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Gene Expression Profile and Angiogenic Marker Correlates of Response to Neoadjuvant Bevacizumab (BV) Followed by BV plus Chemotherapy in Breast Cancer

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Abstract

Purpose—Identify biomarkers and gene expression profile signatures to distinguish patients with partial response (PR) from those with stable disease (SD) and progressive disease (PD).

Experimental Design—Twenty patients with inflammatory breast cancer and one with locally advanced breast cancer received one cycle of bevacizumab (BV) followed by six cycles of BV plus docetaxel-doxorubicin before surgery. Baseline angiogenic/tumor markers were examined by immunohistochemistry (IHC) and gene expression profiles were measured by Agilent Whole Human Genome arrays. All were assessed for clinical response.

Results—Fourteen patients (67%, 95% CI 43% – 85.4%) had PR, 5 had SD, and 2 had PD. Expression of CD31 and PDGFR-beta in the tumor vasculature by IHC was significantly associated with response (PR vs. SD/PD; CD31 median: 33.5 vs. 13.2, $P = 0.0004$; PDGFR-beta median: 5.9 vs. 0.6, $P = 0.01$). Tumor VEGF-A demonstrated a trend towards association with response (2.65 vs. 0.25, $P = 0.04$). pVEGFR2(Y996), pVEGFR2(Y951), MVD, Ki67, apoptosis, grade, ER, HER-2/*neu*, and p53 were not associated with response. Twenty-six of 1339 Gene Ontology (GO) classes at gene transcriptional level were differentially expressed between patients with PR and SD/PD ($P < 0.005$). Representative significant GO classes include *spindle* (11 genes; $P = 0.001$), *vascular endothelial growth factor receptor activity* including PDGFR-beta (5 genes; $P = 0.002$), and *cell motility* including CD31 (80 gene; $P = 0.005$).

Conclusions—Baseline CD31, PDGFR-beta and GO classes for VEGFR activity and mitosis were significantly associated with response to BV followed by BV plus chemotherapy.

Keywords

Bevacizumab; CD31; Gene expression Profiling; Inflammatory Breast Cancer; VEGF-A

Introduction

Inflammatory breast cancer (IBC) is an extremely aggressive subtype of locally advanced breast cancer (LABC) accounting for 1% to 6% of all breast cancers (1). A combined modality treatment (chemotherapy, surgery and radiation therapy) is still the mainstay to manage patients with IBC. The overall response rate to therapy is approximately 60 to 80% but 15-year overall survival is still only 20% (2). Therefore, it is imperative to identify biomarkers, gene expression profile signatures to single out subsets of patients who will respond to therapy and have an improved survival.

Given the fact that IBC is a highly angiogenic disease characterized by dermal lymphatic invasion and increased intra-tumoral microvessel density (MVD), a combination of anti-angiogenic therapy plus chemotherapy is a sound approach (3). It has been known that vascular endothelial growth factor (VEGF) plays a central role in the process of angiogenesis that leads to the growth and metastasis of tumors (4). Bevacizumab (BV) is a humanized monoclonal antibody directed to all forms of VEGF-A, which therefore targets the angiogenic pathway (Avastin, Genentech Inc., South San Francisco, CA).

In an open-label, randomized, phase III trial of paclitaxel plus BV *versus* paclitaxel alone in metastatic breast cancer, the addition of BV significantly improved progression-free survival (median: 11.8 *vs.* 5.9 months, $P < 0.0001$) and increased the objective response rate (36.9% *vs.* 21.2%, $P < 0.001$). However, the overall survival was similar in two treatment arms (26.7 *vs.* 25.2 months, $P = 0.16$) (5). The results are very encouraging but underscore an urgent need in searching functional biomarkers to predict patient response to BV plus chemotherapy.

Gene ontology (GO) is a term used to describe the genes in their associated biological processes, cellular components and molecular function (<http://www.geneontology.org>). Molecular pathway is defined as a series of actions among molecules in a cell that leads to a certain end point or cell function. It is of interest to explore and identify the differentially expressed genes within a biological context between responders (partial response, PR) and non-responders (stable disease, SD plus progressive disease, PD).

In our pilot trial, 21 patients with IBC and LABC were treated with neoadjuvant BV followed by BV plus docetaxel and doxorubicin chemotherapy and the objective response rate was 67% (95% CI 43% – 85.4%) (6). The aim of the current study was to identify biomarkers, GO category or molecular pathway signatures that distinguish the responders from non-responders. A total of 1339 GO categories that each contained 5 to 100 genes, and 171 BioCarta pathways in 20 baseline tumor biopsies (one had an inadequate biopsy) were evaluated for response. In this study, we also investigated tumor VEGF-A, CD31, PDGFR- β , pVEGFR2(Y996), pVEGFR2(Y951), microvessel density (MVD), Ki67, apoptosis, grade, ER, HER-2/*neu*, and p53 at baseline in relation to response to BV followed by BV plus chemotherapy.

