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Tumoral angiogenesis and breast cancer

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Abstract Breast cancer (BC) is the most common neoplasm in women in Western countries. Tumoral angiogenesis (TA) is essential for the growth and spread of BC cells. There are at least 6 different angiogenic growth factors associated with TA in BC. The major mediator of TA is vascular endothelial growth factor (VEGF), a homodimeric heparin-binding glycoprotein. VEGF signals through VEGF receptor-2 (VEGFR-2), the major VEGF signalling receptor that mediates sprouting angiogenesis. Recently, different antiangiogenic agents have shown efficacy in the treatment of advanced BC. Bevacizumab, a humanised monoclonal antibody against VEGF, in combination with taxanes improves progression-free survival and overall response rate in first-line therapy. Other new antiangiogenic agents, called multi-kinase inhibitors (sunitinib and pazopanib), are under investigation. Finally, a schedule of treatment called metronomic chemotherapy, with antiangiogenic activity, has also demonstrated efficacy in the treatment of advanced BC.

Keywords VEGF · Sunitinib · Bevacizumab · Metronomic chemotherapy · Pazopanib

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Introduction

Tumoral angiogenesis (TA) is an essential process in the progressive growth of neoplasms and the production of metastasis [1]. Angiogenesis consists of a series of linked and sequential steps that ultimately leads to the development of a neovascular blood supply to the tumour mass [2]. Infiltrating immune cells, adjacent normal tissue or/and tumour cells secrete angiogenic growth factors, which then bind to specific receptors on endothelial cells. This ligand-receptor interaction leads to endothelial cell proliferation, migration, invasion, and eventually capillary tube formation.

The proangiogenic process is balanced by the activity of antiangiogenic molecules that are necessary for homeostatic processes. When the activity of the proangiogenic molecules exceeds the activity of the antiangiogenic molecules, new blood vessel formation occurs [3].

Extensive laboratory data suggest that angiogenesis plays an essential role in development, invasion and metastasis in breast cancer (BC). In BC, TA is regulated by at least 6 different proangiogenic factors. The major mediator of TA is vascular endothelial growth factor (VEGF). VEGF is a homodimeric heparin-binding glycoprotein that exists in at least four isoforms due to alternative splicing of the primary messenger RNA transcript. The isoforms are designated $VEGF_{121}$, $VEGF_{165}$, $VEGF_{189}$ and $VEGF_{205}$ according to the number of amino acids each protein contains. There are at least four other members of the VEGF family (Table 1) [4]. The aforementioned VEGF is referred to as VEGF-A. VEGF binds to specific receptors (VEGFR), and induces endothelial cell mitosis, invasion and eventually capillary tube formation [5]. VEGFR are expressed on endothelial cells and some neoplastic cells. Three VEGF receptors (VEGFR) have been identified: VEGFR-1, VEGFR-2 and VEGFR-3 (Fig. 1) [6].

VEGF-A signals mainly through VEGFR-2, which is expressed at elevated levels by endothelial cells engaged

Table 1 The family of VEGF molecules

Type of VEGF	Mechanism of action
VEGF-A	Physiologic and tumour angiogenesis
VEGF-B	Vasculogenesis and activation of invasive enzymes on endothelial cells
VEGF-C	Lymphangiogenesis and tumour angiogenesis
VEGF-D	Angiogenesis
VEGF-E	Endothelial cell mitosis and angiogenesis

in angiogenesis and by circulating bone marrow-derived endothelial progenitor cells. The role of VEGFR-1 is a mystery with respect to VEGF-mediated angiogenesis. It binds VEGF with approximately 10 times the affinity of VEGFR-2 binding, but its signal-transducing properties are extremely weak [7].

VEGF expression is increased in advanced BC, and it is known that high VEGF levels are associated with poor clinical outcome and impaired response to tamoxifen or chemotherapy [8]. Some studies have shown that VEGF status is a significant predictor of relapse in primary BC patients receiving adjuvant endocrine treatment with tamoxifen [9]. VEGF expression level in the tumour tissue was also a significant predictor of relapse-free survival in node-negative BC patients treated with radiotherapy. Therefore, high vascularisation of BC is a prognostic and predictive factor.

