IMMUNOHISTOCHEMICAL DETECTION OF THE VASCULAR ENDOTHELIAL GROWTH FACTOR: VALUE, LIMITS, SCORING, AND TECHNICAL APPROACHES

Amalia Raluca Ceausu, Pusa Nela Gaje, Anca Maria Cimpean

[1]Victor Babes University of Medicine and Pharmacy, Department of Microscopic Morphology/Histology, Angiogenesis Research Center Timisoara, Romania

ABSTRACT

Vascular endothelial growth factor (VEGF), the main pro-angiogenic molecules is a useful immunohistochemical marker in both pathological diagnosis and research. The aim of current minireview was to underline some features on value, limits and technical approaches. VEGF is maintained as an important prognostic, diagnostic or therapeutic marker for some tumor types such as: oral and head and neck squamous cell carcinoma, gastric and colorectal adenocarcinoma, hepatocellular carcinoma, non-small lung cell carcinoma and breast cancer. Main limits of VEGF values are the heterogeneity of analysis methods of the immunohistochemical reaction. The immunohistochemical scoring of VEGF is oriented in two main directions: visual examination by the pathologist and computer-assisted image analysis. Future studies which focus on VEGF reliable measurements are essential for the application in clinical trials.

Key words: immunohistochemistry, vascular endothelial growth factor

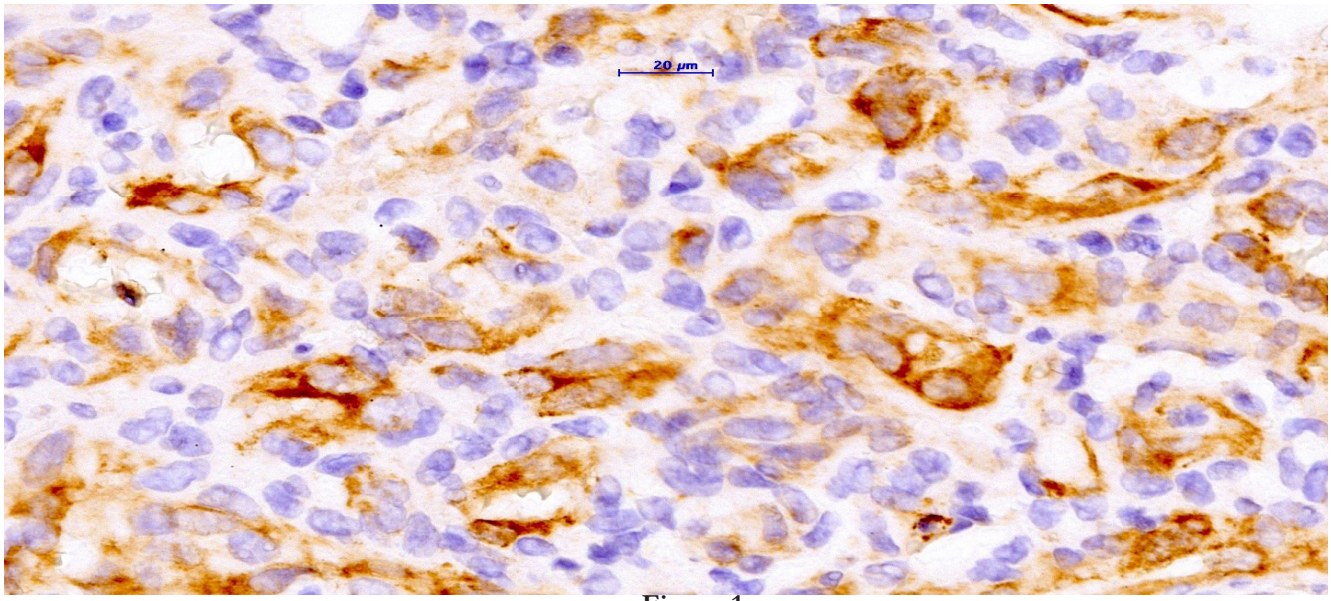

Figure 1.
VEGF immunoexpression inside cells of thymic hemangioma.