Patients and Methods

Patients, tumor core biopsies, and evaluation for response

Study design, patient enrollment, toxicity, and clinical evaluation have been described in detail (6). Patients were eligible if they were diagnosed with IBC or LABC. IBC was defined as histologically proven invasive adenocarcinoma with clinical signs of diffuse erythema and edema involving more than half of the breast with or without an underlying tumor mass. LABC was classified as stage IIB, IIIA, IIIB or IIIC breast cancer utilizing 2002 American Joint Committee on Cancer staging guidelines (7). Twenty patients with IBC and one with LABC were enrolled from October 2001 to August 2004. The study was approved by the Institutional

Review Board of the National Cancer Institute. All patients signed informed consent before enrollment.

Tumor biopsies were taken by mammotome or by either 16 or 18 gauge needles on ipsilateral lymph nodes. A set of biopsies were snap-frozen in liquid nitrogen and stored at -80°C , and another set were immediately fixed in formalin and subsequently sent to the Laboratory of Pathology, Clinical Center, National Institutes of Health for paraffin-embedding and tumor confirmation. The presence of tumor was evaluated on hematoxylin and eosin stained paraffin-embedded sections (6). Fifty to 100% tumor was present in 14 of the 20 biopsies and 20 to 30% was present in 6 of the 20 biopsies at baseline.

Patients were treated with one cycle of BV at 15 mg/kg followed by six cycles of BV plus doxorubicin at 50 mg/m² and docetaxel at 75 mg/m² every three weeks prior to surgery. All measurable, evaluable, and nonevaluable lesions were accounted in the tumor assessment. Radiographic tumor assessments according to the Response Criteria in Solid Tumors (RECIST) were made at baseline and after C1, C4, and C7 (8).

Immunohistochemistry and quantitative analysis

Tumor VEGF-A, CD31, pVEGFR2(Y996), pVEGFR2(Y951), MVD, Ki67, apoptosis, grade, ER, HER-2/*neu*, and p53 were examined on formalin-fixed, paraffin-embedded biopsy sections using a standard avidin-biotin-peroxidase complex indirect immunoperoxidase procedure, and quantitatively analyzed as previously described (6,9,10). Antibodies used were appropriately validated prior to their application to biopsy sections (6). As for PDGFR- β staining, the antibody to PDGFR- β was from Cell Signaling Technology, Inc. (Danvers, MA) and applied to biopsy sections at a dilution of 1:100. A breast cancer specimen was used as the positive control; mouse isotype immunoglobulins were used as the negative controls (Zymed Laboratories, South San Francisco, CA). Expression levels of CD31 and PDGFR- β were quantitatively analyzed with the assistance of an Automated Cellular Imaging System (ACIS; DAKO, Carpinteria, CA). To compare expression levels objectively, a hot spot feature of ACIS was used to identify sites of vessel staining in the tumor area for CD31 and to identify sites of tumor stroma and tumor cell staining for PDGFR- β where three areas were scored using an x60 tool or a free-scoring tool. Staining index (SI) was used to report levels of CD31 and PDGFR- β expression. SI was expressed as the percentage of staining multiplied by staining intensity after subtracting the tissue readouts of the corresponding negative control for each marker per 100.

Statistical analysis of marker expression data

A Wilcoxon rank sum test was used to analyze the association between clinical response (PR vs. SD/PD) and expression of markers such as tumor VEGF-A, CD31, PDGFR- β , pVEGFR2 (Y996), pVEGFR2(Y951), MVD, Ki67, or apoptosis. Fisher's exact test was used to analyze the association between grade, ER, HER-2/*neu*, or p53 and clinical response. To determine if changes in CD31 differed significantly from zero after BV treatment alone from baseline, the relative change in value of CD31 SI parameter [(post-C1 – baseline)/baseline] $\times$ 100% was evaluated using a Wilcoxon signed rank test after determining that this measure was less dependent on the baseline than was the absolute difference (post-C1 - baseline). All *P* values were two-tailed and have not been adjusted for multiple comparisons.

