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Angiogenesis and Antiangiogenesis in Triple-Negative Breast cancer[1]

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Abstract

Several data support a central role for angiogenesis in breast cancer growth and metastasis. Observational studies have demonstrated that microvascular density (MVD) is a prognostic factor in invasive breast cancer, whereas others reached the opposite conclusion. Vascular endothelial growth factor is the most important angiogenic factor with proven significance in breast cancer, as it has been assessed in both experimental and clinical studies. Triple-negative breast cancer (TNBC) is a type of breast cancer which lacks estrogen, progesterone, and HER-2/neu receptors. MVD in both basal-like and TNBC is significantly higher than in non–basal-like and non-TNBC. In breast cancer and other malignancies, the development of agents that inhibit tumor angiogenesis has been an active area of investigation. In TNBC, clinical trials combining targeted agents and chemotherapy have failed to show substantial survival improvement. There is evidence that patients with TNBC may have a greater probability of obtaining some kind of clinical efficacy benefit from bevacizumab-based therapy.

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Angiogenesis in Breast Cancer

Breast carcinoma is a heterogeneous tumor made up of different cell clones, with different growth rates and metastatic potentials. The most important parameters which determine the prognosis of breast carcinoma are thought to be tumor size and grade, presence of lymph node metastasis, hormonal receptor status, and c-erb2 [1]. Breast tumors have been classified based on their gene expression profile and immunohistochemical expression of hormone receptors, HER2, cytokeratin 5/6, epidermal growth factor receptor (EGFR), p53, and BCL-2 [2].

Several data support a central role for angiogenesis in breast cancer growth and metastasis. Observational studies have demonstrated that microvascular density (MVD) is a prognostic factor in invasive breast cancer, whereas others reached the opposite conclusion [3–5]. Breast cancer with high MVD have been found to have significant association with larger tumor size, high grade, lymph node metastasis, and poor prognosis [6–9]. Gasparini et al. [10] found a significant correlation between MVD and metastatic disease, recurrence-free survival, and overall survival (OS) in early breast cancer patients independent of their lymph node status. In a systematic review of the literature and meta-analysis, Uzzan et al. [11] found a statistically significant inverse relationship between angiogenesis, assessed by MVD, and survival, confirming that human invasive breast cancer is an angiogenesis-dependent malignancy.

Angiogenesis is important in the transformation of hyperplastic *in situ* epithelium to invasive carcinoma. In 1970, Gullino's group [12–15] observed that experimental breast cancer in rat and mouse gave rise to marked breast angiogenic activity that was lacking in adult

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gland. Moreover, just like the hyperplastic and dysplastic breast lesions more frequently subject to malignant change, premalignant lesions also induce a strong vasoproliferative response long before any morphological sign of malignant transformation can be observed. Normal murine mammary tissues only rarely induce neovascularization, whereas tissues from 30% of hyperplastic alveolar nodules and from 90% of murine mammary tumors are strongly angiogenic [14]. Multiple angiogenic factors are commonly expressed by invasive human breast cancers; at least six different proangiogenic factors, including acidic and basic fibroblast growth factor (bFGF), transforming growth factor beta-1 (TGFβ-1), platelet-derived endothelial cell growth factor, placenta growth factor, and pleiotrophin, have been identified, with the 121–amino acid isoform of vascular endothelial growth factor (VEGF) predominating [16].

Among the inflammatory cells involved in breast cancer, mast cells play a crucial role favoring angiogenesis and tumor progression. Mast cells are attracted in the tumor microenvironment by stem cell factor secreted by tumor cells and produce several angiogenic factors, including VEGF, bFGF, interleukin-8, and TGFβ-1, as well as matrix metalloproteinases, which promote tumor vascularization and invasiveness, respectively [17]. Kankkunen et al. [18] observed that significant increases in mast cell counts in breast carcinoma versus benign lesions are due to tryptase-containing mast cells. Moreover, tryptase-positive mast cells are significantly more numerous in the zone of invasion than elsewhere in malignant lesions. Breast cancer patients with metastases in the axillary nodes reveal greater numbers of mast cells in all nodes examined compared with patients without metastasis [19]. We have demonstrated that angiogenesis increases in parallel with the number of tryptase-positive mast cells and that their values are significantly higher in sentinel lymph nodes with micrometastases compared with those without [20].