In BC, VEGF is expressed throughout the tumour lifecycle, but other factors come into play at later stages, such as basic fibroblast growth factor (b-FGF), transforming growth factor beta-1 (TGF-ß1), placental growth factor (PlGF), platelet-derived endothelial cell growth factor (PD-ECGF) and pleiotrophin [10].

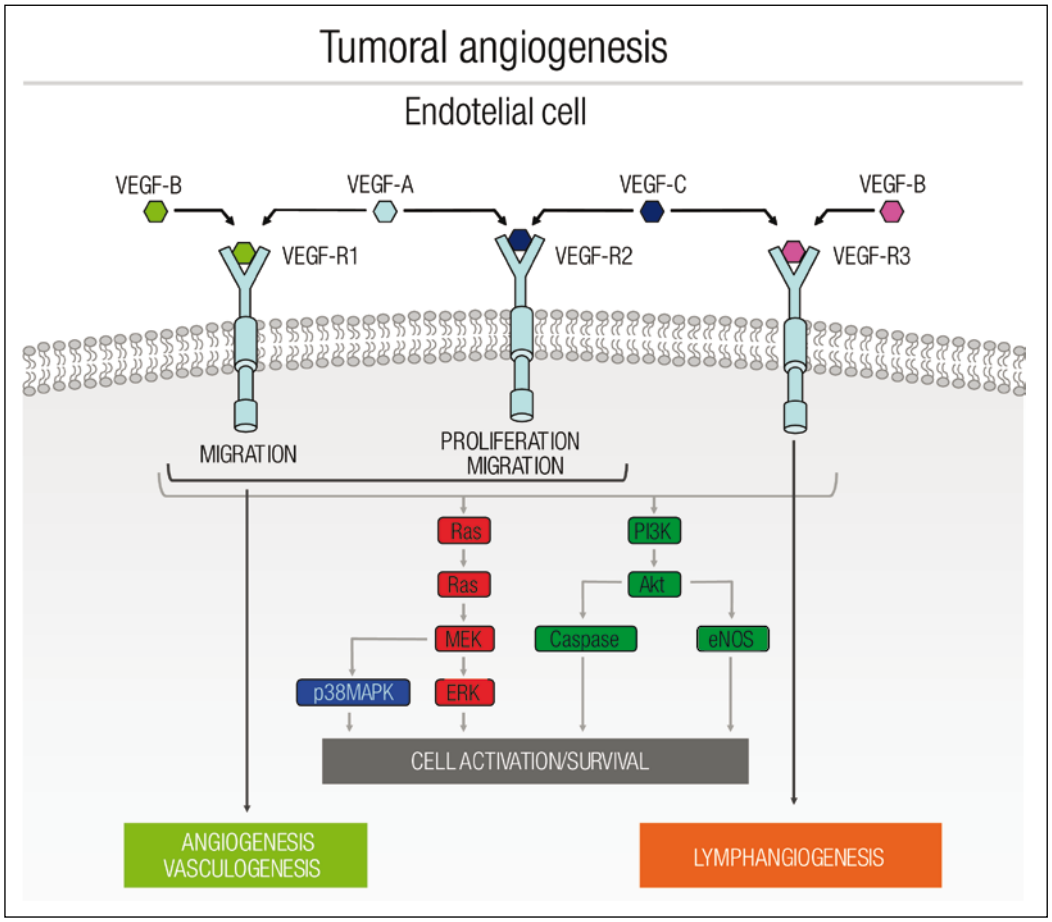

Fig. 1 Interaction of VEGF molecules and VEGF receptors. The major mediator of TA is VEGF-A. VEGF-A signals through VEGFR-2, the major VEGF signalling receptor that mediates TA. The role of VEGFR-1 is unclear. The binding of VEGF to VEGFR-2 leads to a cascade of different signalling pathways, resulting in the upregulation of genes involved in mediating the proliferation and migration of endothelial cells and promoting their survival and vascular permeability. Binding of VEGF-C to VEGFR-3 mediates lymphangiogenesis

