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The diagnostic value of VEGF expression in the renal parenchyma tumors

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Abstract

Tumor angiogenesis emerged as an important concept in cancer therapy over two decades ago, and was extensively studied by the discovery of VEGF family members. VEGF, also known as vascular permeability factor, is a generic name for VEGF-A, which is one of the members involved in angiogenesis. VEGF is the most important angiogenic factor, with significant effects on tumor angiogenesis. Tumor expression of VEGF was not the first angiogenesis indicator, but a growing number of studies have demonstrated that VEGF could be a prognostic factor, independent even from microvascular density, which is increased by its expression. Renal parenchyma tumors are a heterogeneous group of malignancies, difficult to classify or monitor, which prompts for the assessment of novel markers useful for the investigation of tumor histogenesis or prognostic assessment. VEGF expression in renal parenchyma tumors is poorly studied, with most of the articles published so far focusing on antiangiogenic usage in renal carcinoma therapy. The aim of this study is to detect the expression pattern of VEGF in renal parenchyma tumors by immunohistochemistry.

Keywords: angiogenesis, renal carcinomas, immunohistochemistry, VEGF.

Introduction

VEGF is a growth factor member of the platelet derived growth factor (PDGF) dimeric glycoprotein superfamily. It is the most important angiogenic factor, with significant effects on tumor angiogenesis, including that from renal parenchyma tumors. Tumor angiogenesis, promoted by the so-called angiogenic switch, begins in most cases by synthesis and activation of VEGF by the tumor cells or the inflammatory cells of the stroma, such as macrophages or mast cells.

VEGF expression is regulated by different factors such as cytokines, other growth factors, hormones, but mostly hypoxia. Its effects at the vascular level include induction of endothelial cells division, their migration, enhancing their survival through protection against apoptosis and slowing of their aging. Its effects are mediated through interaction with transmembrane tyrosinkinase receptors present on the surface of the endothelial cells, VEGFR and neuropilins [1–4].

At present, the complete cancer therapy includes anti-angiogenic products as well, with VEGF receptors as some of the most efficient targets of this therapy [5–10].

Literature data show a correlation between VEGF secretion and von Hippel–Lindau gene inactivation, usually on conventional renal carcinomas with familial aggregation [11–15]. Although the importance of VEGF overexpression in tumor angiogenesis is widely accepted, its prognostic importance is still subject for debate [16]. Serum levels of VEGF in patients with renal carcinoma seems to have no prognostic significance, unlike that of IL-6 which indicates a worse outcome [17, 18].

Renal parenchyma tumors are a group with heterogeneous members, with major difficulty in classification and histogenesis. The evolution of these tumors is highly unpredictable, as there are few reliable prognostic markers. The tumors usually grow slow, with areas of massive necrosis and calcifications, and sometimes have abrupt evolution. This behavior suggests an involvement of VEGF and angiogenesis, at least in the latest phases of their evolution.

Materials and Methods

Forty-seven renal parenchyma tumors specimens from patients admitted between 1999 and 2004 were selected from the archive of the Urology Clinic of the Emergency County Hospital, Timişoara, were primary processed, performed morphological diagnosis and pretreated for immunohistochemistry. Briefly, the specimens were fixed in 4% buffered formalin, embedded in paraffin and sectioned at 3–5 μm. The slides were then dewaxed and rehydrated and either stained with the usual Hematoxylin–Eosin (HE) stained slides, according to WHO classification. Additional staining for the morphological diagnosis or pretreated for immunohistochemistry. Morphological diagnosis was performed on HE stained slides, according to WHO classification. Additional slides were immunohisto-chemically stained with the polyclonal anti-VEGF antibody VG1. Briefly, slides were dewaxed and rehydrated in baths of benzene and then ethylic alcohol with decreasing concentration, then antigen retrieval was performed by microwave heating at pH 8 before blocking the endogenous peroxydase. Then the primary

antibody (1:400 dilution) and EnVision working systems were applied, followed by nuclear counterstaining with Lillie's Hematoxylin. The slides were then examined at a Nikon i80 microscope, with the tubular system of the nephron and collecting tubes as internal positive control. The assessment of the staining was by a positive cells number score, as following: 0 for the negative cases, +1 for the cases with less than 10% positive cells, +2 for the specimens with positive cells between 10% and 50%, and +3 for the tumors with more than 50% positive cells. The positive cells had a granular cytoplasmic staining pattern, with concentration in the perinuclear space. The external positive control was represented by slides of undifferentiated gastric carcinoma, which were intensely positive. After staining, the slides were dehydrated and mounted with Canada balsam. Statistic data analysis was performed with SPSS software package, version 17.