VEGF in Breast Carcinoma

VEGF is the most important angiogenic factor with proven significance in breast cancer, as it has been assessed in both experimental and clinical studies. VEGF mRNA and/or protein expression has been detected at low level in normal human mammary gland [21]. VEGF induces tumor cell proliferation in mice models of breast cancer, and increased tumor proliferation is observed in transgenic mice with VEGF165 targeted to mammary epithelial cells under the control of mouse mammary tumor virus promoter 23 or in xenograft mice model generated by the injection of mammary tumor cells transfected with a VEGF165 or VEGF189 plasmid [22]. VEGF has been measured in sera and was detected at higher levels in sera of patients with stage III breast cancer as compared with stage I or II breast cancer and in healthy subjects [23,24]. Several studies have found an inverse correlation between VEGF expression and overall survival in both node-positive and node-negative cancer [10,25]. Moreover, increased VEGF expression has been associated with impaired response to tamoxifen or chemotherapy in patients with advanced breast cancer [26]. In fact, the two antiestrogens tamoxifen and toremifene, which are both used in the treatment of breast cancer, did not inhibit the estrogen-induced increase of VEGF mRNA expression.

An elevated number of VEGF receptor-3 (VEGFR-3)–positive cells were found in invasive breast cancer, and the expression of VEGFR-3 becomes upregulated in the endothelium of angiogenic blood vessels (Figure 1) [27]. The HER2 subtype is one of the most aggressive molecular variants of breast cancer, frequently associated with lymph node metastasis and poor prognosis. The aggressive behavior of these tumors may be explained in part by VEGF-C expression in tumor cells (Figure 1) [28].

In human breast cancer, the expression of VEGF correlates with mutant p53, and the combination of both mutated p53 and high VEGF levels has been associated with poor outcome [29]. The role of VEGF, hypoxia inducible factor-1 alpha (HIF-1α), and MVD in BRCA-1-2 carrier and BRCAX breast cancer has been retrospectively evaluated [30], and an increase of VEGF, HIF-1α expression, and MVD (Figure 2) in BRCA-1-2 carrier and BRCAX compared with the sporadic control group has been demonstrated. Other authors have demonstrated a relationship between BRCA-1 mutation and VEGF and HIF-1α expression in breast cancer patients [31,32] and their prognostic significance [33,34]. Bos et al. [35] investigated the correlation between the level of HIF-1α overexpression and VEGF, MVD, estrogen receptor, and p53 expression and demonstrated that the level of HIF-1α increases as the pathologic stage increases and is higher in poorly differentiated and more aggressive lesions than in the corresponding type of well-differentiated lesions. Increased levels of HIF-1α are associated with increased proliferation and increased expression of estrogen receptor and VEGF. Moreover, MVD and HIF-1α phenotype have a prognostic value in lymph node–positive and –negative breast cancer [36–38] and in invasive ductal carcinoma of the breast [9,11].

Angiogenesis in Triple-Negative Breast Cancer

Triple-negative breast cancer (TNBC) is a type of breast cancer which lacks estrogen, progesterone, and HER-2/neu receptors. Its features include young age, advanced stage at presentation, unfavorable histopathology, grade III, high proliferative index, lack of tubule formation, and high rate of metastases to distant organs, including lung and brain [39]. TNBC may be dissected into distinct subsets, including the basal-like and claudin-low subtypes, both of which have unique genetic characteristics and treatment responses. TNBC causes mortality when it metastasizes, and the average survival of advanced TNBC is 2 months, much shorter than the duration of survival observed in other subtypes of advanced breast cancer. Therapeutic treatment options for grade IV TNBC are very limited and often unsuccessful.