Table 2 Antiangiogenic agents in the treatment of BC

Agent	Mechanism of action	Administration
Bevacizumab	Humanised monoclonal antibody against VEGF-A	IV
Sunitinib	Multi-kinase inhibitor: VEGFR, PDGFR	PO
Pazopanib	Multi-kinase inhibitor: VEGFR, PDGFR, C-Kit	PO
Metronomic chemotherapy	Damage DNA or disrupt microtubules	PO/IV

In addition, hypoxia, a characteristic of solid tumours, is an important inducer of VEGF. Its effect is mediated through the hypoxia-inducible transcription factors (HIF) 1α and 2α [11, 12]. During BC carcinogenesis, the expression of HIF-1α increases proportionally in the gradation from ductal hyperplastic lesions, well differentiated ductal carcinomas in situ (DCIS), well differentiated invasive BC, poorly differentiated DCIS and poorly differentiated invasive carcinomas. Increased level of HIF-1α expression is significantly correlated with an increased level of VEGF expression.

In normoxia, three prolyl hydroxylases (prolyl hydroxylase-1, -2 and -3) hydroxylate HIF-1α at two proline residues in its oxygen-dependent degradation domain, with oxoglutarate from the Krebs cycle, ascorbate and Fe^{2+} leading to recognition and binding of the α domain by the von Hippel-Lindau protein. This interaction, and through binding of elongin C via von Hippel-Lindau ß domain in turn, leads to ubiquitination and targeting for degradation through the proteasome. In conditions of hypoxia, however, molecular oxygen is not available for hydroxylation, which results in HIF-1α stabilisation and translocation to the nucleus, where it binds to HIF-1α and consensus hypoxia response elements on gene promoters. Co-activators and polymerases are recruited and transcriptional activation of several gene pathways that are involved in angiogenesis, glycolysis, erythropoiesis and apoptosis occurs. The asparagine hydroxylase, factor inhibitor of HIF-1 and CITED4 (CBP p300-interacting transactivator 4), which interfere with co-activator binding, provide a further level of control [13, 14].

Antiangiogenic therapies in breast cancer

The VEGF ligand-receptor system is the target of most of the novel antiangiogenic agents. Some new antiangiogenic agents, called multi-kinase inhibitors, also have activity against other receptors, such as epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor (PDGFR) and C-KIT, in addition to VEGFR (Table 2).

Bevacizumab and breast cancer

Bevacizumab is a humanised monoclonal antibody against VEGF, which inhibits TA [15]. In addition to its direct antiangiogenic effects, bevacizumab may also improve the delivery of chemotherapy by altering tumour vasculature and decreasing the elevated interstitial pressure in tumours.

In a phase 1 and phase 2 study that tested three different doses of bevacizumab monotherapy (3, 10 or 20 mg per kilogram of body weight every 2 weeks) in 75 patients with previously treated metastatic BC, the objective response rate (ORR) was 9.3% and 17% of patients had a response or were stable at 22 weeks. The dose of 10 mg per kilogram was suggested for further trials [16]. In a phase 3 trial, the addition of bevacizumab to capecitabine in patients previously treated with anthracyclines and taxanes significantly increased the ORR (9.1% vs. 19.8%, p=0.001), but not progression-free survival (PFS=4.2 vs. 4.9 months; *hazard ratio* [HR] for disease progression=0.98) or overall survival (OS=15.1 vs. 14.5 months) [17].

In a recent randomised phase 3 trial (E2100 trial), Miller et al. [18] compared the efficacy of paclitaxel plus bevacizumab with paclitaxel alone, as initial treatment for advanced BC. The primary end point was PFS and OS was a secondary end point. The study included a total of 722 patients with advanced BC. Paclitaxel plus bevacizumab significantly prolonged PFS as compared with paclitaxel alone (median, 11.8 vs. 5.9 months; HR for progression, 0.60; p<0.001) and increased the ORR (36.9% vs. 21.2%; p<0.001). The OS rate, however, was similar in the two groups (median, 26.7 vs. 25.2 months; HR, 0.88; p=0.16). Grade 3 or 4 hypertension (14.8% vs. 0.0%, p<0.001), proteinuria (3.6% vs. 0.0%, p<0.001), headache (2.2% vs. 0.0%, p=0.008) and cerebrovascular ischaemia (1.9% vs. 0.0%, p=0.02) were more frequent in patients receiving the combination therapy.