RNA isolation, cRNA synthesis and labeling, microarray hybridization, scanning and image analysis

RNA isolation and amplification were performed, and fluorescent cRNA was synthesized from total RNA using the low-input RNA fluorescent linear amplification kit according to the manufacturer (Agilent Technologies, Inc., Santa Clara, CA). The kit uses Cy5-CTP (633 nm,

test channel) and Cy3-CTP (532 nm, reference channel) as the fluorescent dyes. One microgram of total RNA was used for the amplification and labeling. All tumor biopsy samples were labeled with Cy5 and compared against a Universal Human Reference RNA sample (UHR) from Stratagene (La Jolla, CA). A single pool of UHR probe was generated and used for the entire experiment. For all hybridizations, 750 ng of labeled cRNA sample was used for both Cy5 and Cy3 channels. Hybridizations were done on Agilent's Whole Human Genome arrays that consist of ~40,000 genes. After hybridization, the arrays were scanned by the Agilent Scanner, producing raw image files. The Agilent feature-extracted Software produces .xml file that contains the processed data for each array. The resulting array data was then uploaded into BRB-ArrayTool for data analysis.

Spot filtering, normalization, data selection

The spots were excluded if Cy5 and Cy3 intensity were below 100. If one intensity was below 100 it was increased to 100 before computing the expression ratio. Background-corrected intensities were used to calculate log2 transformed ratios, which was used to report gene expression at the transcriptional level. Subsequently, each array was normalized using its median over the entire array. To perform analysis, the number of genes was reduced by filtering out genes where greater than 50% of the values were missing and those in which fewer than 20% of samples showed less than a 1.5 fold change in either direction from the gene's median value. A set of 11216 genes remaining was used for further data analyses.

Statistical analyses of gene expression profiling data

To identify individual genes differentially expressed between the responders and nonresponders, we used a *t*-test on the log-ratios with a $P < 0.001$ significance level threshold. The identification of differentially expressed GO classes between the responders and non-responders was performed using a functional class scoring analysis as described by Pavlidis et al (11). GO classes with a *P* value less than 0.005 for the average log *P* (LS) or Kolmogorov-Smirnov (KS) statistic were reported. Functional class scoring is a more powerful method of identifying differentially expressed gene classes than the more common over-representation analysis or annotation of gene lists based on individually analyzed genes. Functional class scoring analysis for GO classes and molecular pathways was performed with BRB-ArrayTools (<http://linus.nci.nih.gov/BRB-ArrayTools/>). GO classes and pathways differentially expressed between the responders and non-responders with a *P* value less than 0.005 for LS or KS statistic were reported.

Results

Patient characteristics, clinical response, and biomarkers

Patient characteristics have been described previously (6). Fourteen of the 21 patients (67%, 95% CI 43% – 85.4%) had a clinical PR, 5 had SD, and 2 had PD. Among the tumor and angiogenic markers studied, as shown in Table 1, CD31 expression in the tumor vasculature was significantly higher in the responders than non-responders (median: 33.5 vs. 13.2, $P = 0.0004$); as was PDGFR- β (median: 5.9 vs. 0.6, $P = 0.01$). Tumor VEGF-A at baseline was higher in the responders than non-responders, demonstrating a trend (median: 2.65 vs. 0.25, $P = 0.043$). Shown in Fig. 1 was the expression of CD31, VEGF-A and PDGFR- β in a representative IBC patient with PR and representative IBC patients with PD and SD. The other biomarkers such as pVEGFR2(Y996) and pVEGFR2(Y951), MVD, Ki67, and apoptosis were not associated with response (Table 1). In addition, grade ($P = 1.00$), ER ($P = 1.00$), HER-2/*neu* ($P = 0.27$) and p53 status ($P = 1.00$) were not associated with response.

Gene ontology categories and response to BV followed by BV plus combination chemotherapy

Twenty-six of the 1339 GO categories were differentially expressed between the responders and non-responders at $P < 0.005$ level (Table 2). Among these significant GO classes, the class for *vascular endothelial growth factor receptor activity* ($P < 0.002$) is of interest. The representative genes at the transcriptional level in the class included the platelet-derived growth factor receptor PDGFR- α and - β . PDGFR- α at baseline was higher in patients with PR than those with SD/PD (1.438 vs. 0.919, $P = 0.07$). Likewise, PDGFR- β at baseline was higher in patients with PR than those with SD/PD (8.085 vs. 4.372, $P = 0.04$). Further, expression of PDGFR- β was confirmed at the protein level by IHC (Table 1 and Fig. 1).

In the GO class for *cell motility, locomotion or localization of cell*, the platelet/endothelial cell adhesion molecule CD31 is a representative of the differentially expressed genes. Expression of CD31 was higher in the responders than non-responders at the transcriptional level (19.85 vs. 12.56, $P = 0.03$). This was confirmed at the protein level by IHC (Table 1 and Fig. 1). In the class for *spindle*, one representative gene is CDC16, a cell cycle-regulated ubiquitin ligase, which is a part of cyclin degradation system that controls exit from mitosis. Patients with PR had low CDC16 as compared to those with SD/PD (0.65 vs. 1.15, $P = 0.001$).