INTRODUCTION

Immunohistochemistry is currently a basic method in both pathological diagnosis and research. Immunohistochemical methods used for the routine diagnosis in pathology are well standardized, with only little differences related to the clone of the antibody and manufacturer. In research, mainly when new or fewer usual antibodies are used, the procedures are often not very clear and this aspect frequently leads to controversial results, and often, non-convincing microscopical images. Growth factors often fall in this category, and one of them, vascular endothelial growth factor (VEGF), the main pro-angiogenic molecules known at present time, is not quite easy to make evident by immunohistochemistry and to score it correctly. In the current minireview we show some technical peculiarities, the best score, limits and value of the method.

VALUE

Over 70 years ago, it was proposed the involvement of a diffusible factor in the development of the vasculature in the normal and pathological conditions. It was showed the interrelation with the normal retinal vasculature and with pathological neovascularization from various diseases such as proliferative diabetic retinopathy and also other disorders (1). The demonstration of the role of this factor in tumor cell growth (2, 3) was followed by the hypothesis of antiangiogenic therapy proposed by Judah Folkman in 1971. (4). Later on, in 1989, Napoleone Ferrara identified, isolated and cloned for the first time, the vascular endothelial-derived growth factor (VEGF), one of the most important pro-angiogenic factors (5).

VEGF (VEGF-A) is a member of a family that contains VEGF-B, VEGF-C, VEGF-D, VEGF-E and Placental GF (PlGF). There are many isoforms of VEGF with angiogenic potential: VEGF 121, 145, 165, 183, 189, 206. On the other hand, some inhibitory isoforms of angiogenesis were described: VEGF165b (6) and VEGF-Ax (7).

A high level of VEGF-A, IL 10 and prostaglandine E2 accompanied a hypoxic tumor microenvironment. VEGF-A has some major roles in the tumor microenvironment and antitumor immunity: the angiogenesis stimulation, the killing of T cells by apoptosis intermediated by FasL (expressed on tumor ECs) -Fas (expressed on T cells) relation, the recruitment and the survival of immunosuppressive T cells by the multifunctional endothelial receptor CLEVER-1/ stabilin-1 pathway, the influence on the intercellular adhesion molecule and vascular cell adhesion protein (8,9,10,11). VEGF-A was associated with reduced dendritic cell differentiation from hematopoietic progenitors, the recruitment of immature myeloid cells, the M1 (pro-inflammatory) and M2 (immunosuppressive) phenotype of TAMs and the enhanced expression of PD 1 (12,13,14,15,16,17,18).

Data from the literature demonstrated the prognostic value of VEGF in some tumor types and no significant prognostic value in others, such as melanoma. Overexpression of VEGF, detected by immunohistochemistry was associated with an unfavorable impact on overall survival and on disease free survival in patients with oral and head and neck squamous cell carcinoma (19, 20). The role of VEGF as a prognostic marker was demonstrated in the tumors of the digestive area. Thus, in the gastric adenocarcinoma were found a high VEGF-A level than those in paracancerous tissues. A significant correlation with TNM stage, tumor size, lymph nodes status and lymphovascular invasion was noticed (21). The survival of the patients was significantly longer in the patients with negative or weak expression of VEGF in comparison to those with moderate to high expression on the section in the case of patients with colorectal carcinoma. In this tumour type, VEGF could be considered as a prognosis predictor. The overexpression of VEGF was noticed to the patients with liver metastasis of colon carcinoma (22). It was shown that VEGF plays an important role in the development, growth, metastasis and angiogenesis in advanced hepatocellular carcinoma patients (23).

In non-small lung cell carcinoma, higher VEGF expression was noticed in the adenocarcinoma than squamous cell carcinoma. It was found a significant correlation between the VEGF expression and poor survival outcome after surgery (24). VEGF expression on survival remains unclear and the prognostic significance of VEGF expression in breast cancer was controversial. The diagnostic value of the VEGF was described in the breast cancer. It was useful for cancer diagnostics in stage I and II and in the differential diagnosis between benign and malign cases. The immunoexpression of VEGF was found in the majority of the breast cancer patients. VEGF expression was correlated with other parameters, such as: size, high histologic grade, estrogen and progesterone receptor negativity, human epidermal growth factor receptor-2 over-expression, and lymph node metastasis (25).

The same controversial value of VEGF as a prognostic marker in non- Hodgkin lymphoma was found. But, the implication of VEGF pathway, particularly by stromal cells in the angiogenesis in these tumors type was proposed (26). VEGF prognostic value is not significant in melanomas, the VEGF expression was described in 20% of cases (27).

