ORIGINAL PAPER

Vascular endothelial growth factor A (VEGF A) as individual prognostic factor in invasive breast carcinoma

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Abstract

VEGF is a potent mitogen for endothelial cells and also acts in an autocrine and paracrine manner for development of tumor cells in breast cancer. Correlations between VEGF and some clinicopathologic findings were largely studied but results were controversial. Our purpose was to find if VEGF could be used as individual prognostic factor in invasive breast carcinoma. We included in our study 35 cases of invasive breast carcinoma, which were immunostained for VEGF using monoclonal antibodies anti-VEGF clone VG1. The assessment of VEGF expression used a scoring system, which included an intensity parameter correlated with percent of positive tumor cells. We found positive correlation between ductal invasive carcinoma type of breast cancer and VEGF expression. In addition, presence of inflammation associated with breast malignancies had a significant correlation with VEGF positive staining. Because of these correlations found in our study, we concluded that VEGF could not be used as individual prognostic factor in invasive breast carcinoma.

***Keywords*:** invasive breast carcinoma, immunohistochemistry, prognosis, VEGF.

Introduction

Angiogenesis, the formation of new blood vessels is essential for tumor expansion and the vascular endothelial growth factor (VEGF) is one of the most potent angiogenic growth factors known. VEGF is expressed in a variety of normal and tumor cells. For mammary gland, the expression of VEGF was found in normal conditions in the epithelial cells lining the ducts but the expression was weak and inconstant. Many malignant tumors of the breast are positive for VEGF in both neoplastic epithelial cells and stromal cells. Cancer progression is dependent on the development of a rich vascular network that supplies nutrients to the growing tumor [1–4]. VEGF is produced by many tumor cells including breast cancer cells [5, 6] and some data suggest that VEGF content of tumor cells is correlated with the prognosis of patient with breast cancer [7, 8]. In addition, expansion of many endocrine dependent tumors including breast cancers depends on sex-steroid hormones. Presence of both estrogen and progesterone receptors influences the progression of the disease and it was suggested that these receptors could control the angiogenic factors via their respective ligands [6, 9]. Regulation of VEGF is under both transcriptional and translational control [10]. Some studies revealed that VEGF acts as a growth stimulator and a proliferation factor for mammary cancer cells [11] but the mechanism of this interaction is not fully understood.

The correlation of VEGF expression in breast cancers with molecular and clinico-pathologic findings from thymidine phosphorilase [12] to serum VEGF level [13] is discussed for a long time. In 2000, Gasparini G [15] reviewed the studies concerning VEGF involvement in the prognosis of breast cancer. He concluded that VEGF levels support the notion that human breast cancer is angiogenic dependent but in most of the described studies, VEGF was not correlated with the conventional prognostic factors.

Our study included age, tumor size, histopathology, tumor grade, TNM staging, and inflammation as parameters for correlation with VEGF expression in breast cancer. All these analysis were made to find if VEGF could be used as an individual prognostic factor in invasive breast carcinoma. Any correlation between one of the parameters described above and VEGF expression failed to prove the useful of VEGF expression as individual prognostic factor.

Material and methods

Histopathology

Our study included 35 cases of breast tumors. Patients aged between 26 and 81-year-old were clinically evaluated. Biopsies from primary tumors and 1–44 lymph nodes were obtained from each case by open surgery and were fixed in neutral buffered formalin for 24 hours.

Sections of 5 μm thickness were stained with routine Hematoxylin–Eosin for histopathologic diagnosis.

Immunohistochemistry

Immunohistochemistry was applied on primary tumors biopsies for all cases using monoclonal antibodies anti VEGF, clone VG1. Before incubation with primary antibody, we performed heat induced epitope retrieval for 15 minutes with high pH antigen retrieval solution (DAKO Cytomation, DK). Endogenous peroxidase inactivation in 3% aqueous hydrogen peroxide for 5 minutes was followed by incubation with primary antibody anti VEGF, dilution 1:25 for 1 hour at room temperature. To complete imuunohistochemical stain we applied LSAB+ working system and DAB as chromogen. Counterstained was performed using modified Lille's Hematoxylin.

VEGF scoring

We applied for all cases VEGF scoring system described and used by Raica M *et al.* [15] for VEGF interpretation in renal cell carcinoma. Briefly, they noted negative staining with 0, weak positive (+1, weak reaction in less than 10% of tumor cells, Figure 1), moderate positive (+2, weak-moderate reaction in 10–50% of tumor cells, Figure 2) and intense positive (+3, strong or moderate intensity in more than 50% of tumor cells, Figure 3).

