Angiogenesis: bFGF and VEGF in breast carcinoma

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ABSTRACT

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Angiogenesis, or neovascularization, is a complex process leading to formation of new blood vessels from the pre-existing vascular network of the tissue. Angiogenesis plays a central role in various physiological and pathological conditions, including embryonic development, reproduction, inflammation and wound healing, infertility, heart diseases, ulcers, rheumatoid arthritis, diabetic blindness and cancer. It is a multistep process involving EC activation, basement membrane and extracellular matrix (ECM) degradation, EC proliferation, migration and differentiation, synthesis of new basement membrane and maturation of new blood vessels. Tumor vasculature is considered to be of an 'immature' nature with series of structural abnormalities. There are reciprocal paracrine interactions between ECs, tumor cells, stroma and ECM. Angiogenesis plays a key role in transformation of normal to malignant cell, tumor progression and metastasis. It is similar to the metastatic process in that it requires EC attachment, proteolysis, and locomotion to proceed. A close relationship exists between the tumor and ECs invasiveness of the tissue. The switch to the angiogenic phenotype involves a change in the local equilibrium between positive and negative regulators of the growth of microvessels. Basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) are positive regulators of angiogenesis. Intimate cross-talk exists among bFGF and the different members of the VEGF family during angiogenesis, lymphangiogenesis, and vasculogenesis. A substantial body of experimental evidence supports the hypothesis that angiogenesis and angiogenic factors may be strong prognostic and predictive factors in breast carcinoma. This article reviews the current knowledge on angiogenesis and its positive regulators: bFGF and VEGF.

KEYWORDS: *Neovascularisation, Pathologic; Breast Neoplasms; Fibroblast Growth Factor 2; Vascular Endothelial Growth Factors; Angiogenesis Inducing Agents*

INTRODUCTION

Angiogenesis, or neovascularization, is a complex process leading to formation of new blood vessels from the pre-existing vascular network of the tissue (1). The major role of the vasculature is its transport function: the supply of oxygen and nutrients to cells and removal of waste products from cells (2). Angiogenesis plays a central role in various physiological and pathological conditions, including embryonic development, reproduction, inflammation and wound healing, infertility, heart diseases, ulcers, rheumatoid arthritis, diabetic blindness and cancer (3,4).

The first step of this multistep process is EC activation consisting of increasement of their mitotic rates and up-regulation of receptors for angiogenic factors, urokinase receptors, integrins and proteases (5). Secretion of proteolytic enzymes (e.g. metalloproteinases [MMPs] and enzymes of plasminogen pathway) is responsible for basement membrane and extracellular matrix (ECM) degradation. In order to form new vessels, ECs have to proliferate and migrate in a certain direction. Migrated ECs have to differentiate, and synthesize a new basement membrane around newly formed capillary tubes. Sprouts formed from different parental vessels eventually have to merge to form a vessel along which transport of blood can occur and pericytes have to be recruited to assume their perivascular position. Finally, active neovascularization requires vascular adhesion molecules (e.g. integrins and catherins), that permit the linkage of new vessels with the pre-existing ones to give origin to intratissue vascular network (1,2).

TUMOR VERSUS NORMAL TISSUE ANGIOGENESIS

Tumor angiogenesis has properties somewhat different from normal angiogenesis. The regulatory mechanisms, which "turn-off" neovascularization, do not function normally (6). Tumor vasculature is considered to be of an 'immature' nature and despite the active angiogenesis in some tumor edges, series of structural abnormalities are often found in overall tumor vasculature. Some of the characteristics often found in tumor vasculature are: high turnover rate of ECs, gaps in the endothelial lining and basement membrane, low amounts of pericytes, a heterogeneous, sometimes chaotic organization and distribution of the tumor vasculature, an increased vascular permeability and intravascular coagulation. In tumors blood flow, oxygen pressure and tissue pH values are on average mostly lower than in normal tissues, which lead to changes in tumor microenvironment (e.g. hypoxia, acidosis and free radicals). Because of the absence of lymphatic vessels, interstitial pressure is often high in tumors, leading to further transport problems. These phenomena cause vascular compression, necrosis and reduce the blood supply. The onset of vascularization gradually makes a tumor more inaccessible to drugs (2,7).

