelet-derived growth factor in renal disease. J Am Soc Nephrol 2008; 19: 12-23.
[26] Alpers CE, Seifert RA, Hudkins KL, Johnson RJ and Bowen-Pope DF. PDGF-receptor localizes to mesangial, parietal epithelial, and interstitial cells in human and primate kidneys. Kidney Int 1993; 43: 286-294.
[27] Liu G, Changsirikulchai S, Hudkins KL, Banas MC, Kowalewska J, Yang X, Wietecha TA, Volpone J, Gilbertson DG and Alpers CE. Identification of platelet-derived growth factor D in human chronic allograft nephropathy. Hum Pathol 2008; 39: 393-402.
[28] Akai Y, Iwano M, Kitamura Y, Shiiki H, Dohi Y, Dohi K, Moriyama T and Yonemasu K. Intraglomerular expressions of IL-1 alpha and platelet-derived growth factor (PDGF-B) mRNA in experimental immune complex-mediated glomer-ulonephritis. Clin Exp Immunol 1994; 95: 29-34.
[29] Morikawa S, Baluk P, Kaidoh T, Haskell A, Jain RK and McDonald DM. Abnormalities in pericytes on blood vessels and endothelial sprouts in tumors. Am J Pathol 2002; 160: 985-1000.
[30] Chen G, Gharib TG, Huang CC, Taylor JM, Misek DE, Kardia SL, Giordano TJ, Iannettoni MD, Orringer MB, Hanash SM and Beer DG. Discordant protein and mRNA expression in lung adenocarcinomas. Mol Cell Proteomics 2002; 1: 304-313.