MVD in both basal-like and TNBC is significantly higher than in non–basal-like and non-TNBC [40]. Both basal-like and TNBC are no different in terms of the initial route of dissemination from non–basal-like and non-TNBC in that such dissemination occurs through lymph vessels [38]. Patients with operable TNBC show significantly higher levels of VEGF and shorter survival times [41]. Compared with non-TNBC, patients with TNBC had significantly higher intratumoral VEGF levels and significantly shorter recurrence-free survival and OS with a shorter time from diagnosis to relapse and from relapse to death [41]. Levels of VEGF correlated with poor outcome irrespective of tumor size, nodal status, histologic grade, age of patient, or type of relapse. Ray et al. [42] showed that VEGF induction in TN MDA-MB-231 breast cancer is regulated by serum amyloid A activating factor 1 transcription factor. Finally, it has been shown that TNBC correlates with higher lymphatic MVD and expression of VEGF-C and -D [43].

Antiangiogenesis in Breast Cancer

In advanced breast cancer, bevacizumab improved response rate but not progression-free survival (PFS) or OS when added to second-line capecitabine [44]. However, another phase III study demonstrated

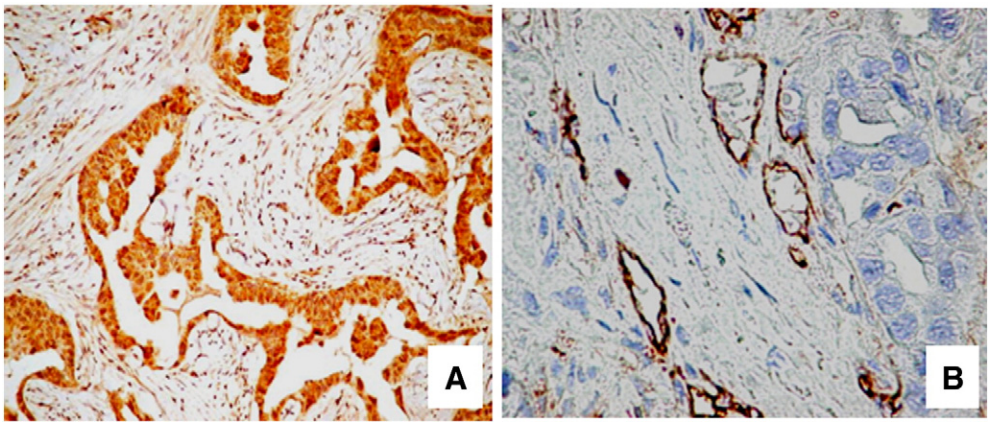

Figure 1. Immunohistochemical expression of VEGF-C (A), and VEGFR-3 (B) in bioptic samples of human breast cancer (courtesy of Prof. Anca Maria Cimpean).

that the addition of bevacizumab to paclitaxel resulted in extension of PFS, but not OS, in metastatic breast cancer [45].

Three further phase III trials of bevacizumab in combination with chemotherapy in HER-2–negative metastatic breast cancer demonstrated an extension of PFS, but no effect on OS, when compared with chemotherapy alone [46–49]. The Ribbon 2 trial investigated the combination of bevacizumab with the physician's choice of capecitabine, a taxane (paclitaxel, nab-paclitaxel, or docetaxel), gemcitabine, or vinorelbine as second-line treatment for metastatic HER2 breast cancer [46]. As consequence of these disappointing results, the Food and Drug Administration withdrew its approval for bevacizumab in this indication. The efficacy of bevacizumab plus chemotherapy as neoadjuvant therapy for primary breast cancer compared with neoadjuvant chemotherapy alone has been reported [50].

In all studies of VEGFR tyrosine kinase inhibitors (TKIs), no OS benefit and no PFS effect have been seen, and adjuvant therapy was not beneficial. Sunitinib had shown single-agent activity in the treatment of metastatic breast cancer [51], and three phase III studies examining the addition of sunitinib to chemotherapy and one comparing single agent sunitinib to chemotherapy all failed to demonstrate improvement in PFS or OS [48,49,52–54].

In neoadjuvant trials, a 5% improvement in pathological complete response has been described when bevacizumab was combined with chemotherapy [55,56]. Axitinib has significant benefits only in patients who have previously received paclitaxel [57]. The pazopanib plus paclitaxel group has a significantly longer PFS than the paclitaxel-only group [58].