Statistical analysis

The data were analyzed using the *Stat Plus 2007* software package. The correlation between the immunohistochemical expression of VEGF and clinico-pathologic findings including age, tumor size, histopathology, grade, lymph node metastasis, TNM status and invasion was determined. $P<0.05$ was considered to be statistical significant.

Results

We analyzed 35 cases of breast tumors. Medium size of the biopsies varied between 1.5 to 14 cm. By light microscopy, on routine Hematoxylin–Eosin stain, we identified 31 cases (88.6%) of infiltrating ductal carcinoma, two lobular carcinoma (5.7%), and two papillary type carcinoma (5.7%). From 31 cases of infiltrating ductal carcinoma, 18 (51.42%) were associated with foci of intraductal carcinoma. Concerning the tumor grade, 15 cases were G3, 17 cases were evaluated as G2 and only three cases presented microscopic criteria for G1.

VEGF was positive in 30 cases (87.15%) in the cytoplasm of tumor cells with granular pattern. Using VEGF scoring described above, we found 18 cases with +3 pattern, seven cases with +2, and other five cases noted with +1. The intensity of staining was heterogeneous but most of the cases showed moderate to intense expression of VEGF.

In some cases, the periphery of tumors was more intensely stained than the core of the tumors. Positive cells were also found in the stroma of the tumors with spindle or macrophage like pattern. In addition, isolated blood vessels had positive endothelium for VEGF. In all VEGF positive subgroups, most of the positive cases for VEGF aged over 50 years old. The distribution of VEGF positive cases linked to the age is shown in Table 1.

Table 1 – ***Age related distribution of VEGF positive cases***

	20–29-year-old	30–49-year-old	50–81-year-old
VEGF (+3)	0	8	10
VEGF (+2)	1	0	6
VEGF (+1)	0	2	3
Total	*1*	*10*	*19*

By statistical analysis we found no significant correlation ($p = 0.1438$) between age and VEGF expression for patients with breast cancer. Another parameter studied was the size of the tumors (Figure 4).

A tumor size of 4–8 cm was found in 29 cases (83.4%). Half of these cases had a score for VEGF noted as +3. Three of six tumors with size over 9 cm expressed low levels of VEGF which were scored as + 1, two were negative and only one expressed high levels (+3) of VEGF. Despite of an apparent correlation between VEGF expression and tumors size between 4 to 8 cm, p-value calculated for these parameters was $p = 0.1597$.

From 31 cases of infiltrating ductal carcinoma, 27 (87.1%) were positive for VEGF. We observed a significant correlation between histopathologic type and VEGF expression ($p = 0.0328$). Tumor grade and VEGF expression was summarized in the Table 2.

Table 2 – ***Distribution of VEGF positive cases linked to the tumor grade***

	G3	G2	G1	Total
VEGF score (+3)	10	8	0	*18*
VEGF score (+2)	1	5	1	*7*
VEGF score (+1)	2	1	2	*5*
VEGF score (0)	2	3	0	*5*
Total	*15*	*17*	*3*	*35*

VEGF expression linked to the tumor grade revealed that most of positive cases for VEGF with intense staining (+3) were G3 (28.57%) and G2 (22.85%) but a significant correlation between this two parameters was not demonstrated ($p = 0.542$ for G3 and $p = 0.397$ for G2 subgroups).

Tumor stage of TNM system was included in our study and correlated separately with VEGF expression. Twenty-four positive cases for VEGF have been classified as T1c and T2 stage. The stage noted as T4a included only two cases one of them negative for VEGF and another weak positive (+1). For T4b stage, the VEGF expression was similar with those of T1c and T2 stages. No correlation was found between VEGF and T component ($p = 0.91$).

Number of lymph nodes with metastasis was not correlated with VEGF expression in our study ($p = 0.613$). Thirty cases had tumor-associated inflammation. From this subgroup, 25 specimens were positive for VEGF. Correlation between the presence of inflammation and VEGF expression was significant ($p = 0.0395$).

