Arch. Biol. Sci., Belgrade, 65 (2), 487-491, 2013 DOI:10.2298/ABS1302487D

EXPRESSION AND POSSIBLE SIGNIFICANCE OF VASCULAR ENDOTHELIAL GROWTH FACTOR IN NON-HODGKIN LYMPHOMA

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Abstract - There is little information about the exact trigger mechanisms of vascular endothelial growth factor (VEGF) expression in hematolymphoid malignancies. The aim of this study was to investigate the expression of VEGF in malignant non-Hodgkin lymphoma (NHL), in terms of its immunohistochemical distribution and clinicopathological significance. We evaluated the distribution in tumor cells, macrophages and in non-tumoral cells. VEGF immunoreactivity was estimated according to this score: 0 (0% positive cells); 1 (<10%); 2 (10-30%); 3 (>30%). Histopathological evaluation revealed 18 cases of diffuse type lymphoma and 3 cases of follicular type lymphoma. VEGF was positive in 95.23% of cases and in one case, VEGF was negative in all cell types. We noticed a significant correlation in VEGF expression between tumor cells and macrophages (p=0,001), tumor cells and non-tumoral cells (p=0,002). In non-Hodgkin lymphoma, the main mechanism of angiogenesis seems to be dependent on the VEGF pathway and its expression particularly by stromal cells.

Key words: Angiogenesis, macrophages, VEGF, non-Hodgkin lymphoma, tumor microenvironment

INTRODUCTION

Angiogenesis is required for tumor growth and metastasis and is an important component in the control of cancer progression (Folkman, 2002). Hematologic malignancies are a group of heterogenous diseases with a wide range of features and many disease-associated variables that make the response to treatment differ considerably among patients. Angiogenesis-associated parameters are important for prognosis in NHL, and VEGF and its receptors (VEGFR) are an important target for antiangiogenic therapy. The role of angiogenesis might vary in lymphoma subtypes because the prognostic value of microvessel density and the expression of angiogenesis-related molecules differ between lymphoma subtypes (Giles, 2001).

Lymphoma growth and progression is potentiated by two angiogenic mechanisms: autocrine stimulation of tumor cells through the expression of VEGF and VEGFR by lymphoma cells, and paracrine influences of a proangiogenic tumor microenvironment through neovascular transformation and the recruitment of circulating bone marrow-derived progenitors (Ruan et al., 2009).

VEGF is the major angiogenic factor and together with its receptors regulates different aspects of vascular angiogenesis and lymphangiogenesis. It regulates several endothelial cell functions, including mitogenesis, permeability, vascular tone, and the production of vasoactive molecules. The activity of VEGF is mediated through three receptor tyrosine kinases: VEGFR-1 (Flt-1), VEGFR-2 (KDR/Flk-1),

and VEGFR-3 (Flt-4). VEGFR-1 is expressed on endothelial cells and monocytes and mediates cell motility, hematopoiesis and cell recruitment. The proliferative and mitogenic activities of VEGF, as well as vascular permeability, are mediated primarily through VEGFR-2, and VEGFR-3 mediates lymphangiogenesis (Koster et al., 2005).

Tumor cells produce VEGF-A and other angiogenic factors, bFGF, P1GF, VEGF-C that promote neoangiogenesis through sprouting angiogenesis of mature resident endothelial cells, and vasculogenesis from the recruitment of bone marrow-derived progenitor cells. VEGF-A also supports the survival, proliferation and migration of lymphoma cells that express VEGFR-1 and VEGFR-2 in an autocrine fashion. The tumor stroma made of fibroblasts, inflammatory and immune cells provides additional angiogenic factors (Ruan et al., 2009).

Taking into account the particular implications of angiogenesis and VEGF in the growth and development of malignant lymphomas, we investigated the expression of VEGF in malignant non-Hodgkin lymphoma in terms of its immunohistochemical distribution and clinicopathological significance. We evaluated the distribution in tumor cells, macrophages and in non-tumoral cells.

MATERIALS AND METHODS

Twenty-one specimens of lymph node biopsies were included. Specimens were fixed in buffered formalin and embedded in paraffin, based on the conventional histological technique. Step sections, 5 mm thick, were prepared for each case. Initial sections were stained with HE for the pathological diagnosis. Histopathological evaluation revealed 18 cases of diffuse type lymphoma and 3 cases of follicular type lymphoma. Additional sections from each case were stained for VEGF. After heat-induced epitope retrieval in citrate buffer pH 9 for 15 min (with PT link modules; DakoCytomation, Denmark) the immunohistochemical technique used was based on an avidin-biotin method using LSAB+ working system that followed incubation with primary antibody VEGF (VG-1 clone, ready-to-use, 30 min incubation time at room temperature, Labvision/Neomarkers, Fremont, CA, USA). 3,3'-diaminobenzidine was used as chromogen. Staining of nuclei was performed with modified Lille's hematoxylin. The entire immunohistochemical procedure was performed with a DakoCytomation Autostainer. We evaluated the distribution in tumor cells, macrophages and in non-tumoral cells. VEGF immunoreactivity was estimated as the percent of positive cells according to this score: 0 (0% positive cells); 1 (<10%); 2 (10-30%); 3 (>30%). Microscopic images were captured in JPEG format, using a Nikon Lucia G program of analysis of the microscopic image (Nikon, Tokyo, Japan). The local research ethics committee approved the protocol of the study, and informed consent was obtained from all subjects according to the World Medical Association Declaration of Helsinki.