Antiangiogenesis in TNBC

Potential approaches in TNBC disease have included targeting VEGF, EGFR tyrosine kinases, and poly(ADP-ribose) polymerase 1. In the subgroup analysis, patients with TNBC had considerable improvement in overall response rate (ORR) and in the E2100 and the Avado trials [46,50], but no significant improvement was observed in the Ribbon 1 trial [49,50]. A meta-analysis was performed that included 621 patients with TNBC enrolled in these three trials. A significant improvement was observed in PFS and ORR with the combination therapy; however, no OS benefit was observed [59]. In the Ribbon 2 trial, in the subgroup analysis of 159 patients with TNBC, compared with chemotherapy alone, the addition of bevacizumab significantly improved ORR and PFS. There was a trend toward improved OS [60].

In the neoadjuvant setting, randomized studies of bevacizumab in combination with chemotherapy have yielded conflicting results. In the GeparQuinto trial, the rate of pathological complete response (pCR) was significantly higher with the addition of bevacizumab to the epirubicin plus cyclophosphamide followed by docetaxel regimen [61]. The benefit was restricted to the TNBC subpopulation, compared with a pathological complete response (pCR) rate of 7.7% and 7.8% with and without the addition of bevacizumab, respectively, among 1262 patients with HRþ tumors. However, these findings were not reproduced in the NSABP B-40

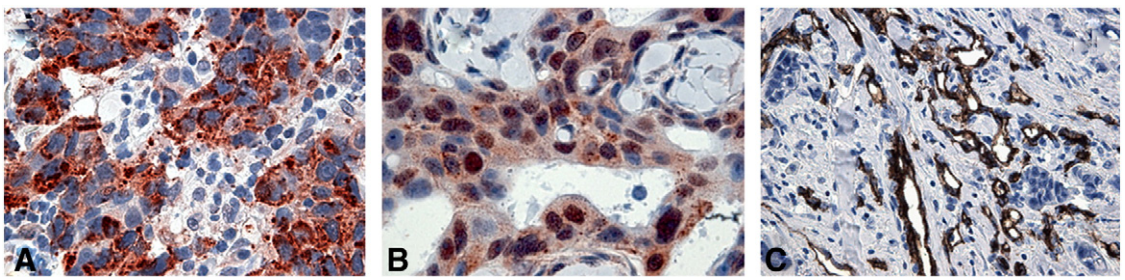

Figure 2. Immunohistochemical expression of VEGF (A), HIF-1α (B), and CD31 (C) in bioptic samples of human breast cancer.

trial [51]. In patients with metastatic TNBC, single-agent sunitinib led to a worse PFS than standard care in a phase II study [61].

Concluding Remarks

In breast cancer and other malignancies, the development of agents that inhibit tumor angiogenesis has been an active area of investigation. Strategies to inhibit tumor vessel growth include the use of bevacizumab, a monoclonal antibody targeting VEGF-A, and small-molecule TKIs. These targeted agents have been studied in combination both as monotherapies and in combination with cytotoxic chemotherapy. Combination of angiogenesis inhibitors with standard chemotherapy regimens in metastatic breast cancer so far has resulted in modest clinical efficacy, and TKIs have not shown efficacy in breast cancer treatment until today.

As TNBC cannot be treated with either hormonal therapy or anti-HER2 agents, standard chemotherapy is based on anthracycline and taxane combinations for the first line of treatment, followed by capecitabine at the time of progression [62]. In TNBC, clinical trials combining targeted agents and chemotherapy have failed to show substantial survival improvement. However, with chemotherapy alone, the residual disease risk in the breast and lymph nodes remains substantially higher, between 30% and 40% [63]. There is evidence that patients with TNBC may have a greater probability of obtaining some kind of clinical efficacy benefit from bevacizumab-based therapy [51,60].

Future antiangiogenesis trials should be more regimen, dose, and patient specific because these treatments act like targeted therapies in breast cancer and need to be more individualized. In this context, in order to provide a truly targeted therapy, it is vital to identify subsets of patients who display predictive markers for response and select these patients for treatment accordingly.

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