Results

Thirty-two (68.08%) of the tumor specimens were positive for VEGF and 15 (31.92%) were negative. The intensity of the staining was compared with the staining of the collecting tubes from the adjacent medulla, which was considered +3. In the majority of the cases the density of tumor positive cells was lower than the one of the collecting tubes, and the intensity of staining followed the same pattern (Figure 1, a and b). The heterogeneity of the staining was common in tumor specimens, usually with random distribution of positive cells in the tumor mass (Figure 1, c and d).

All of the specimens had collecting tubes from the medulla as internal positive control. Fifteen specimens were negative, 16 were scored +1, 11 had +2 score and only five cases were +3, with more than 50% positive cells.

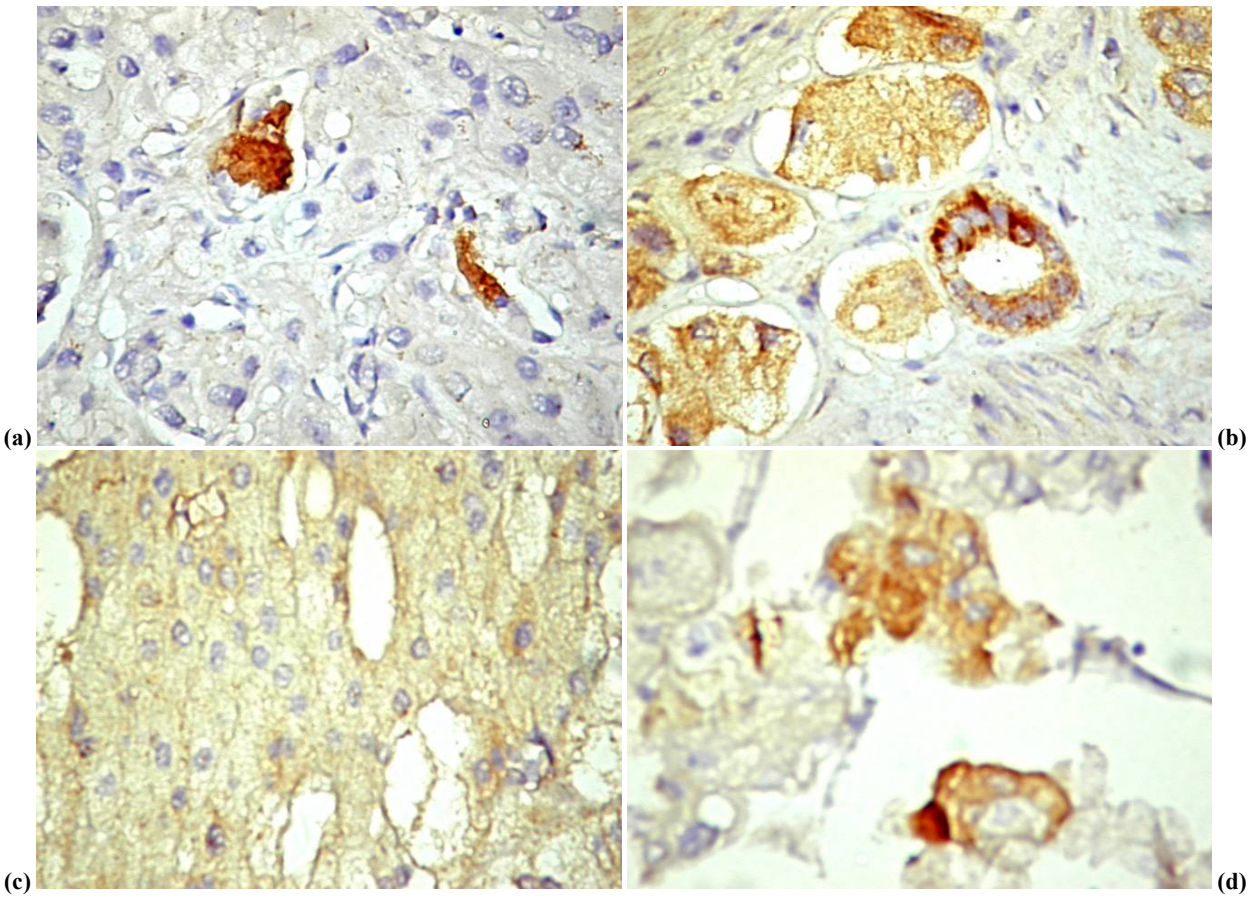


Figure 1 – ***(a) VEGF immunostaining (×400) in renal parenchyma tumors. Low density of intensely positive tumor cells. (b) VEGF immunostaining (×400) in renal parenchyma tumors. Low intensity VEGF staining in tumor cells compared with those of the collecting tubes. (c) VEGF immunostaining (×400) in renal parenchyma tumors. Low intensity staining. (d) VEGF immunostaining (×400) in renal parenchyma tumors. Heterogeneous staining with moderate intensity.***

The intensity of the staining differs from one case to another and is heterogeneous in the same specimen as well. In some of the cases, most cells were weakly stained, with groups of moderately stained cells (Figure 2, a and b). In other cases, we have noticed the association between intensely stained groups of tumor cells and a negative background, which was scored +3 due to the number of positive cells (Figure 3).