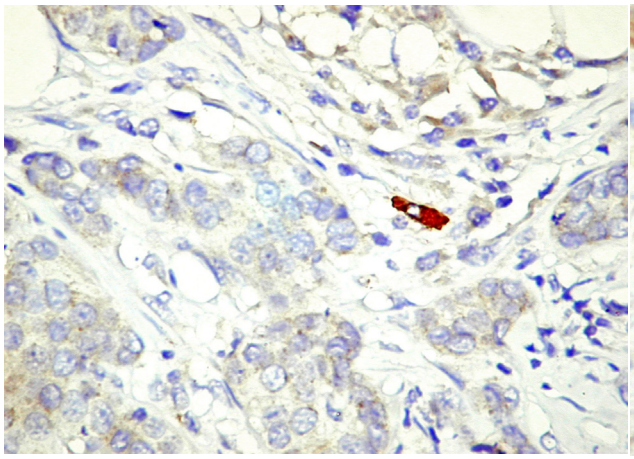

Figure 1 – ***Positive staining for VEGF noted with +1***

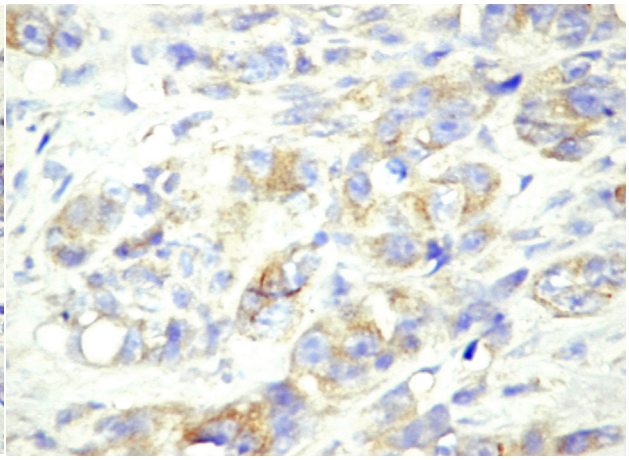

Figure 2 – ***VEGF expression scored as +2 in invasive breast cancer***

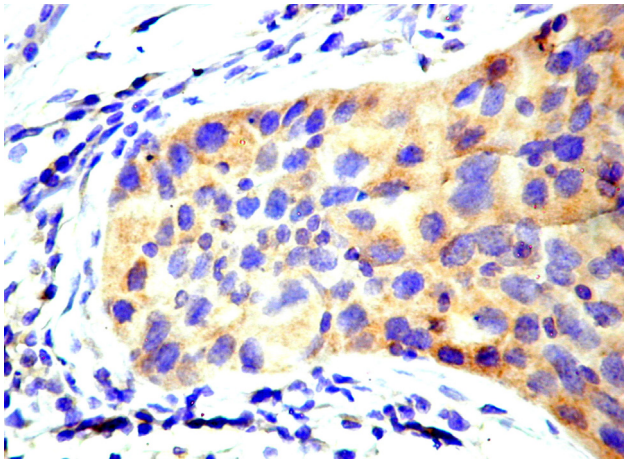

Figure 3 – ***VEGF immunostaining pattern with +3 score***

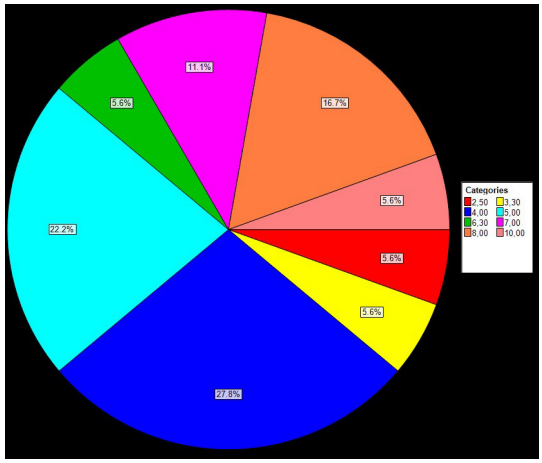

Figure 4 – ***Percentage of breast tumors subgrouped by size***

Discussion

VEGF is a potent angiogenic growth factor [16] up regulated in many tumors [17]. It acts through receptor mediated pathways [18] and represents a target for antiangiogenic therapy, first approved as neoadjuvant treatment in human colon tumors with metastasis [19, 20] and recently in advanced non-small cell lung carcinoma [21].

In many other tumors like ovarian malignancies [22], and breast cancer [23] anti-VEGF therapy is still a subject of debate. It is well known that VEGF is involved in tumor angiogenesis mainly as a mitogen for endothelial cells of tumor blood vessels. The presence of its receptors (VEGFR1 and VEGFR2) both on endothelial cells and tumor cells [24] suggested that VEGF may play additional roles in tumor pathology like involvement in autocrine dependent tumor cell proliferation and invasion [25].

Another problem is represented by the scoring systems used for VEGF assessment in breast cancer. There were proposed and used different methods, which included only the intensity of immunostaining scored as weak or strong [26], or more than three parameters [27] without using of positive cells percentage. VEGF scoring system used in our study is simple and more reliable and made a better correlation between the intensity of staining and percent of positive cells in breast cancer.