REGULATION OF ANGIOGENESIS: MOLECULES, CELLS AND EXTRACELLULAR MATRIX (ECM)

Angiogenic factors, such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), angiopoetin-1, angiogenin, TGF-alpha, TGF-beta, TNF-alpha, IL-8, etc., mediate positive regulation of angiogenesis (1). These factors not only induce mitosis

and/or migration of ECs, but can also be involved in the induction of specific proteases like the collagenases and constituents of plasminogen activator complex. All this actions exert bFGF and VEGF. On the other hand, some angiogenic factors do not stimulate cell division or even inhibit division of ECs, like TNF-alpha, TGF-alpha and angiogenin (2). They stimulate angiogenesis indirectly, by e.g. attracting and activating inflammatory cells and stromal cells to produce other, directly acting angiogenic factors (1,2).

Antiangiogenic factors, such as trombospondin-1 (TSP-1), angiostatin, endostatin, IL-12, TGF-beta, plasminogen activator inhibitor (PAI), tissue metalloproteinases inhibitors (TIMPs) and heparinases, are negative regulators of angiogenesis and they counteract the action of angiogenic factors, in normal situations leading to a balance between angiogenesis and anti-angiogenesis. Secretion of antiangiogenic factors from a primary tumor into the circulation is the reason why the distant metastases angiogenesis may be suppressed by primary tumor and may become apparent only after its removal (8).

There are reciprocal paracrine interactions between ECs, tumor cells, stroma (mast cells, monocytes and macrophages) and ECM (5). Some of the angiogenic factors are expressed as latent precursor molecules, which are activated by specific proteolytic cleavage. Other angiogenic factors (such as the members of the FGF family and some variants of VEGF) are immobilized by binding to heparin-like molecules in ECM, but are released by proteolytic processes as well. Thus, by inducing extracellular proteolysis angiogenic factors may activate or mobilize themselves or each other (2). Neoplastic cells may produce several angiogenic factors, but vascular ECs also secrete growth factors and cytokines that stimulate tumor cells to proliferate or attract and stimulate inflammatory cells (4). Cells that make up the ECM around tumors secrete angiogenic and anti-angiogenic factors. Furthermore, the ECM may act as a reservoir for these factors. bFGF is sequestered in the ECM and is released by the action of proteases produced by tumor or ECs. These proteases also produce a degradation of ECM facilitating capillary migration (5).

ANGIOGENIC SWITCH

Balance between angiogenesis and anti-angiogenesis can shift towards angiogenesis after wounding, during the menstrual cycle, or during tumor growth (2). Angiogenic switch in ECs is induced by secretion of specific EC growth factors produced by tumor or stromal cells (1). Only some clones of a primary tumor become angiogenic. In fact, angiogenic activity of primary solid tumors presents intra and inter-individual heterogeneity (7). An angiogenic cell shed from a primary tumor is more likely than a nonangiogenic cell to develop into detectable metastasis. Tumor cells that are not angiogenic may become dormant micrometastasis until they eventually switch to the angiogenic phenotype (8). In prevascular phase a tumor may persist for years and is usually associated with limited tumor growth and few or no metastases. The vascular phase is usually followed by rapid tumor growth, bleeding and the potential for metastases (9).

The spontaneous angiogenic switch in human tumors can be driven by: 1) angiogenic oncogenes which up-regulate expression of pro-angiogenic proteins, and/or down-regulate expression of angiogenesis inhibitors; 2) tumor-associated hypoxic conditions; 3) fibroblasts which can be induced by tumor cells to elaborate pro-angiogenic proteins ; and 4) bone marrow derived progenitor ECs which traffic to tumors (10).

Actually, the switch from the avascular to a vascular phase of tumor is regulated by multiple biochemical and genetic mechanisms (4). Hypoxic and glycolytic pathways are intricately involved in angiogenesis regulation. This is obviously important clinically because as a tumor grows, areas of necrosis almost always develop. Many of hypoxia-regulated genes (e.g. VEGF gene, Glut-1 gene) in tumor cells are controlled by hypoxia-inducible factor-1 (HIF-1). It binds to hypoxia response elements of target genes and increases their expression or half-life of their mRNA. At genetic level several oncogenes and tumor suppressor genes have been involved in regulation of angiogenesis. For example, erb-B2 oncogene, the EGF receptor, and its ligands EGF and TGF-alpha, are up-regulated and prognostic in breast cancer, and may induce production of angiogenic factors (5). Certain oncogenes, such as proto-c-met, down regulate TSP-1, promoting neovascularization and tumor progression. Other oncogenes including ras, fos, HER-2/neu, Src, and raf stimulate angiogenesis by upregulating VEGF (1).