The areas with few intensely stained tumor cells against a negative background were frequent (Figure 4).

This aspect shows that only a small number of tumor cells have angiogenic phenotype, which is correlated with the low progression rate and high incidence of necrosis and calcification of these tumors. VEGF expression was not correlated with the histopathological type nor with the nuclear grade.

In the cases with isolated intensely positive cells, the latter are concentrated in close vicinity of tumor blood

vessels (Figure 5, a and b). The tumors with high score were intensely stained, either with homogenous or heterogeneous pattern (Figure 6, a and b).

In papillary carcinomas, we have noticed some particular aspects of the staining patterns. First of all, the intensity of the staining was significantly higher in the adjacent parenchyma (Figure 7).

In one case, the staining was negative in the tumor, but some isolated cells from the connective axes with macrophage/mast cells morphology were positive (Figure 8a). Another aspect is the presence of positive cells only at the proliferation front with most of the other cells negative (Figure 8b).

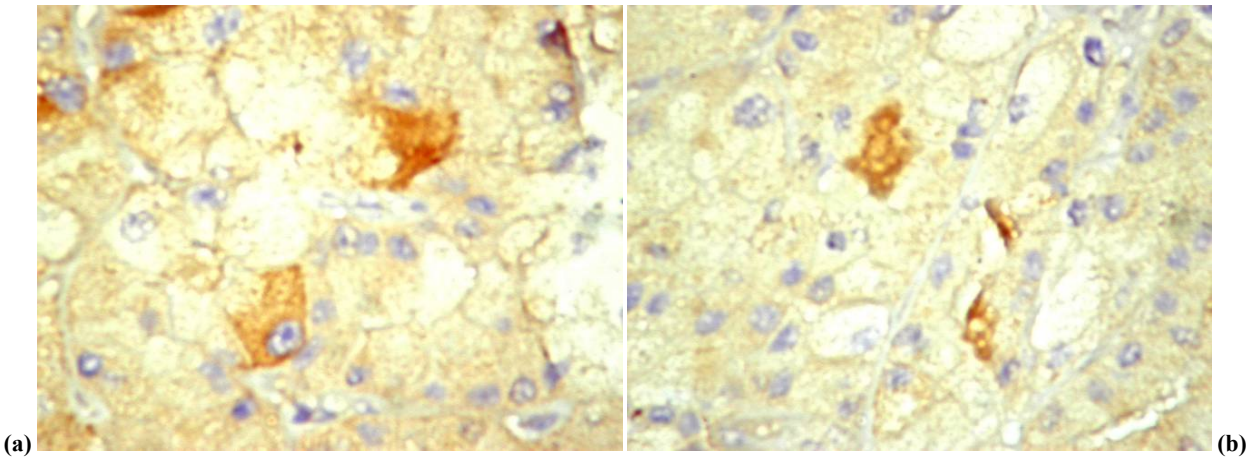


Figure 2 – ***(a) Renal carcinoma. Intensely stained tumor cell groups or isolated cells against a weakly stained background (VEGF immunostaining, ×400). (b) Renal carcinoma. Intensely stained tumor cell groups or isolated cells against a weakly stained background (VEGF immunostaining, ×400).***