We found positive cells for VEGF in 87.15% from all studied cases. Previous studies reported heterogeneous percent of positive cases for VEGF in breast carcinoma (Table 3).

Table 3 – ***Review of literature concerning the percent of positive cases in VEGF studies for breast carcinoma***

Author	Year	Cases	%VEGF	References
Adams J *et al.*	2000	53	80	[28]
Rivkin SE *et al.*	2004	125	37	[29]
Konecny GE *et al.*	2004	611	58.80	[30]
Wei-guo Xu *et al.*	2007	88	70.45	[31]

A variability of positive cases could be derived from different clones for VEGF used to immunostain the breast cancer specimens.

High number of positive cases for VEGF sustains the concept that this growth factor is involved in the development of breast tumors by different mechanism. Predominance of VEGF intense staining for most of the cases over 50-year-old reported in the present study may be explained by estrogen-mediated regulation of VEGF.

Previous experimental studies showed that MCF7 human breast cancer cells were found to express four VEGF mRNAs: VEGF121, VEGF165, VEGF145 and VEGF189 with high expression of the first two isoforms [32].

The gradual decrease observed in VEGF mRNA levels during incubation with estrogen, as well as the significant increase of VEGF protein expression during tamoxifen treatment strongly imply that estrogen does not regulate VEGF expression via conventional ER-induced transcription of this growth factor. Overexpression of ERα in tumors initiated from Ishikawa human endometrial cancer cells was found to inhibit the expression of VEGF and $\alpha_v ß_3$ integrin, as well as to suppress the degree of vascularization and, hence, to limit tumor growth [33].

A recent study showed that estrogen acts as an angiogenic switch in breast cancer cells by down regulating soluble VEGFR-1 (s-flt) which is believed to sequester available VEGF, although whether this is a direct or an indirect effect of hormone remains to be determined [34, 35]. Despite of a large body of literature that describes the effects of sex steroids on regulation of VEGF in breast cancer cells the overall picture on sex-steroid regulation of VEGF remains confusing [36].

Tumor size is a frequently used parameter in breast cancer diagnosis and treatment. Some previous studies reported that VEGF gene polymorphisms may be associated with breast cancer [37] and other malignant tumors [38, 39]. Although, there are controversies concerning this subject.

Findings of Langsenlehner U *et al.* [40] do not support the hypothesis that VEGF polymorphisms are associated with breast cancer risk. They found only one statistical significant association between VEGF genotypes and haplotypes and tumor characteristics (-634C allele and small tumor size; $p<0.001$). This might be explained by our findings concerning the lack of significant correlation between tumor size and VEGF expression and also could explain tumor size distribution of most of our cases between 4 to 8 cm.

At least four polymorphisms in the VEGF gene have been associated with changes in VEGF expression levels: -2578C/A, -1154G/A and -634G/C are all located in the promoter region; and +936C/T is located in the 3'-untranslated region.

In 2006, Jacobs EJ *et al.* studied the association of this VEGF gene polymorphism and histopathology of breast cancer. They found that +936C allele, which is also hypothesized to increase VEGF expression, was not clearly associated with invasive breast cancer but it was associated with reduced risk for *in situ* cancer in postmenopausal women [41]. These data partially support our results about a significant association of VEGF expression with ductal invasive carcinoma type of breast cancer.

In 2006, Vogl G *et al.* reported a significant correlation between VEGF, tumor grade and stage of the breast tumors [42].

One year later, Balasubramanian SP [43] confirmed the correlation between VEGF expression and high tumor grade by VEGF single nucleotide polymorphisms and haplotypes in breast cancer.

Our results partially support with previous findings. Despite of predominance of G2 and G3 positive cases for VEGF, we found no correlation between high tumor grade and VEGF expression. Our study failed to prove any correlation between tumor stage and the same growth factor. In addition, lymph node status had no correlation with VEGF expression in studied samples.

Strong correlation between presence of inflammation and VEGF expression reported in this study suggested that inflammatory cells and tumor stromal cells are also potential source of additional VEGF and we consider that this aspect have to be discussed more largely for breast tumors in subsequent studies.

Conclusions

We consider that VEGF scoring system applied in our study, which include both intensity score combined with percent of tumor positive cells had better characterize the VEGF expression in human breast tumors.

Some findings like VEGF expression in tumor stromal cells remain to be discussed separately due to the heterogeneity of stromal cells, which express VEGF in breast malignancies.

The use of VEGF expression as individual prognostic factor in breast cancer failed to be proved in present study because there were found correlation with histopathology and associated inflammation but not with tumor stage and grade.

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