ROLE OF ANGIOGENESIS IN TUMORS

Angiogenesis plays a key role in transformation of normal to malignant cell, tumor progression and metastasis (7). Angiogenic activity is an early event and is precedes the development of mammary carcinoma (4). In the late 1960s, Folkman was the first to describe the critical role of angiogenesis in tumor growth (5). Most tumors in humans persist in situ for months to years without neovascularization but then become vascularized when a subgroup of cells in the tumor "switches" to an angiogenic phenotype. In the prevascular phase, the tumor is rarely larger than 2-3 mm^3. Such asymptomatic lesions are sometimes clinically undetectable (8). Neoplastic tissue usually exceeds the oxygen diffusion limit when tumor cell layers accumulate to a thickness of approximately 150–200 μ from a nearest open microvessel. Tumor cells beyond this limit undergo apoptosis (10). Cells in prevascular tumors or dormant metastases may replicate as rapidly as those in expanding, vascularized tumors, but without the growth of new vessels the rate of proliferation of such cells reaches equilibrium with their rate of death (8). Thus, tumors are truly dependent on angiogenesis for their own growth (2). Since the transformation and tumor progression of breast cancer are coordinated multistep processes and besides angiogenesis other endocrine, autocrine, and genetic factors are implicated (4). Furthermore, angiogenesis is essential for metastasis, as blood vessels provide an escape route for disseminating tumor cells establishing connections to existing vasculature (2).

ANGIOGENESIS AND METASTASIS

Metastasis of epithelial tumors occurs when the epithelial cells no longer respect the basement membrane boundary to underlying stromal compartment. Metastasis is not under precisely the same regulation as tumor growth, although its progressive deregulation sometimes occurs in parallel with deregulated proliferation. This is one of the reasons why breast cancer is an unpredictable disease. Even small tumors can already be metastatic. Metastasis depends on four major processes: attachment (to basement membrane and subsequently to stromal matrix), proteolysis (of basement membrane and subsequently stromal matrix), motility and angiogenesis. These four processes are required for local invasion of tissue, lymphatics, and blood vessels. Angiogenesis is similar to the metastatic process in that it requires EC attachment, proteolysis, and locomotion to proceed. Metastasis temporally occurs parallel with angiogenesis (11). A close relationship exists between the tumor and ECs invasiveness of the tissue being mediated by similar proteolytic enzymatic pathways (5). Degree of development of the capillary network around the tumor is proportional to metastases (11).

BASIC FIBROBLAST GROWTH FACTOR IN BREAST CARCINOMA

Fibroblast growth factors family and basic FGF

Mammalian FGFs family consists of 22 members and most of them are secreted glycoproteins (12). FGFs are pleiotropic factors acting on different cell types, including ECs, following interaction with heparin sulfate proteoglycans (HSPGs), tyrosine kinase FGF receptors (FGFRs) and integrins (3). The FGF ligands depending on the cell type or stage of maturation produce diverse biological responses that include proliferation, growth arrest, differentiation or apoptosis (12).

Basic FGF is synthesized by several cell types including tumor cells fibroblasts, ECs and macrophages (2,13). Human bFGF gene encodes multiple bFGF isoforms which lack a leader sequence for secretion and are released in limited amounts by an alternative secretion pathway (3).

Basic FGF is involved in different stages of angiogenesis: basal lamina and ECM degradation, migration, proliferation and morphogenesis of ECs, and vessel maturation. Basal lamina and ECM degradation are mediated trough induction of proteases of plasmin-plasmiongen activator system e.g. urokinase plasminogen activator (uPA) and its receptor, matrix metalloproteases (MMPs), and their inhibitors PAI-1 and TIMPs leading to fine modulation of the proteolytic balance. Endothelial cell migration and proliferation are limited

by lateral cell–cell adhesion and ECM interactions that, in turn, are mediated by cadherin and integrin receptors. Basic FGF regulates the expression of different integrins and cadherins. FGFs can promote both EC scattering (through cadherin down-regulation and integrin modulation and cell-cell, cell-ECM adhesion disturbance), that is required during the first steps of the angiogenic process, and the formation of the cell–cell interactions (through cadherin redeployment and gap junction upregulation) required for vessel maturation. FGFs initially promote the disruption of the basal lamina by inducing protease production and lately, FGFs may induce the production of various ECM components by ECs and also pericyte recruitment, contributing to the maturation of the new vessels (3).