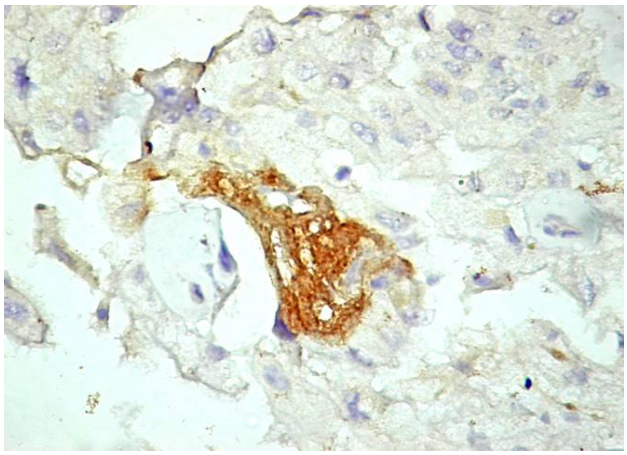


Figure 3 – ***Renal carcinoma. Focal intensely positive reaction with high intensity against a negative background (VEGF immunostaining, ×400).***

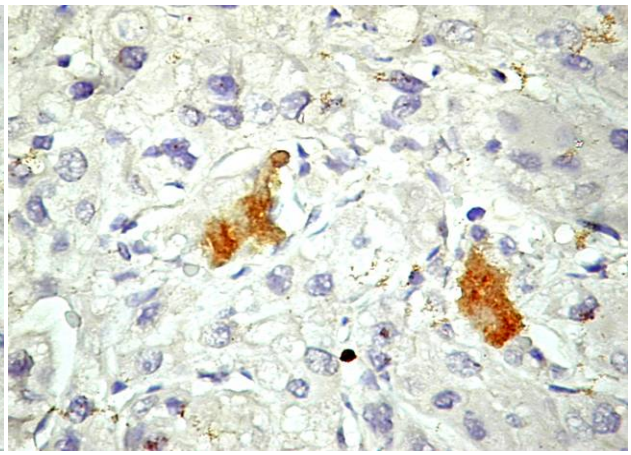


Figure 4 – ***Rare intensely positive cells and cell groups. Most of the tumor cells are negative (VEGF immunostaining, ×400).***

