The Involvement of PDGF-B/PDGFRβ Axis in the Resistance to Antiangiogenic and Antivascular Therapy in Renal Cancer

ALIN ADRIAN CUMPĂNAS[1], ANCA MARIA CIMPEAN[2], OVIDIU FERICIAN[1], RALUCA AMALIA CEAUSU[2], SIMONA SARB[2], VLAD BARBOS[2], ALICE DEMA[3] and MARIUS RAICA[2]

Departments of [1]Ortophedic Surgery, Traumatology and Urology and [3]Pathology, "Victor Babes" University of Medicine and Pharmacy Timisoara, Timisoara, Romania; [2]Department of Microscopic Morphology/Histology, Angiogenesis Research Center, Timisoara, Romania

Abstract. *Background/Aim: Studies developed in the field of platelet-derived growth factors/platelet-derived growth factor receptors (PDGFs/PDGFRs) inhibition have focused on the therapeutic effects on tumor cells, neglecting their potential effects on tumor blood vessels. We herein propose a differential and critic assessment of platelet-derived growth factor B (PDGF-B) and platelet-derived growth factor receptor β (PDGFRβ) in renal cell carcinoma, correlated with the four main vascular patterns previously reported by our team. Materials and Methods: PDGF-B and PDGFRβ were evaluated on 50 archival paraffin embedded specimens related to vascular endothelial growth factor (VEGF), its inhibitory isoform VEGF165b and vascular patterns. Results and Conclusion: Our results support the involvement of VEGF165b in the phosphorylation of PDGFRβ with an inhibitory effect on endothelial proliferation and migration. The simultaneous action of PDGF-B/PDGFRβ and VEGF165b on the same type of receptor may explain the resistance to antiangiogenic therapy, which depends on the degree of modulation of PDGFRβ phosphorylation.*

The platelet-derived growth factor B/platelet-derived growth factor receptor β (PDGF-B/PDGFRβ) system is involved in both tumor angiogenesis and progression (1-3). Nowadays, the main target of several therapies is the inhibition of platelet-derived growth factor (PDGF) family members and their related receptors (4, 5). Unfortunately, the resistance developed to anti-platelet-derived growth factors/platelet-derived growth factor receptors (PDGFs/PDGFRs) therapies is already known and accepted; however, the mechanisms of this resistance is not fully understood yet.

Renal cancer treated with PDGF/PDGFRs inhibitors has initially a positive response, reflected by the decrease in tumor size and suppression of distant metastasis (6-8). Despite these encouraging initial results, 3-4 years after initiation of therapy, patients with renal cancer are diagnosed with multiple distant metastases, frequently in advanced stages (9, 10). Most preclinical and clinical studies developed in the field of PDGFs/PDGFRs inhibition were focused on the therapeutic effects of these targeted therapies on tumor cells, neglecting their potential effects on tumor blood vessels. This is a paradox as the receptors and their ligands are known to be expressed by both endothelial cells and perivascular smooth muscle cells.

Our previous work identified four patterns of tumor blood vessels in renal cancer (11), with a predominance of mature tumoral and peritumoral blood vessels, characterized by the presence of different amounts of perivascular smooth muscle cells surrounding the newly formed vascular structure. As a paradox, this fact has not been previously well-studied in correlation with the expression of PDGF-B and PDGFRβ. Only one recent study suggests that the overexpression of PDGFRβ is involved in the suppression of the tumor's vessels progression (12).

Based on previously described evidence and controversies, we propose a differential and critic assessment of PDGF-B and PDGFRβ immunohistochemical expression in renal cell carcinoma, in correlation with the four main vascular patterns previously reported by our team (11). These factors have been evaluated in the normal tissue neighboring the malignant tumor, the tumor cells and the tumor vessels. One particular aspect of the present study can be considered the PDGF-B and PDGFRβ evaluation in close relationship with other angiogenic and antiangiogenic factors, some of them being known to have a strong impact on renal cell carcinoma progression and metastasis [vascular endothelial growth factor (VEGF) or others not yet studied in renal cell carcinoma (VEGF165b)].