RESULTS

Histopathological evaluation based on a routine hematoxylin and eosin method revealed 18 cases of diffuse non-Hodgkin lymphoma and 3 cases of follicular non-Hodgkin lymphoma.

VEGF expression was present in 95.23% of cases, both follicular and diffuse type of non-Hodgkin lymphoma. In only one case was the VEGF negative in all evaluated three cell types.

In all three cases of follicular non-Hodgkin lymphoma we noted a similar VEGF expression in macrophages and tumor cells – the value +2 according to our score. The VEGF expression values in non-tumor, non-macrophage cells ranged from 0, 1, 3 according to our score.

In follicular non-Hodgkin lymphoma we noticed a heterogeneous expression of VEGF in terms of distribution and intensity of reaction. VEGF positive tumor cells and macrophages were observed predominantly at the periphery of the lymph node. The macrophages presented the maximum intensity expression of VEGF with a granular cytoplasmatic pattern (Fig. 1).

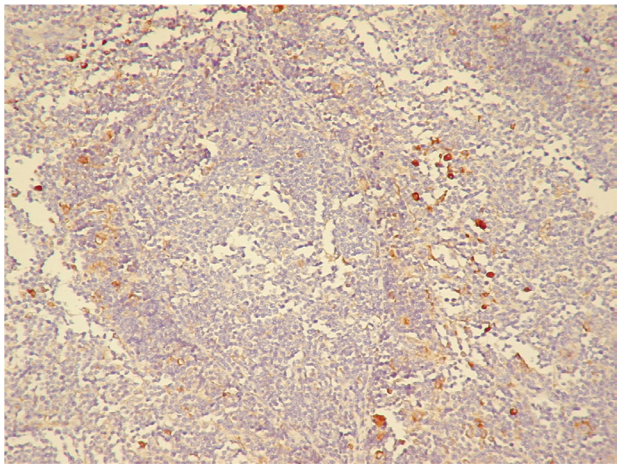

Fig. 1. A heterogeneous expression of VEGF in terms of distribution and intensity of reaction in a follicular non-Hodgkin lymphoma, magnification X100