The therapeutic value of VEGF started with the use of Bevacizumab, the humanized monoclonal antibody. It was approved by the FDA for patients with metastatic colorectal cancer, non-small cell lung cancer, recurrent glioblastoma multiforme and metastatic breast cancer in combination with chemotherapy and renal cell carcinoma in combination with interferon alpha (28). Together with Bevacizumab, on the extracellular VEGF domains, acts VEGF Trap (anti-VEGF compound, engineered by combining domains from VEGFR-2 and VEGFR-1) and Pegaptanib (the VEGF-A aptamer— RNA or DNA oligonucleotides which bind proteins with high affinity and high specificity). The intracellular signaling pathways of VEGF receptors are targeted by tyrosine kinase inhibitors (imatinib, erlotinib, gefitinib, sorafenib and sunitinib) approved for the use in the solid tumors (28).

The used of bevacizumab together with oxaliplatin/ fluoropyrimidine improved the median survival times of up to 2 years (29) in first-line therapy and to 1 year in second-line therapy (30). The success of the treatment with Bevacizumab was not so high in patients with stage II and III CRC. The progression-free survival and overall response rate were found to favor the bevacizumab group in patients with non -small cell lung cancer (31). A statistically significant overall survival benefit was noticed for the patients who received bevacizumab containing therapy in renal cell carcinoma. A favorable progression-free survival was shown in the patients with glioblastoma, receiving bevacizumab as part of first-line treatment, but not an overall survival compared with the control cohort.

Approved by the FDA for the treatment of patients with HER2-negative, metastatic breast cancer in combination with paclitaxel in 2008, Bevacizumab was removed in 2010 from the breast cancer indication therapy. A continued progression-free survival rate with a trend in overall survival improvement was noticed in the ovarian cancer after bevacizumab use (31).

LIMITS

The analysis methods of the immunohistochemical reaction are different and the resulting score could be associated with high dose of subjectivity. The parameter

used for the efficiency of angiostatic compounds -microvascular density is not always usually in routine hospital practice. The clinical consequence of the treatment which interfere with VEGF function are: hypertension, proteinuria, arterial and venous thrombotic events, hemorrhage, gastrointestinal perforation.

Some factors are considered responsible for the anti-angiogenic therapy resistance, one of the major limitation of this type of therapy, such as: redundancy in growth factor signaling, recruitment of bone marrow derived cells, stromal cells, intussusceptive microvascular growth, vasculogenic mimicry and vessel co-option, redundancy in growth factor signaling, increased invasiveness and metastasis, endothelial heterogeneity (32).

SCORING

The immunohistochemical evaluation of VEGF was oriented in two main directions: visual examination by the pathologist and computer-assisted image analysis. In the first situation, two parameters are evaluated: intensity of the immunoreaction and the quantity of VEGF positive cells. Some data based on the evaluation of VEGF intensity only, by calculated of the mean immune reactive score of the three independent observers. The values score was negative, weak, medium and strong and VEGF expression subclasified as low and high grade (33, 34). Other authors used the quantitative methods for VEGF evaluation (35, 36, 26). One of the most used method is the semiquantitative one, which combine the intensity reaction score and quantity of positive cells. The values score in this case were: mild (intensity value =1 and less than 10% positive cells), moderate (intensity value= 2 and 10-50% positive cells) and intense (intensity value=3 and more than 50% positive cells) (37). Methods for computer-assisted image analysis of VEGF-A have been described also (38, 39).

TECHNICAL APPROACHES

The VEGF A antibodies found in the suppliers are rabbit polyclonal or mouse monoclonal anti human. One of the most used clones for VEGF A was VG 1, agreed in our lab also. This is a mouse monoclonal VG1 to VEGF A, which detects the 121, 165 and 189 VEGF isoforms. The heat induced epitope retrieval was recommended, with pH 6 or pH 9 retrieval solution for 15-20 minutes. In our lab a pH 9 retrieval solution for 20 minutes was preferred for the automated system. The recommended dilutions varied between 1:25 and 1:250 and the incubation time in our lab was 30 minutes. The incubation time for the visualization system, used by us, in the automated way was 15 minutes. The evaluated immunoreaction pattern was granular cytoplasmic. Positive controls could be represented by kidney tissue, especially tubular structures of the kidney.

CLOSING REMARKS

Future studies which focus on VEGF reliable measurements are essential for their validation and application in routine hospital practice and clinical trials.

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