Correspondence to: Anca Maria Cimpean, MD, Ph.D., Professor of Histology, Department of Microscopic Morphology/Histology, Angiogenesis Research Center, Pta Eftimie Murgu 2, 300041 Timisoara, Romania. Tel: +40 720060955, e-mail: ancacimpean1972@yahoo.com

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Materials and Methods

This was a retrospective study that included fifty archival paraffin-embedded specimens of renal carcinomas selected for our purpose. The selection included the resubmission to the histotechnologist for sectioning and routine hematoxylin and eosin staining. Three independent pathologists re-evaluated the histopatology (including Fuhrman score) and selected the slides for subsequent immunohistochemistry. All specimens were automatically processed by using Bond Max System for immunohistochemistry (Leica Microsystems, Newcastle, UK). All the steps of the immunohsitochemical procedures were carefully selected to be compatible with biotin-free visualization system (Bond Refine Detection System, DAB; Leica Microsystems) that was used after incubation with the primary antibodies. We detected the presence and distribution of platelet-derived growth factor BB (PDGF-BB) by using mouse anti-PDGF-BB monoclonal antibody (clone F-3, dilution 1:100, 20 minutes incubation time at room temperature; Santa Cruz Biotechnology, Dallas, TX, USA) and its corresponding receptor PDGFRβ (polyclonal, P20, dilution 1:30, with the same time of incubation like PDGF-BB). Previous immunostainings of the same cases included the detection and characterization of four main vascular patterns (reticular, diffuse, fasciculated and trabecular) (11), based on the co-localization of CD34 and smooth muscle actin. PDGF-BB and PDGFRβ evaluations were done in close relationship with the vascular patterns previously described, including VEGF (13) and VEGF165b (unpublished) expression in renal cell carcinomas, both of them being part of a previous characterization of the cases. We correlated the expression of PDGF-BB/PDGFRβ with endothelial proliferation assessed by the presence of Ki67 and also with Fuhrman score.

Microscopic data and image acquisition was performed by using Axio Zoom Imager A2 research microscope (Zeiss, Jena, Germany). Statistical analysis used SPSS software, version 17 (SPSS Inc, Chicago, IL, USA); *p*-value less than 0.05 was considered statistically significant.

Results

The expression of PDGF-B was both cytoplasmic and nuclear. Both normal peritumoral and tumor tissue expressed PDGF-B with the dual nuclear and cytoplasmic localization.

In the normal renal tissue, PDGF-B was expressed in both cortex (Figure 1A) and medulla (Figure 1B). In the renal cortex, PDGF-B was constantly expressed by renal corpuscles and cortical tubules of normal kidney tissue adjacent to the tumor. Inside the renal corpuscles, in endothelial cells and podocytes, the expression of PDGF-B was restricted to the nucleus. In the cortical tubules, PDGF-B expression was heterogeneous, with both nuclear and cytoplasmic pattern, and with different intensity depending on the type of cortical tubules. Proximal tubules expressed intensely PDGF-B with a combined nuclear-cytoplasmic pattern (Figure 1C). In the distal cortical tubules, the type of expression that prevailed was with a cytoplasmic pattern with scattered nuclear expression. There was expression heterogeneity in the tubular epithelial cells, both along the same tube and also between tubes. In the renal medulla, both tubular epithelial cells and glomerular endothelial cells expressed PDGF-B with a predominant nuclear pattern. Moreover, the expression was homogeneous and constant among tubular epithelial cells from the same or different renal tubules.