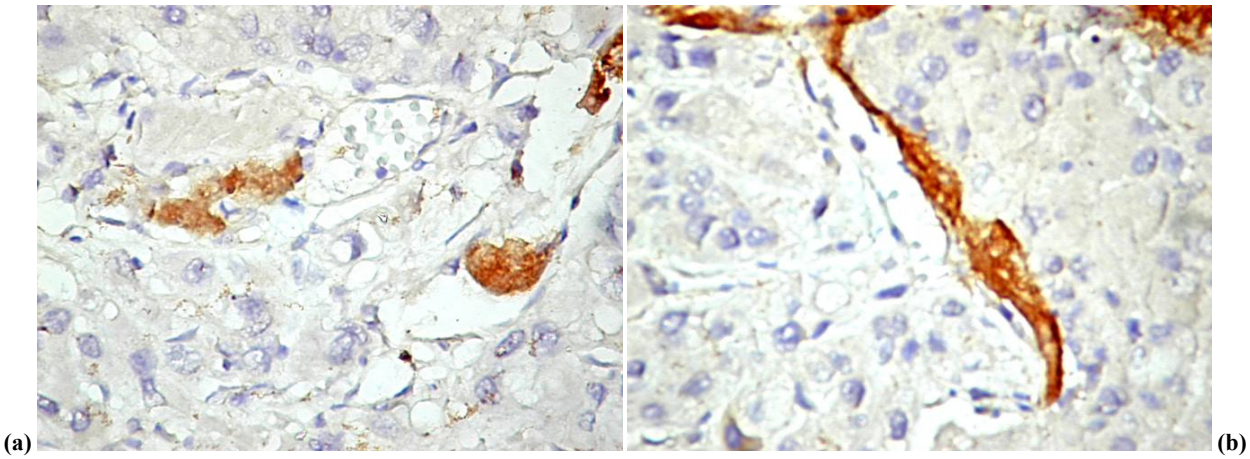


Figure 5 – ***(a) Small groups of intensely positive cells, close to blood vessels (×400). (b) VEGF positive tumor cells cord on one side of a blood vessel (×400).***

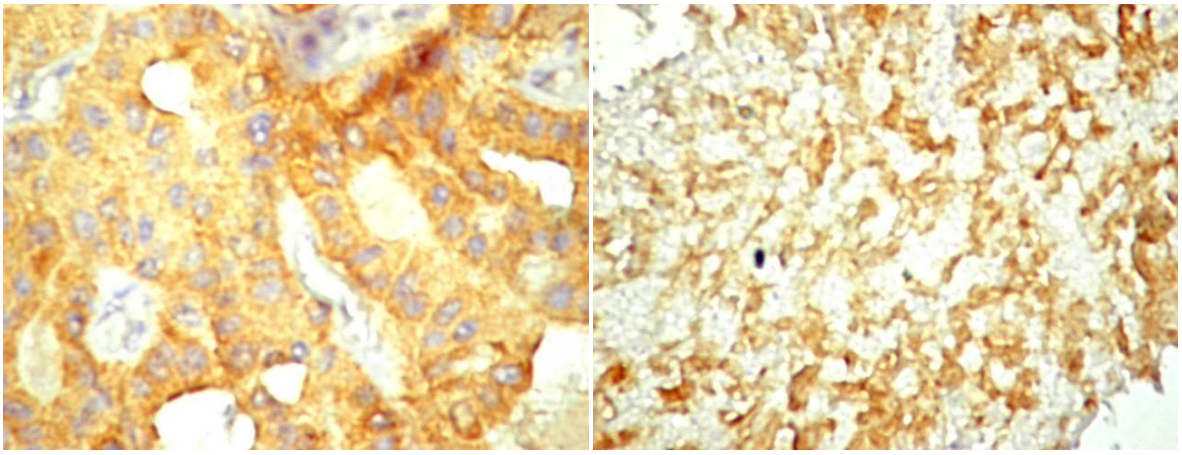
(a)  (b)

Figure 6 – ***(a) Intensely positive renal carcinomas. Homogenous diffuse pattern (VEGF immunostaining, ×400). (b) Intensely positive renal carcinomas. Heterogeneous diffuse pattern (VEGF immunostaining, ×400).***

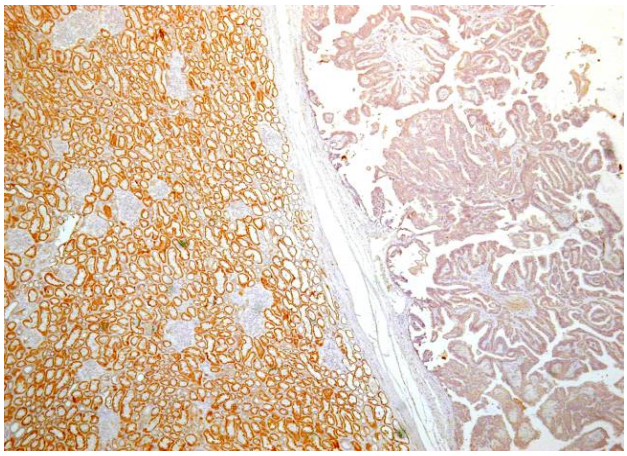


Figure 7 – ***VEGF immunostaining, papillary carcinoma. The normal renal parenchyma is intensely stained, while the tumor is weakly stained or negative (×6).***

