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PATTERNS OF THE VASCULAR ENDOTHELIAL GROWTH FACTOR IMMUNE-HISTOCHEMICAL EXPRESSION IN LUNG CARCINOMAS

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Abstract. Vascular endothelial growth factor (VEGF) has been demonstrated as the main angiogenic molecule in vitro and in vivo. The involvement of VEGF in the prognosis and progression of different types of tumors has been demonstrated by many publications. In the present study we have investigated the patterns of the immunohistochemical expression of VEGF in lung carcinoma. We have investigated 42 biopsies from patients admitted with different histological types of lung carcinoma. The aim of this investigation was to describe the incidence and models of distribution of the positive reaction for VEGF in lung carcinoma. The reaction for VEGF has been evaluated using a scale from 0 (negative) to 3 (strong positive), based on the percentage of positive cells. We found a strong expression for VEGF in cases with squamous cells carcinoma, adenocarcinoma and hepatoid carcinoma, and negative reaction in small cell and large cell carcinomas. Our data suggest that the incidence and intensity of VEGF expression in lung carcinoma can be a good indicator of antiangiogenic therapy based on humanized monoclonal antibody against VEGF.
Keywords: lung carcinoma, vascular endothelial growth factor (VEGF), angiogenesis

INTRODUCTION

The discovery of vascular endothelial growth factor (VEGF), the most powerful angiogenic molecule, is a story extended on decades, and it came to a practical endpoint with the introduction of bevacizumab in the clinical practice. Judah Folkman identified in 1971 a soluble substance, named tumoral angiogenic factor (TAF) with mitogenic properties for endothelial cells, responsible for the appearance of new capillary vessels. In 1983, Harold Dvorak had identified and purified part of the vascular permeability factor (VPF). In 1989, Napoleone Ferrara and his Genentech team were able to isolate a protein with mitogenic effects on endothelial cells that was called vascular endothelial growth factor (VEGF) (Ribatti, 2007). Further tests have shown that VPF and VEGF have the same properties. VEGF gene produces 5 kinds of messenger RNA that encodes different VEGF variants, classified according to the molecular weight and biological properties (Neufeld, 1999). VEGF family includes 5 distinct molecules with structure of a homodimeric glycoprotein: VEGF-A, B, C, D and placenta-like growth factor, which bind the specific tyrosine-kinase receptors VEGFR-1, 2, 3. The main effects of VEGF is mediated by action of the complex VEGF and its cognate receptor located on the surface endothelial cells.

The involvement of VEGF in the progression, metastasis, and prognosis was demonstrated by many studies, like in cancer of the lung, kidney, colorectal or glioblastoma (Fontanini et al., 1998; Ishigami et al., 1998; Chaudry et al, 2001).

Yuan et al (2000) have found that levels of VEGFmRNA in the tumor tissue from patients with non-small cell lung cancer were significantly higher than the adjacent lung tissue. They have shown that lung adenocarcinomas showed higher levels of VEGFmRNA as compared with squamous cell carcinoma. This fact may explain in part the high metastatic potential of lung adenocarcinomas. Yuan and Bremnes (2006) noticed that overexpression of VEGF has been associated with decreased survival in lung cancer. Jarzynka et al (2006) have found a significant correlation between increased expression of VEGF and the tumor progression in lung carcinoma.

Although there are many studies on the expression of VEGF in lung cancer, only few of them characterized the immunohistochemical expression related to the pathological forms. Moreover, there are no definite data regarding the relationship between the expression of VEGF and conventional factors of prognosis. Therefore, the aim of this study was to investigate the expression of VEGF in different pathological types of lung carcinomas, and to define particular patterns that could be used as target for antiangiogenic therapy.