A recent analysis of E2100 trial evaluated the association of VEGF genotype with efficacy and toxicity .The VEGF-2578 AA genotype was associated with a superior median OS in the bevacizumab arm when compared with the alternate genotypes combined (HR=0.58; 95% CI 0.36–0.93; p=0.023). The VEGF-1154 A allele also demonstrated a superior median OS with an additive effect of each active allele in the combination arm but not the control arm (HR=0.62; 95% CI 0.46–0.83; p=0.001). Two additional genotypes, VEGF-634 CC and VEGF-1498 TT, were associated with significantly less grade 3 or 4 hypertension in the combination arm when compared with the alternate genotypes combined (p=0.005 and p=0.022, respectively) [19].

The AVADO trial presented at the 2008 American Society of Clinical Oncology (ASCO) annual meeting showed a clinical benefit for the combination of two different doses

of bevacizumab (7.5 mg/kg or 15 mg/kg IV every 3 weeks) plus docetaxel in advanced BC [20].

Bevacizumab in combination with a taxane (paclitaxel or docetaxel) should be the first-line therapy of most patients with recurrent or advanced BC, previously treated with anthracyclines and/or taxanes. But more studies are needed in order to establish the optimal dose of bevacizumab.

Bevacizumab is under investigation in neoadjuvant and adjuvant settings. Maintenance therapy with bevacizumab may be effective in the sub-group of patients with triple negative phenotype (BEATRICE trial).

Small-molecule tyrosine kinase inhibitors and breast cancer

An alternative approach to VEGF inhibition involves small-molecule tyrosine kinase inhibitors. These agents inhibit not only the VEGFR, but also other receptors in the split kinase domain superfamily of receptor tyrosine kinases, including the PDGFR. PDGFR is expressed in pericytes, which serve as structural supporting cells for endothelial cells.

Sunitinib is an oral multitargeted tyrosine kinase inhibitor that inhibits VEGFR, PDGFR, stem cell factor receptor (KIT) and colony-stimulating factor-1 receptor [21–23]. Single-agent sunitinib has demonstrated antitumour activity in several preclinical BC models, both alone and in combination with chemotherapy [24].

A phase II, open-label, multicentre study evaluated sunitinib monotherapy in patients with metastatic BC. In this study 64 patients previously treated with an anthracycline and a taxane received sunitinib 50 mg/day in 6-week cycles (4 weeks on, then 2 weeks off treatment). Seven patients achieved a partial response, with a median duration of 19 weeks, giving an ORR of 11%. Three additional patients (5%) maintained stable disease for at least 6 months. Median time to progression (TTP) and OS were 10 and 38 weeks, respectively. Notably, responses occurred in triple-negative tumours and HER2-positive, trastuzumab-treated patients. Treatment was associated with increases in plasma VEGF and decreases in soluble VEGFRs and KIT. The most common adverse events were fatigue, nausea, diarrhoea, mucosal inflammation and anorexia, but most adverse events were mild to moderate (grade 1 to 2) in severity and were effectively managed with dose delays or reductions [25].

Pazopanib is an oral angiogenesis inhibitor targeting VEGFR, PDGFR and c-kit. A study presented in ASCO 2008, called VEG20007 [26], evaluated the efficacy and safety of dual pathway inhibition with pazopanib (antiangiogenic agent) plus lapatinib (anti-HER2 agent) in patients with HER2-positive previously untreated advanced BC. In this study patients were randomised to receive pazopanib 400 mg/day plus lapatinib 1000 mg/day or lapatinib alone (1500 mg/day) for 12 weeks. The primary endpoint was progressive disease rate (PDR) at week 12 in HER2-positive (FISH-positive) patients. Thirty-two patients received pazopanib plus lapatinib and 30 patients lapatinib alone. The PDR was 19% and 27% in the pazopanib plus lapatinib arm and lapatinib arm, respectively. The ORR was 44% in patients receiving the combination therapy vs. 30%. Reduction in target lesions occurred in 73% of patients with pazopanib plus lapatinib vs. 43% of those in the lapatinib arm. The most common adverse events (pazopanib plus lapatinib vs. lapatinib) were diarrhoea (63 vs. 57%), rash (22 vs. 20%) and nausea (22 vs. 17%).