Basic FGF-binding molecules (FBMs) and signaling pathways

Basic FGF-binding molecules (i.e. proteins, polysaccharides, and lipids) are present as free molecules in body fluids (e.g. heparin/HSPG, TSP-1, FGFR-1), associated to ECM (HSPG, TSP-1), or anchored to EC membrane (e.g. HSPG, integrins, FGFRs). FGFs exist mainly as immobilized molecules bound to this molecules, so changing the FBMs' status from an immobilized to a free form exerts opposite effects on the biological activity of FGFs. Some FGF binders are also able to interact with FGF-binding sites/receptors present on the surface of ECs, possibly exerting agonist/antagonist effects. The most important FBMs are FGFRs, heparin/HSPGs and integrins (3). The FGF receptors (FGFRs) are transmembrane tyrosine kinases that belong to the immunoglobulin (Ig) superfamily. The extracellular domain, which includes the FGF binding site, contains two or three Ig loops. The two membrane proximal loops bind the FGF ligand, resulting in the formation of a complex containing at least two FGFs, two FGFRs and the glycosaminoglycan moiety (14). Upon ligand binding, receptor dimers are formed and their intrinsic tyrosine kinase is activated causing autophosphorylation of the receptors. Signaling complexes are assembled and recruited to the active receptors resulting in a cascade of phosphorylation events. The best understood FGF signal transduction pathways are: the RAS-MAP kinase pathway, the P-I-3 kinase-AKT pathway, and the PLC-gamma pathway. Primary target proteins of the FGF signaling cascades are transcription factors such as the Ets, AP-1, and ATF/CREB proteins. FGF can cause cell-specific changes in gene transcription using several mechanisms: transcription factor activation, new synthesis of a novel transcription factor, chromatin remodeling. FGF signaling causes cell-specific modifications of nucleosomal histones and recruitment of negative transcriptional regulators (12).

Interplay between angiogenesis, bFGF and inflammation

Inflammation may promote FGF-dependent angiogenesis, since inflammatory cells can express bFGF. Moreover, cell damage, osmotic shock, shear stress and inflammatory mediators can induce the release of bFGF from ECs that, in turn, will stimulate angiogenesis by an autocrine mechanism of action. Conversely, by interacting with ECs, bFGF may amplify the inflammatory and angiogenic response by inducing vasoactive effects, the recruitment of an inflammatory infiltrate and increasement vascular permeability. Besides this indirect action of bFGF on inflammation and angiogenesis mediated through activation of ECs (e.g. increased expression of VEGF, nitric oxide (NO) and vascular adhesion molecules), bFGF acts also directly. Pro- or anti-inflammatory activity of FGFs may be contextual and may explain, at least in part, the reduced leukocyte adhesion and transendothelial migration observed in experimental tumors that, nevertheless, are characterized by the presence of proangiogenic tumor-associated macrophages (3).

Clinical significance of bFGF

Basic FGF is involved in tumorigenesis, angiogenesis and metastasis (13). Stromal-derived fraction of bFGF is the predominant form in breast tumors (1,13). Loss of staining of tumor bFGF may be related to greater liability due to lack of binding to proteoglycans (15). It is possible that stromal bFGF could interact in paracrine manner with its receptor located in the tumor epithelium (13).

bFGF levels are significantly higher in tumor cytosols compared to normal breast tissue. This implies an involvement of bFGF in breast cancerogenesis (13,17). But also greater extractability may be characteristic of bFGF in breast cancer (15).

Until now two studies have reported that the patients with high levels of bFGF, with either node-positive or negative breast tumors, had a significantly better outcome than those with low bFGF levels (13,16). But there are controversial data concerning clinical significance of bFGF in breast cancer. One study has shown that high levels of bFGF had a significantly longer disease-free survival (DFS) than patients with low bFGF. Their results indicate that low bFGF levels in breast carcinoma are an independent prognostic indicator of poor prognosis and disease recurrence (16), while the other study shows the opposite results (18). Other studies with either node-positive or negative breast tumors reported negative results on the clinical significance of bFGF (1,13,17,19).