MATERIAL AND METHODS

We included in our study 42 biopsies from patients with different types of lung carcinoma. Specimens were fixed in buffer formalin and paraffin embedded. Five μm thick step sections were performed from each case. Slides from each case were stained with haematoxylin-eosin method for the pathologic diagnosis. VEGF expression was detected by immunohistochemistry, and we used anti-VEGF antibody. Slides were dewaxed and

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rehydrated, treated by heat-induced epitope retrieval in citrate buffer pH9 for 15' (with PT link module, Dako Cytomation, Denmark) and endogenous peroxidase was blocked with hydrogen peroxide 3%. It was followed by incubation with primary antibody, VG-1 clone, ready for use (Labvision 1⁄4 Neomarkers, Fremont, CA, USA) for 30 minutes at room temperature. The LSAB+- HRP working system was used, followed by visualization with 3,3 diamino-benzidine dyhidrochloride. Nuclei were stained with Lillie's modified haemotoxylin (Dako, Glostrup, Denmark). The full immunohistochemical procedure was performed with DakoAutostainer Plus (DakoCytomation). Image acquisition and analysis were performed using Nikon Eclipse E 600 microscope and Lucia G software for microscopic image analysis. Immunoreaction for VEGF has been evaluated according to the following score: 0 (0% positive cells), 1 (< 10% positive cells), 2 (10-30%), 3 (> 30%). 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

By histophatologic examination, we evaluated 5 cases of small cell carcinomas, 5 adenocarcinomas, 1 case of large cell carcinoma, 1 case of hepatoid carcinoma, 27 squamous cell carcinomas and 3 cases of neuroendocrine lung carcinomas.

Concerning the VEGF immunohistochemical assessement in lung adenocarcinomas we noticed the presence of VEGF as a granular cytoplasmic pattern, and with an intensity scored as +2 (10-30% positive cells) and +3 (over 30% positive cells). Expression of VEGF with moderate intensity (+2) was found in 2 cases (figure 1) representing 40% from studied lung adenocarcinomas. Three from the 5 adenocarcinomas included in the study were had a VEGF expression in tumor cells scored as +3 (figure 2).

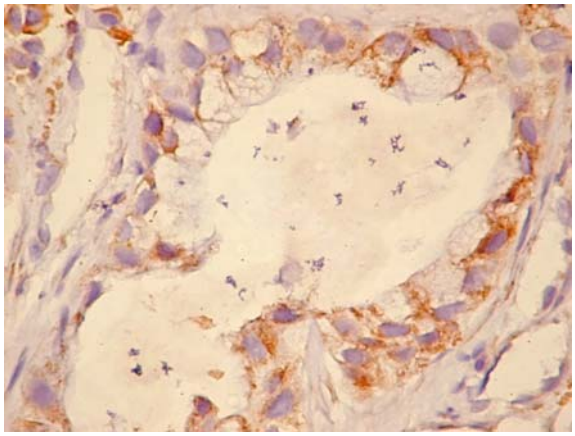

Figure 1- lung adenocarcinomas, score +2- VEGF expression with homogeneous intensity in the 10-30% tumor cells; VEGF immunostaining, x400

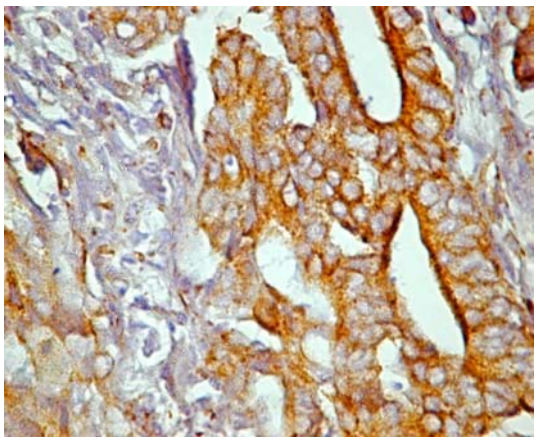

Figure 2- Lung adenocarcinoma, score +3- expression of VEGF in >30% tumor cells; VEGF immunostaining, x400

One of the 5 cases of small cell lung carcinomas was negative for VEGF, two were scored as +1-(40%), and other two had moderate intensity expression (figure 3). No cases with high VEGF expression were found for this histopathologic type.