Metronomic chemotherapy and breast cancer

Recently there has been considerable interest in a new schedule of chemotherapeutic drug administration that can inhibits neoplastic angiogenesis. The rationale is based on the fact that virtually all classes of cancer chemotherapeutic drugs are designed to damage DNA or disrupt microtubules of dividing cells, and endothelial cell division takes place during new blood vessel formation, including tumour angiogenesis [27]. The results of recent experimental studies have suggested that frequent administration of certain cytotoxic agents at low doses (a tenth to a third of the maximum tolerated dose), known as metronomic chemotherapy, increases the antiangiogenic activity of the drugs [28]. The advantage of this strategy is lower toxicity and risk of emergence of drug-resistant tumour cells than conventional administration [29]. The clinical efficacy and antiangiogenic effect of low-dose, metronomic administration of cyclophosphamide and methotrexate have been demonstrated. In a trial with metastatic breast carcinoma a total of 153 patients were enrolled, five of which demonstrated complete remission and 25 partial remission. The proportion of patients who achieved prolonged clinical benefit was 15.7% (95% CI 9.9–21.4%). Median TTP for patients with prolonged clinical benefit was 21 months [30].

In the study conducted by Orlando et al., the combination of trastuzumab and metronomic chemotherapy was effective and minimally toxic in HER2-positive advanced BC patients. The clinical benefit in all patients and in patients with disease resistant to previous trastuzumab therapy was 46% (95% CI 24–68%) and 27% (95% CI 6–61%), respectively. Median TTP was 6 months and median duration of treatment was 5 months [31].

In a more recent phase II study, metronomic chemotherapy with capecitabine and cyclophosphamide in combination with bevacizumab was effective in advanced BC and was minimally toxic. Forty-six patients received metronomic oral capecitabine (500 mg thrice daily) and cyclophosphamide (50 mg daily) plus bevacizumab (10 mg/kg every 2 weeks). The ORR was 48% (95% CI 33–63%), with one complete response (CR; 2%). In addition, 19 patients (41%) had stable disease. Median TTP was 42 weeks (95% CI 26–72 weeks) in the study. Higher baseline circulating endothelial cells were correlated with ORR (p=0.02),

clinical benefit (p=0.01) and improved TTP (p=0.04) [32].

Finally, it is known that weekly administration of paclitaxel (80–90 mg/m^2 IV weekly) has an antiangiogenic activity, with better PFS and OS in advanced BC.

Conclusion

Angiogenesis is the generation of new blood vessels from the existing vasculature. It consists of multiple coordinated, sequential and interdependent steps. The major mediator of TA is VEGF-A. VEGF-A signals through VEGFR-2, the major VEGF signalling receptor that mediates sprouting angiogenesis. The role of VEGFR-1 is much less clear. The binding of VEGF to VEGFR-2 leads to dimerisation of the receptor, followed by intracellular activation of the PLCγ-PKC-Raf kinase-MEK-mitogen-activated protein kinase (MAPK) pathway and subsequent initiation of DNA synthesis and cell growth, whereas activation of the phosphatidylinositol 3'-kinase (PI3K)-Akt pathway leads to increased endothelial-cell survival. Activation of src can lead to actin cytoskeleton changes and induction of cell migration.

In BC, VEGF is expressed throughout the tumour life cycle, but other factors come into play at later stages, such as b-FGF, TGF-ß1, PlGF, PD-ECGF and pleiotrophin.

Different antiangiogenic agents may improve the outcome of patients with advanced BC. Bevacizumab in combination with taxanes improves PFS and ORR in first-line therapy. Other new antiangiogenic therapies, such as multikinase inhibitors (sunitinib and pazopanib) or metronomic chemotherapy, also have demonstrated efficacy in the treatment of advanced BC.

Conflict of interest The authors of this article indicated no potential conflicts of interest.

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