Furthermore, it was experimentally proved that high bFGF levels were significantly related to high estrogen receptor (ER) levels (13). Our preliminary data confirm the experimental data above. In fact, we assessed the finding that bFGF positively correlates with ER (r=0.291, P=0.035) in the node-negative group of ER-positive patients, particularly grading T1 (Figure 1).

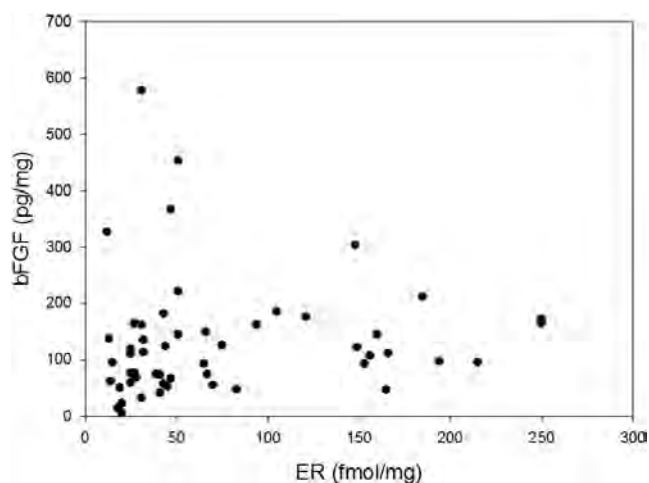**Figure 1.** Correlation between bFGF and ER protein levels (r=0,291, P=0,349) in ER-positive T1 group of node-negative patients with primary breast cancer

Other authors have also found the positive correlation between these two tumor proteins. Similarly, they found that high bFGF levels were significantly related to good prognostic features such as low grade and small tumor size (13). This suggests that bFGF correlates with ER level in early estrogen-dependent phases of breast cancer.

The relationship between tumor microvessel counts and bFGF levels implies no direct involvement between bFGF and angiogenesis. bFGF may be one of multiple factors that synergize with other growth factors such as VEGF to enhance angiogenesis (13).

FGFs can exert their effects on ECs via a paracrine mode consequent to their release by tumor and stromal cells (e.g. inflammatory cells) and/or by their mobilization from the ECM. On the other hand, bFGF may also play an autocrine role in ECs (3). FGF expression by tumor cells is not only significant in view of a possible increase in tumor angiogenesis, but may also serve as an autocrine growth stimulation for tumor cells themselves. Considering the dual effect of bFGF on tumor growth (by increasing tumor angiogenesis and tumor growth as an autocrine mechanism), anti-bFGF may be effective in the treatment of tumors (2).

VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) IN BREAST CARCINOMA

Vascular endothelial growth factor, also called vascular permeability factor (VPF) or vasculotropin, induces multiple processes directly involved in angiogenesis: EC survival, mitogenesis, migration, differentiation and self-assembly, but it is also involved in vascular permeability, immunosuppression and mobilization of endothelial progenitor cells from the bone marrow into the peripheral circulation (2,20-22).

The VEGF family consists of six members: VEGF-A (VEGF), VEGF-B, VEGF-C, VEGF-D, VEGF-E (viral), and placenta growth factor (PIGF) (20). There are several molecular variants of the VEGF protein called: VEGF 189, 165, 121, 206 and 145. The binding of VEGF to its high affinity receptors is dependent on the presence of heparin or heparin-like molecules on EC surface (2).

VEGF family members signal by binding to receptor tyrosine kinase family: VEGFR-1 (fms-like tyrosine kinase-1, flt-1), VEGFR-2 (kinase insert domain-containing receptor (KDR), fetal liver kinase (flk)), and VEGFR-3 (flt-4) (20). VEGF binds to Flt-1 and flk-1/KDR protein which are two homologous transmembrane proteins, with seven extracellular immunoglobin-like (IgG-like) domains and a tyrosine kinase domain interrupted by a so-called kinase insert. IgG-like domains and a kinase insert sequence are also found in FGF-receptor family (2).

Upon binding to its receptor, VEGF initiates a cascade of signaling events that begins with dimerization of two receptors and then autophosphorylation of each other by the tyrosine kinase domain to form the active receptors. This is followed by activation of numerous down-stream proteins, including phospholipase C-gamma/protein kinase C (PKC), Ras pathway members, phosphatidylinositol-3 kinase (PI3K), mitogen-activated protein kinase (MAPK), and others to manifest end point function, such as an increase in vascular permeability, cell survival and proliferation, and migration. Among the many transcription factors, Sp1, HIF-1, Stat3, and AP-1 appear to be the key factors in regulation of VEGF expression (20).