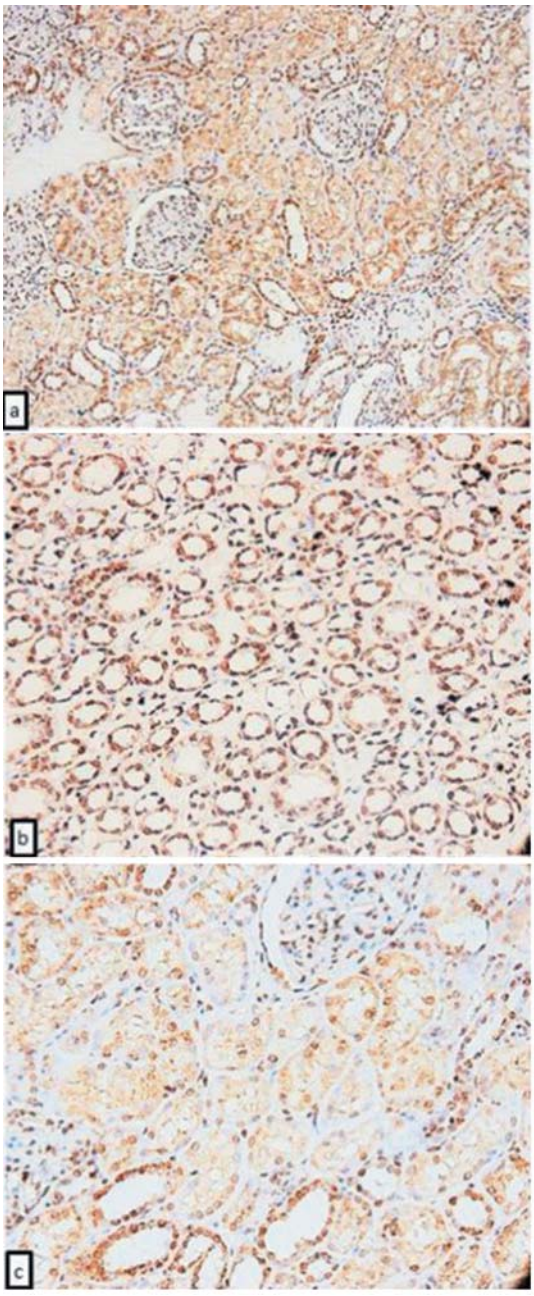

Figure 1. *PDGF-B expression in normal renal parenchyma, adjacent to the tumor. Renal cortex has a heterogeneous expression at the level of renal corpuscle and tubular components (a). Cells, lining renal tubules, express PDGF-B with both nuclear and cytoplasmic pattern (b). Predominant intense nuclear and weak cytoplasmic expressions characterize renal proximal tubes close to renal corpuscle (c).*