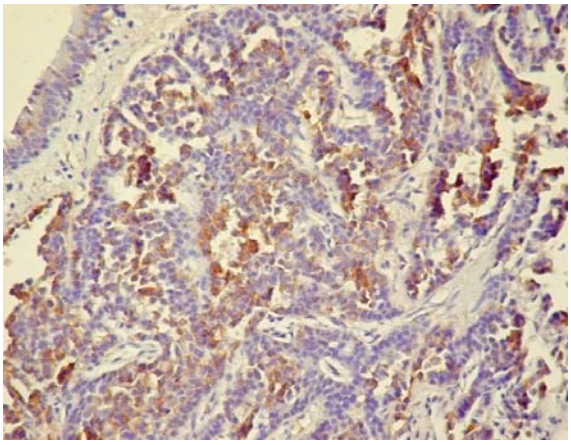

Figure 3- Small cell lung carcinoma, score +2; VEGF immunostaining, x 200

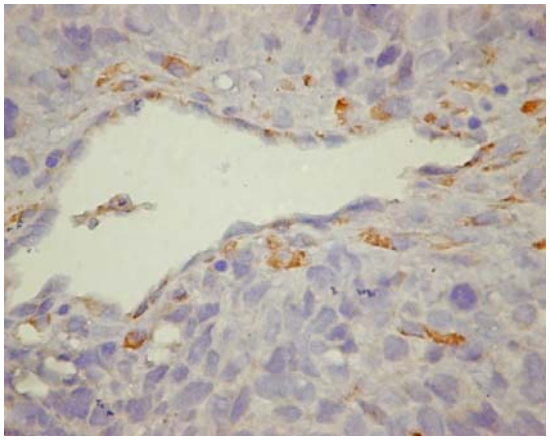

Figure 4- granular cytoplasmic pattern of VEGF in perivascular tumor cells of a large cell lung carcinoma, VEGF immunostaining, x 400

The VEGF granular cytoplasmic expression was preserved, with homogeneous distribution in the cells of the center and periphery areas of the tumor.

The only one case of large cell carcinoma was evaluated with a score value of 2. It was characterized by the presence of 10-30% tumor cells which expressed VEGF. In this situation, the expression of VEGF was noticed with a heterogeneous distribution, predominantly in tumor cells from blood vessels vicinity (figure 4).

Tumor cells of the only case of hepatoid carcinoma of the lung expressed VEGF for more than 30%, and we assessed it as + 3 (figure 5).

In squamous cell lung carcinomas we noticed the predominance of intense expression of VEGF (+3), similar with those from lung adenocarcinomas. From 27 cases of squamous cell lung carcinomas, 15 (55.55%) were graded as + 3, 4 (14.81%) scored as + 2,and 5 (18.51%) as +1.We observed the lack of VEGF expression in 3 cases (11.11%) of squamous cell lung carcinomas. The granular cytoplasmic pattern of VEGF was similar with the expression found in the previously described histopathologic types.

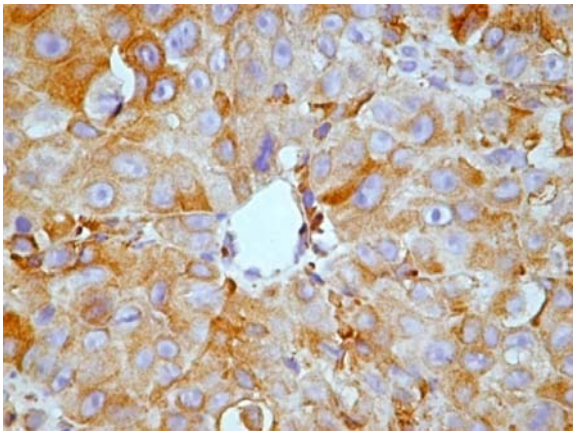

Figure 5- score +3 of VEGF expression in the hepatoid carcinoma case, VEGF immunostaining, x 400

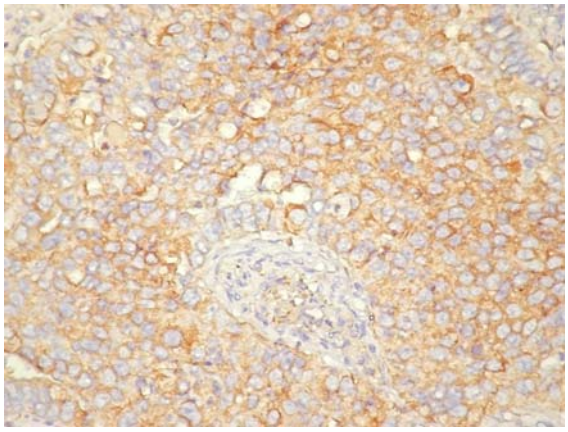

Figure 6- score +3 in the squamous cell lung carcinoma, VEGF immunosatinig, x 200