VEGF is secreted by tumor cells, but also non-malignant host cells (e.g. monocytes, macrophages, host parenchymal organ cells etc.) recruited by the tumor, may produce this growth factor in response to environment stimuli, mainly hypoxia, certain cytokines and estradiol (1).

Spatial distribution of VEGF mRNA during the course of menstrual cycle and expression was seen especially in steroid-responsive cell types suggesting that VEGF expression is hormonally regulated (2). Our preliminary data, presented in Figure 2, reveal significant positive correlation between VEGF and ER protein levels (r=0.314, P=0.04) in the node-negative group of ER-positive patients older than 59 years, all postmenopausal. This confirms literature data that VEGF is estrogen regulated protein (23 and many other), particularly in postmenopausal women where the levels of estrogen are constant and at the lowest level.
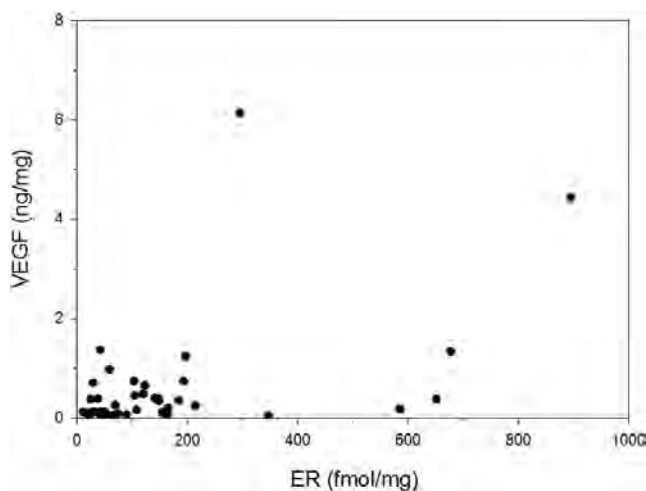

Figure 2. Correlation between VEGF and ER protein levels (r=0,314, P=0,04) in the node-negative group of ER-positive patients older than 59 years, all postmenopausal

VEGF-mediated tumor angiogenic switch-on is regulated by the synergistic cooperation of two signals: the genetic makeup of tumor cells that signals constitutive VEGF expression (constitutive angiogenic signal) and of tumor microenvironmental stimuli that signal inducible VEGF expression (inducible angiogenic signal) (24). The constitutive VEGF signal, which stems from various genetic alterations such as loss of function of tumor suppressor genes and/or gain of function of oncogenes, is a prerequisite and acts as an initiation signal to create a preangiogenic condition for tumor angiogenesis. With an increase in tumor mass and tumor- host interaction comes inducible VEGF expression from both tumor and tumor stromal cells by various stimuli, especially hypoxia, acidosis, and free radicals. This inducible signal cooperates with the constitutive signal, leading to a high level of VEGF production and then tumor angiogenic switch-on (20).

Clinical significance of VEGF

VEGF levels in breast tumors are often higher than in surrounding normal tissue which implicates its role in cancerogenesis (2). High VEGF expression correlates with poor prognosis (17,25). Furthermore, experimentally data (1,17,19) indicate that VEGF seems to be the most powerful prognostic marker to discriminate between high and low risk of disease relapse, whether in node-negative or node-positive, operable breast carcinoma (19).

The patients with high levels of VEGF in tumors are unlikely to benefit from adjuvant conventional treatments. This indicates that VEGF has also a predictive value in breast carcinoma (1). VEGF expression correlates with microvessel density, which indicates direct involvement of VEGF in angiogenesis (13).

CROSS-TALK BETWEEN BASIC FGF AND VEGF

Recently, it was discovered that an intimate cross-talk exists among bFGF and the different members of the VEGF family during angiogenesis, lymphangiogenesis, and vasculogenesis. Indeed, experiments demonstrated that bFGF modulates VEGF expression in ECs, and it upregulates the expression of both FGFRs and VEGFRs in ECs. The two growth factors exert a synergistic effect on tumor blood vessel density. Nevertheless, the two growth factors retain distinct biological properties exerting different biological effects on ECs during angiogenesis. Recent data demonstrate that a FGF/VEGF cross-talk may occur also during lymphangiogenesis mediated by endogenous VEGF-C and VEGF-D upregulation, leading to VEGFR-3 activation (3).