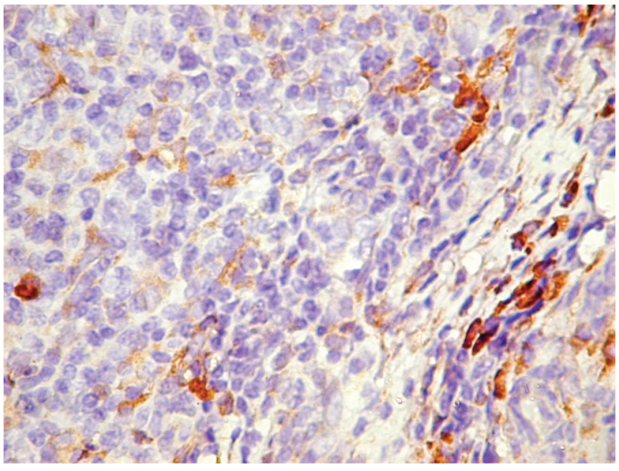

Figure 2b. Immunoexpression of VEGF in macrophages, +3 score, magnification X400

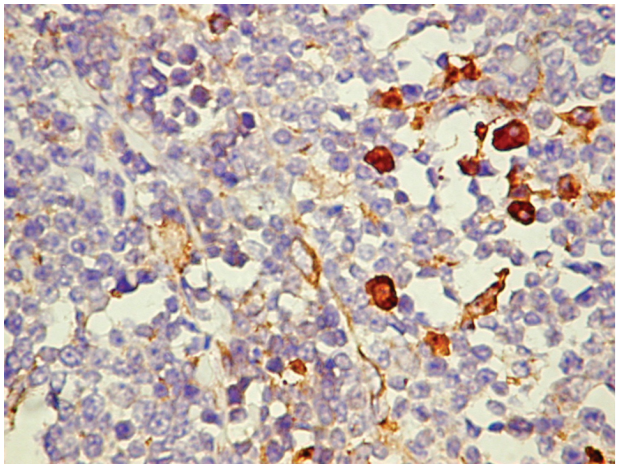

Fig. 2a. Immunoexpression of VEGF in tumor cells, +3 score, magnification X400

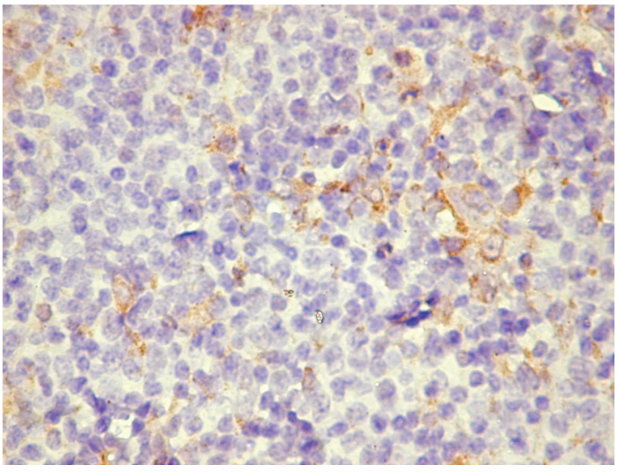

Fig. 2c. Immunoexpression of VEGF in non-tumor non-macrophages cells, +3 score, magnification X400

In diffuse non-Hodgkin lymphoma, we found a similar value +3 in tumor cells, macrophages in 6 cases – 33.33% (Figs. 2a, 2b), +2 in 2 cases – 11.11%, +1 in one case – 5.55%. According to our score, we noticed a +3 value in non-tumor, non-macrophages cells (Fig. 2c). The reaction intensity was higher in macrophages, then in non-tumor, non-macrophage cells of stroma, and tumor cells.

Different values for tumor cells and macrophages (+3 tumor cells, +2 for macrophages – in 6 cases, +2 in tumor cells, +1 in macrophages – in 1 case, +1 in tumor cells, 0 in macrophages – in 1 case). For non-tumor, non-macrophage cells we found the following values: 0 (in 5 cases) 1 (3 cases), and 3 (10 cases).

For all types of lymphoma included in the present study we found a significant correlation in VEGF expression between macrophages and tumor cells ($p<0.001$) and between tumor cells and non–tumor, non-macrophage cells ($p<0.002$).

DISCUSSION

Current data suggest the important prognostic and therapeutic implications of angiogenesis in a variety of malignancies of the hematopoietic system including NHL. Wang et al., (2004) examined angiogenesis in NHL by using *in vivo* and *in vitro* techniques. They demonstrated that human lymphoma cells secrete VEGF and express VEGFR-1 and VEGFR-2, and supported the presence of autocrine VEGFR-1 and paracrine VEGFR-2 mediated pathways in lymphangiogenesis. Another study of angioimmunoblastic T-cell lymphoma showed that there was increased expression of VEGF in lymphoma cells and in endothelial cells. Cellular and circulating levels of VEGF are elevated in hematologic malignancies and are adversely associated with the prognosis (Giles, 2001).

The increased VEGF expression was correlated with extranodal involvement and with decreased survival. In follicular lymphoma, increased vascularization was associated with improved clinical outcome (Koster et al., 2004). VEGF expression was detected in all NHL subtypes, the strongest in peripheral T-cell lymphoma. In follicular lymphoma, patients with diffuse VEGF expression in lymphoma cells had poorer overall survival than those with focal expression (Jorgensen et al., 2007). In follicular non-Hodgkin lymphoma we noticed a heterogeneous expression of VEGF in terms of distribution and intensity of reaction higher at the periphery of the lymph node. We noticed the highest expression levels of VEGF in both tumor cells and macrophages.

According to recent data, the tumor-associated macrophage content is a novel favorable prognostic factor in immunochemotherapy-treated follicular lymphoma patients (Taskinen et al., 2007), whereas a high mast cell score is associated with unfavorable prognosis (Taskinen et al., 2008). We found a significant correlation in VEGF expression between macrophages and tumor cells for all types of lymphoma included in the present study.

In all NHLs, significant correlation was found between vessel count and the number of mast cells ($p < 0.0001$) and between vessel count and the number of VEGF-expressing cells ($p < 0.05$), but not between vessel count and bFGF-expressing cells. A strong correlation was detected between the number of mast cells and the number of VEGF-expressing cells in all non-Hodgkin lymphoma (Fukushima et al., 2001). In our study, we found a significant correlation between VEGF expression in macrophages and tumor cells on the one hand and between tumor cells and the cells of the tumor stroma – non-tumor, non-macrophage cells, on the other hand.

CONCLUSIONS

In non-Hodgkin lymphoma the main mechanism of angiogenesis seems to be dependent on the VEGF pathway and its expression, particularly by stromal cells. Of these, macrophages and non-tumor, non-macrophage cells stand out as important.

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