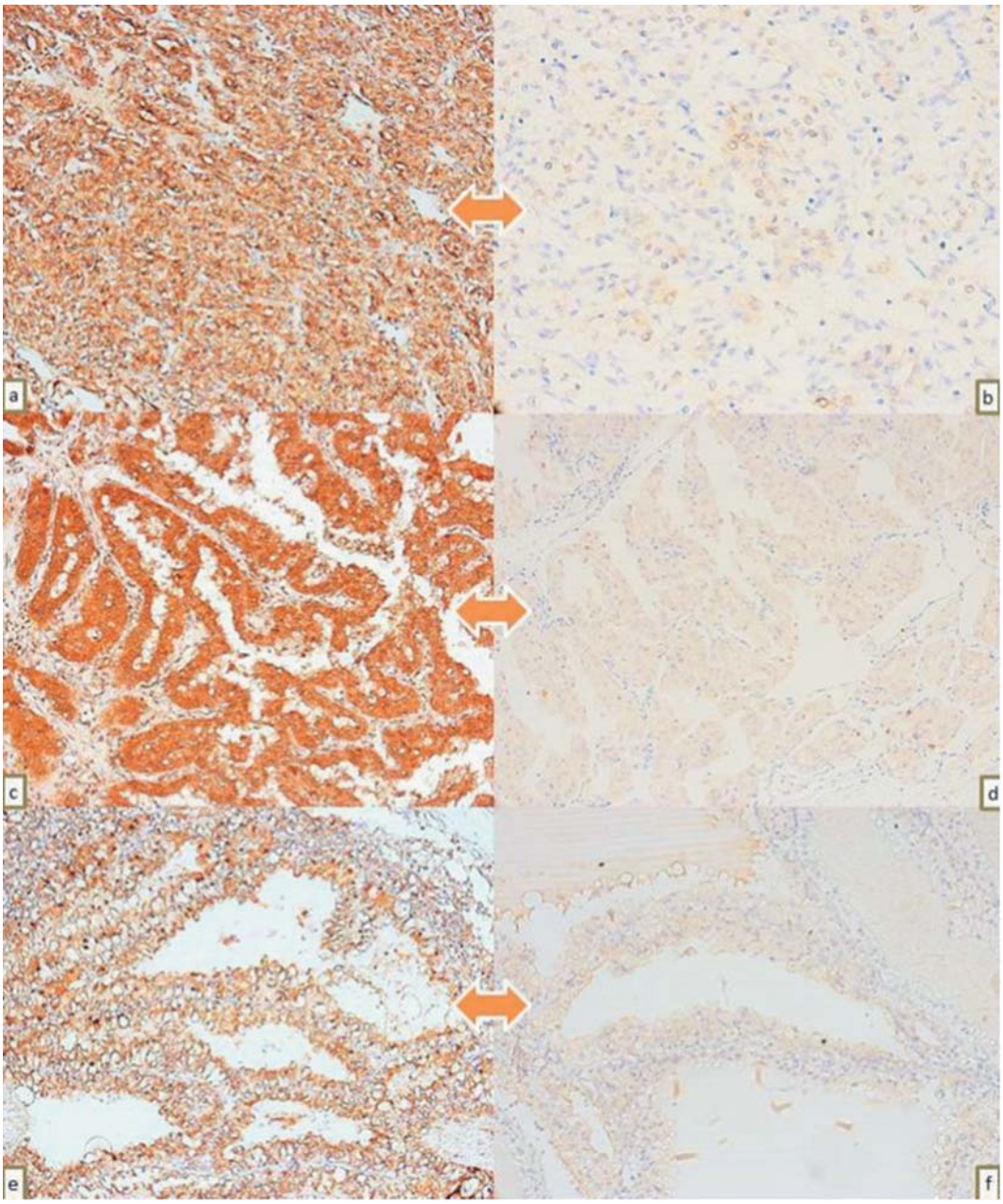

Figure 2. *Comparative and differential expression of PDGF-B (a, c, e) and PDGFRβ (b, d, f) in renal cell carcinomas. Intense PDGF-B expression is observed in all types of RCC, with a highest intensity registered for papillary type (c) with both nuclear and cytoplasmic pattern (e). PDGFRβ had a focal (b) and weak positivity (d) in the same cases, with papillary morphology expression being restricted to the cytoplasm of the tumor cell (f).*

For kidney tumors, we evaluated PDGF-B/PDGFRβ axis in tumor cells and also with emphasis to the four vascular patterns previously defined by our team (11). Briefly, reticular pattern of the vascular network was present in 63% of cases with predominance of mature $CD34^+/SMAct^+$, highly interconnected tumor vessels. Diffuse pattern was observed in 23% of the cases, which was characterized by non-interconnected blood vessels, predominant of mature $CD34^+/SMAct^+$ type as well. Vascular invasion was present in 64% of the cases. Eight percent of the cases had a fasciculate model of blood vessel distribution, all of which were of mature type, and were located in the connective axis of the papillary renal tumors. For this pattern, vascular invasion was found in 50% of the cases. In 6% of kidney tumor cases, trabecular

patterns were observed. These cases were accompanied by the lowest rate of vascular invasion.

PDGF-B was significantly correlated with Fuhrman's score 2, $p=0.038$. We also found significant statistical correlation with the mature blood vessels ($CD34^+/SMAct^+$), $p=0.048$ and with immature blood vessels ($CD34^+/SMAct^-$), $p=0.048$.

Renal cancer cells were also positive for PDGF-B. All studied tumors had positive PDGF-B tumor cells. The expression of this factor was, however, heterogeneous from a tumor to another and, also, within the same tumor type. The heterogeneity observed regarded the pattern of expression, as well as the intensity and distribution of PDGF-B. At the periphery of the tumor, the pattern that prevailed was the combined nuclear and cytoplasmic one. Inside the tumor, this type of expression was maintained; however, the majority of the tumor cells were positive for PDGF-B with cytoplasmic expression, which was intense in most cases (Figure 2A, C).

A particular aspect of PDGF-B expression was represented by the papillary renal cell carcinoma, where the PDGF-B expression was tightly restricted to the cytoplasm of the epithelial component (Figure 2E). Clear-cell carcinoma presented a moderate positive expression, predominantly in cells at the periphery of the tumor.

The beta receptor for PDGF (PDGFRβ) was expressed in approximately one third of the cases. The pattern of expression was strictly cytoplasmic and its intensity ranged from weak to moderate (Figure 2B, D). There were no cases with high intensity of PDGFRβ expression. Moderate intensity was observed only in the papillary renal cancer with a strictly cytoplasmic pattern of expression (Figure 2F).

From the statistical point of view, regarding the correlation between PDGF-B and PDGFRβ with factors previously analyzed, significant statistical data were obtained: PDGF-B was correlated significantly with the Fuhrman's degree of differentiation, $p=0.038$. We also obtained a significant statistical correlation with mature blood vessels (CD34/SMAct), $p=0.048$, and also with immature blood vessels ($CD34/SMAct^-$), $p=0.048$. In the same time, significant statistical results were obtained between the PDGF-B/PDGFRβ ratio and mature/immature blood vessels, $p=0.027$. Last, but not least, PDGF-B was significantly correlated with PDGFRβ in the context of stimulation of endothelial cell proliferation, $p=0.042$. PDGFRβ was significantly correlated with VEGF ($p=0.05$) and with the most often met reticular vascular pattern ($p=0.041$). One of the most significant statistical correlations was obtained between PDGFRβ and VEGF165b, $p=0.028$. VEGF165b was also significantly correlated with the PDGF-B/PDGFRβ ratio ($p=0.05$).