VEGF may be a paracrine mediator for indirectly acting angiogenic agents, such as TGF-beta, bFGF (26), which can induce VEGF expression (20).

In ECs both bFGF and VEGF (in sinergistic way) induce the expression of specific proteases involved in matrix degradation and constituents of plasminogen activator pathway: bFGF induces both urokinase plasminogen activator (uPA) and its inhibitor PAI-1, while VEGF induces tPA (2).
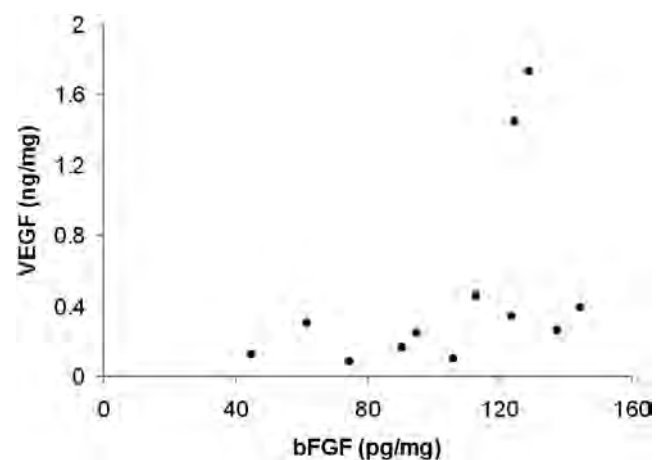

Figure 3. Correlation between bFGF and VEGF protein levels (r=0,65, P=0,02) in the node-negative group of patients younger than 45 years, all premenopausal

Our preliminary data demonstrated that high bFGF levels correlated with high VEGF levels (r=0.65, P=0.02) in the group of patients younger than 45 years, all premenopausal as shown in Figure 3. The positive correlation between these two angiogenic factors was also

found by other authors (19). These finding confirm literature data (3,20) that the crosstalk exists between bFGF and VEGF in their action and signaling pathways. We may notice that this correlation exists in premenopausal women, during the cyclical function of ovaries and fluctuating levels of estrogen, while the correlation between bFGF and VEGF is no more present in postmenopausal women.

Blocking of VEGF and/or bFGF activity therefore may inhibit both tumor progression and metastasis and might have clinical applications (2).

CONCLUSION

Clinical importance and application of studying tumor angiogenesis

The prediction of the development of distant and lymph-node metastases in breast carcinoma is a very relevant prognostic end point (16). The heterogeneous nature of human breast cancer (HBC) is reflected in its widely variable disease course. Our inability to understand fully the biologic heterogeneity of HBC is reflected in the consensus that almost every newly diagnosed HBC patient should be offered adjuvant therapy (16,27). The obvious disadvantage of this recommendation is that the majority of early breast cancer patients are overtreated, in particular those with node-negative disease. Among all node-negative breast cancer patients, 20-30% will experience disease recurrence, while the majority (70%-80%) will have been cured by their primary surgical treatment and, therefore, do not need adjuvant therapy. Therefore, interest must continue in the exploration and validation of more selective prognostic tumor biomarkers that can distinguish node-negative patients' subset with low versus high risk (19).

Furthermore, the development of more valid predictive biomarkers of responsiveness to systemic therapy could be helpful in the tailoring of the optimum treatment for the high vs. low-risk patients (4).

A substantial body of experimental evidence supports the hypothesis that angiogenesis and angiogenic factors may be strong prognostic and predictive factors in breast carcinoma (16). Also, it would be useful to select those patients who are more likely to benefit from antiangiogenic therapy (1).

Angiogenesis can be studied directly, by quantifying the vasculature and indirectly, by measuring the factors involved (5).

There are several ways to assess intratumoral vascularization and to measure angiogenic activity: determining microvessel counts through markers specific for activated/proliferating blood vessels, intratumoral expression of angiogenic peptides and antiangiogenic factors and expression of proteolytic enzymes in tumor stroma (4).

To summarize, experimental data indicate prognostic and predictive value of angiogenesis regulators, particularly bFGF and VEGF, and further researches should be done in this area. These findings and present clinical data may lead us to conclude that antiangiogenic therapy may be the therapy of future in treatment of human breast carcinoma.

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