Neuroendocrine lung carcinomas, were evaluated with an expression of VEGF in tumor cells as a percentage of more than 30% (+3). A case has been negative for VEGF immunoreactivity in tumor cells. VEGF expression in different types of lung carcinomas is summarized in the following table:

Score	0	1	2	3
Lung adenocarcinomas	-	-	2 cases	3 case
Small cell lung carcinomas	1 case	2 cases	2 cases	-
Large cell lung carcinomas	-	1 case	-	-
Squamous cell lung carcinomas	3 cases	5 cases	4 cases	15cases
Hepatoid lung carcinoma	-	-	-	1 case
Neuroendocrine carcinomas	1 case	-	-	2 cases

Table No.1- Relation between score of VEGF expression, histophatological type and number of cases.

DISCUSSIONS

VEGF has been extensively studied in lung adenocarcinomas, but despite all of these efforts, a specific therapy has not been found.

More, not only the expression of VEGF-A, but also those of VEGF- B, C, D were studied in primary tumors and lymph node status in terms of lung adenocarcinomas. Toshiro *et al*., *2000* have noticed that only the levels of VEGF-A and not those of VEGF-C and B were higher in the tumors with larger lymph node metastases (> 1 cm) compared to those with small nodal metastases (< 1 cm). These results support the hypothesis that two members of the VEGF family are involved in metastasis: VEGF-A favors the metastasis growth by angiogenesis and VEGF-C which facilitates invasion of tumor cells in the lymphatic system.

Li *et al., 2009* showed that the prognosis of patients with lung adenocarcinomas is significantly correlated with the angiogenesis, the VEGF and Notch ligand DDL4 expression. Studies of Inda *et al., 2007* have shown differences in the expression of VEGF in adenocarcinomas (82% of cases were positive) and squamous cell lung carcinomas (24% of cases -positive). VEGF expression in adenocarcinomas was much more intense compared to squamous cell carcinomas, but microvascular density did not present significant changes. Our results have shown a maximum score of VEGF expression in both the adenocarcinomas (60%) and squamous cell lung carcinomas (55.55%).

Tanno *et al., 2004* have shown by western blotting the presence of VEGF, VEGF-C and their receptors VEGFR2 and VEGFR3 in five cell lines of small cell lung carcinomas. They noticed that the levels of expression are higher in hypoxic conditions. In our study, it was noticed a score of VEGF with values between 0 and 2, and lack of high expression (+3) in cases of small cell lung carcinomas. *Fontaninni et al., 1998* has shown that p53 and bcl2 controls the development of tumor angiogensis in the non- small cell lung carcinomas marks, through the mediation of VEGF. An immunohistochemical study realized by Takahama *et al., 1998* on 155 primary tumors and 26 metastases, focused on the VEGF expression highlighted that immunostaining for VEGF was present in 86.5% adenocarcinomas, 56.7% of squamous cell lung carcinomas, 100% large cell carcinomas, 66.7% adenosquamous carcinomas, 20% small cell carcinomas. In our study, the small cell lung carcinomas was the only histopathological type which not presented the +3 VEGF expression. In all other types at least one case was scored as +3. The influence of different angiogenic factors involved in lung carcinomas have shown that VEGF plays an important role in non-small cell lung carcinomas compared with other growth factor (Aikawa *et al., 1999*).

The five years survival rate for stage I to IV patients in, according to the TNM classification is 15%, and this rate was improved with only 5% in the last 40 years (Spira *et al., 2004*). Although the multiple pathways involved in the progression and metastasis of non-small cell lung carcinomas have been described, only two of them (the EGF and VEGF pathways) are used as a potential molecular therapeutic targets in clinical practice:.

CONCLUSIONS

Knowledge of the peculiarities of VEGF expression in the different types of lung carcinomas may be useful in addressing future therapeutic practices and finding optimal combinations to allow blocking of tumor angiogenesis eficiently. Our study showed a maximum score (3) of the VEGF expression in squamous cell, adenocarcinomas, hepatoid, neuroendocrine lung carcinomas and the absence of this value in the small and large cell lung carcinomas. In these last histophatological types the VEGF score had values between 0 and 2. The use of drugs targeting the various pro-angiogenic factors or combination of drugs with different mode of action-monoclonal antibodies with tyrosine kinase inhibitors can help more effectively targeting tumor angiogenesis.

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