Discussion

The PDGF-B/PDGFRβ axis is a well-known and accepted mechanism involved in recruiting perivascular smooth muscular cells and pericytes, both during embryonic evolution and in adult life, whenever exists necessity for blood vessels to mature (13). An intriguing field of study is the implication of this axis in tumor angiogenesis as it is known to be a stimulator for the maturation of newly formed vessels (12, 14). This mechanism is responsible for the recruitment of smooth muscular cells, as well as their maturation from progenitors to the state of perivascular mature cells, a fact recently showed by Sheikh and colleagues (15) in vascular alterations from pulmonary hypertension.

The PDGF-B/PDGFRβ axis exerts angiogenic effects acting on proliferation and migration of endothelial cells through tumor mass. This interaction has already been reported in the literature in relation to renal cancer. The VEGF/VEGFR2 axis and the PDGF-B/PDGFRβ axis are expressed in the cytoplasm of tumor cells. Furthermore, there have been differences in the expression of the ligand (VEGF and PDGF-B) depending on the tumor stage and cell types involved as demonstrated by Song and collaborators (12) who reported that VEGF and PDGF-B are intensely expressed in the papillary tumor type compared to clear-cell carcinoma. Our results partially agree with these data since, in our study, the papillary type tumor cells were strongly positive not only for VEGF and PDGF-B but also for PDGFRβ. In addition, the cytoplasmic expression of PDGF-B in tumor cells was constant, albeit heterogeneous, regarding the intensity and the density of the positive cells. This aspect might have clinical implications, mainly on the prognosis of clear-cell renal carcinoma as previous data have shown a decreasing rate of survival and an increasing rate of recurrence after nephrectomy (16). Our results contrast the published data and show a combined nuclear and cytoplasmic expression of PDGF-B, which has been observed in other tumor types as well (17).

Based on the concomitant expression of both PDGF-B and PDGFRβ observed in several cases in the current study, correlated with previous data reported by Heldin about autocrine effects exerted by the PDGF/PDGFRβ axis expressed in tumor cells (18), our findings support a similar mechanism in renal cell carcinomas and can, thus, explain the rapid maturation of the newly formed blood vessels in clear-cell type carcinomas.

Maturation of blood vessels involves inhibition of VEGF-mediated activation of endothelial cells. The inhibitory fraction, VEGF165b, was statistically significant correlated with the co-expression of PDGF-B/PDGFRβ in our study. This correlation, exclusively found for the reticular pattern, supports the claim that early and rapid maturation of the blood vessels in renal tumors is possible due to concomitant inhibition of endothelial cells by VEGF165b and rapid recruitment and maturation of perivascular cells driven by the PDGF-B/PDGFRβ axis.

In 2007, Ball *et al*. (19) showed that VEGF-A stimulates the expression of PDGFRα and -β and, moreover, binds to both types of receptors, thus rendering VEGF-A a potential regulator for the recruitment of both endothelial and perivascular cells.

The interrelation between PDGFRβ and VEGF stimulates endothelial cell migration and proliferation, as well as the recruitment of perivascular smooth muscle cells. At present, the ratio and the interrelations between VEGF165b and PDGF-B are not reported in the literature. Our study indicates that β receptor for PDGF is a possible factor of resistance to antiangiogenic therapy. It has double, but evident, role in the early maturation of the vascular network of renal tumors through the concordant action of PDGF-B and VEGF165b. This might clarify the weak expression of PDGFRβ in the studied renal tumors, a finding that can be explained either by the presence of a highly phosphorylated form of the receptor or the presence of its oversaturation by the above mentioned ligands.

Conclusion

PDGFRβ has a dual role in the pathogenesis and also modulation of angiogenesis in renal tumors. PDGFRβ is directly involved in achieving the reticular pattern of tumor vascularization through the simultaneous action of PDGF-B/VEGF. There are no known data concerning the interrelation between VEGF165b and PDGFRβ.

Our results support the involvement of VEGF165b in the phosphorylation of PDGFRβ with an inhibiting effect on endothelial proliferation and migration. The simultaneous action of PDGF-B/PDGFRβ and VEGF165b on the same type of receptor may explain the resistance to antiangiogenic therapy, which depends on the degree of modulation of PDGFRβ phosphorylation.

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