Of 17 GO categories that were statistically significant by the LS statistic at $P < 0.005$ level, 6.7 false positive categories would be expected by chance corresponding to a false discovery rate of 39.4% (6.7/17). Similarly, of 15 GO classes that were statistically significant by the KS statistic at $P < 0.005$ level, the false discovery rate was expected to be 44.7% (6.7/15).

Decrease in CD31 expression after BV treatment alone

To extend the findings of CD31 at baseline in association with response and to explore the potential relationship between VEGF-A and CD31, we examined CD31 expression in tumor biopsies after one cycle of BV treatment. Interestingly, there was a significant decrease in CD31 expression after BV treatment compared to that of baseline biopsies ($n = 15$ pairs, mean decrease $\pm$ SEM: 41% $\pm$ 11%, $P = 0.007$ by a Wilcoxon signed rank test) (Fig. 2).

Molecular Pathways and Response to Bevacizumab in Combination with Chemotherapy

Of 171 molecular pathways available for analysis, four that are related to T and NK cell activity and one that is related to bioactive peptide induced signaling were found to be associated with response (Supplementary Table 2). The five significant pathways were: *Lck and Fyn tyrosine kinases in the initiation of T cell receptor activation*; *T cell receptor signaling pathway*; *T helper cell surface molecules*; *NO2-dependent IL 12 pathway in NK cells*; and *bioactive peptide induced signaling pathway*. Overall, the responders had higher T and NK cell activity and/or signaling than non-responders. The bioactive peptide induced signaling pathway included microtubule-associated protein tau (MAPT) that has a role in regulating microtubule stability. Of the 5 pathways statistically significant by the KS statistic at $P < 0.005$ level, 0.86 false positive categories would be expected by chance corresponding to a false discovery rate of 17.1% (0.86/5).

Discussion

Through gene expression profiling approach, we identified 26 GO classes that separate the responders from non-responders in patients with IBC and LABC. The GO class for *vascular endothelial growth factor receptor activity* is most likely relevant to the response to BV and chemotherapy. In this GO class, the representative differentially expressed genes are PDGFR- α and - β , which encode cell surface tyrosine kinase receptors for members of the PDGF family. It has been known that VEGF-A binds to both PDGFR- α and PDGFR- β and induces tyrosine

phosphorylation (12). The tumor stroma and/or tumor with high expression levels of PDGFRs could be more dependent on the signaling of VEGF-A and PDGFRs and, thus, more sensitive to the BV blockade of VEGF-A. *CD31* gene encodes a cell adhesion molecule expressed on platelets and at endothelial cell intercellular junctions, which is involved in cell recognition/motility/signaling in the biological process. We found that higher endothelial cell CD31 expression in the tumor vasculature at both gene transcription and translation levels was significantly associated with the response to BV followed by BV plus chemotherapy. Furthermore, a significant decrease in CD31 expression was observed in patient tumors treated with BV alone in this cohort; and a down regulation of CD31 after BV treatment was found in an *in vivo* lung tumor xenograft study (13). These data suggest that VEGF-A regulates the level of CD31 expression in endothelial cells and may play a role in BV treatment and chemotherapy. Moreover, it is worth mentioning the difference between CD31 expression and MVD. MVD is measured by enumerating endothelial marker CD31 or CD34 labeled vessels (vascular hotspot, area with more vessel numbers) in defined tissue/tumor areas (14). In enumerating MVD, all vessels that pass detection threshold are counted by either automated or manual method regardless of levels of CD31 or CD34 expression (6,15). For example, MVD was lower in the tumor from a patient with PR than that from one with PD (no. of vessels/mm²: 280 vs. 383)(6), whereas CD31 expression was higher in the tumor vasculature from the same PR patient than that from the same PD patient (SI: 47.5 vs. 11.7; Fig. 1).

The GO category for *spindle* is more likely relevant to the response to docetaxel. The tumors with low CDC16 expression were associated with better response relative to those with high CDC16 expression. CDC16 protein is a part of the anaphase promoting complex/cyclosome (APC/C) that is composed of eight proteins and functions as a protein ubiquitin ligase. The APC complex is a cyclin degradation system that governs exit from mitosis (16). The cytotoxic activity of docetaxel is exerted by promoting and stabilizing microtubule assembly, while preventing physiological microtubule depolymerization/disassembly. Consequently, the treated cells tend to be arrested in mitosis (17). The higher CDC16 the cells express, the easier the cells exit from mitosis, the more docetaxel may be needed to arrest cells in mitosis.