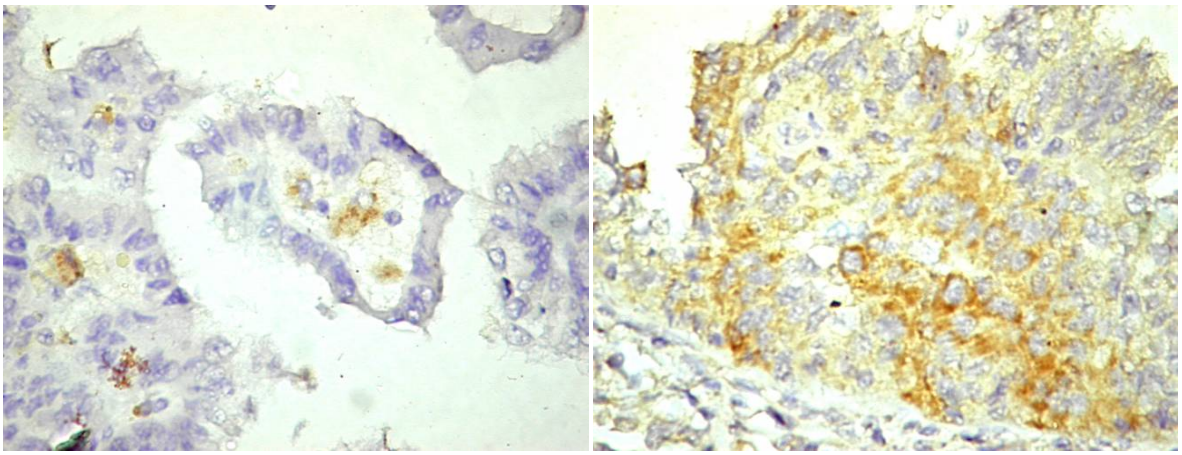
(a)  (b)

Figure 8 – **(*a) Papillary carcinoma, negative for VEGF in tumor cells, but present in isolated cells of the connective tissue axis. (b) Papillary carcinoma. Moderately positive cells from the proliferation front (×400).***

Discussion

The semi-quantitative assessment of VEGF immunostaining yielded similar results with the data from the literature [19]. According to this assessment, 32% of the cases (n=15) had a score of 0 and were considered negative, while the rest of 68% of the cases were positive. These data confirm the ubiquitous expression of VEGF in tumor cells of the renal cells carcinomas. Most of the conventional carcinomas had a significant number of positive blood vessels, while the blood vessels from the normal adjacent parenchyma were negative. Since there are studies that confirm the presence of VEGF receptors mRNA in their endothelial cells [20], we may consider these positive blood vessels as false positive, due to bound VEGF. The lack of correlation between VEGF expression and the pathological type and Fuhrman nuclear grade that we have noticed is similar with the literature data [16, 24]. Our results are in concordance with those of Paradis V *et al.* [19] and Jacobsen J *et al.* [21], which had shown a

positive correlation between VEGF expression and tumor size and differentiation grade and stage, respectively. We have noticed a significant correlation between VEGF expression and microvascular density (p=0.025), which is similar to the results of Gelb AB *et al.* [22] and MacLennan *et al.* [23], but differ to those of Paradis V *et al.* [19], which had identified microvascular density as an independent prognostic factor in renal carcinomas.

In one case of papillary carcinoma, we have noticed some particular aspects of VEGF expression, with positive connective tissue cells with macrophage/mast cell morphology, while tumor cells were negative. Mast cells are a known source of VEGF [25, 26], and might be in this case the only cellular promoters of agiogenesis mediated by VEGF.

We have noticed a large number of vessels devoid of pericytes in the tumor area, compared to the normal adjacent parenchyma, which suggests either vessels frailty, as some authors point out [25–27], or induction of angiogenesis.

Conclusions

VEGF is intensely expressed, with granular cytoplasmic pattern, in the normal renal parenchyma in the nephron tubular system and collecting tubes. 68.08% of the renal parenchyma tumors have expressed VEGF, with heterogeneous, low intensity pattern as compared with the collecting tubes. Positive staining was more intense or present only in the proliferation front. VEGF expression correlates with microvascular density (p=0.025), but is not correlated with pathological type or Fuhrman nuclear grade, so VEGF expression may be considered as an individual prognostic factor.

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