At the individual gene level, there were only few genes significantly ($P < 0.001$) associated with response, which were all within the false discovery rate. Similar to our findings, a study by Hannemann *et al* found no individual gene signature that was associated with response to doxorubicin and docetaxel neoadjuvant chemotherapy in 48 patients with primary breast cancer (18). However, Chang *et al* found a gene expression profile that predicted response to neoadjuvant docetaxel monotherapy in 24 patients with primary breast cancer (19). A 92-gene expression signature was associated with response when less than 25% residual disease present was defined as the sensitive tumors (this cutoff divided the samples into two equal groups). In this study, we used clinical response criteria (RECIST) to classify the responders and non-responders as a closer approach to clinical application.

The analyses that evaluate differential expression of groups of genes where the groups are defined by pathways or GO annotations are more powerful than evaluation differential expression of individual genes for two main reasons. One is that the multiple comparison penalty is much greater for the individual gene analyses because there are so many more individual genes than pathways or GO categories. Secondly, the gene group analyses can be statistically significant if more genes in the group show evidence of differential expression than expected by chance, even if their degree of differential expression does not achieve the stringent level required for statistical significance of individual genes.

Among all the angiogenic and tumor markers studied, expression of CD31 and PDGFR- β was significantly associated with response (discussed earlier). Tumor VEGF-A, the putative BV target, was identified to have a trend towards association with treatment. In a trial with

metastatic colorectal cancer (mCRC), VEGF was found not to be a significant prognostic/predictive factor in patients with mCRC treated with bevacizumab in combination with first-line irinotecan, fluorouracil, and leucovorin chemotherapy. In the study, epithelial and stromal VEGF were assessed together on formalin-fixed paraffin-embedded mCRC surgical specimens by either *in situ* hybridization or IHC (20). The difference between the two studies could be explained by the fact that only tumor VEGF-A, quantitatively analyzed by a digital imaging system, was assessed in our study. The other baseline markers including MVD, consistent with other findings (20), were not associated with response in this study.

We also identified five molecular pathways that were associated with response. Of these five pathways, four were related to host immune response, indicating that levels of intrinsic host immune status may play some roles to the patient outcome. The other pathway identified was the *bioactive peptide induced signaling pathway*. It includes microtubule-associated protein tau which enhances the assembly and stabilization of microtubules (21). Though none of the genes in these pathways was statistically significant at the individual gene expression level, some showed differential expression.

In summary, through biomarker and gene expression profiling approaches, we demonstrate three angiogenic markers, 26 GO categories, and five functional molecular pathways that were differentially expressed between the responders and nonresponders. The key markers in the angiogenesis process that associate or show trends with response to therapy are (1) tumor VEGF-A, the molecular target of BV, (2) PDGFRs, the receptors of VEGF-A, and (3) CD31, an endothelial cell adhesion molecule whose expression may be modulated by VEGF-A. Patients with higher tumor VEGF-A, CD31 and PDGFR- β expression in the tumor vasculature tended to be more likely benefiting from the bevacizumab treatment plus chemotherapy. The findings indicate that bevacizumab may be specifically given as a targeted agent although these data need to be confirmed by future larger perspective cohorts such as National Surgical Adjuvant Breast and Bowel Project (NSABP) B40.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Hance KW, Anderson WF, Devesa SS, et al. Trends in inflammatory breast carcinoma incidence and survival: The surveillance, epidemiology, and end results program at the national cancer institute. *J Natl Cancer Inst* 2005;97:966–75. [PubMed: 15998949]
2. Low JA, Berman AW, Steinberg SM, et al. Long-term follow-up for locally advanced and inflammatory breast cancer patients treated with multimodality therapy. *J Clin Oncol* 2004;22:4067–74. [PubMed: 15483018]
3. McCarthy NJ, Yang X, Linnoila IR, et al. Microvessel density, expression of estrogen receptor alpha, mib-1, p53, and c-erbB-2 in inflammatory breast cancer. *Clin Cancer Res* 2002;8:3857–62. [PubMed: 12473600]

4. Ranieri G, Patruno R, Ruggieri E, et al. Vascular endothelial growth factor (vegf) as a target of bevacizumab in cancer: From the biology to the clinic. *Curr Med Chem* 2006;13:1845–57. [PubMed: 16842197]
5. Miller K, Wang M, Gralow J, et al. Paclitaxel plus bevacizumab versus paclitaxel alone for metastatic breast cancer. *N Engl J Med* 2007;357:2666–76. [PubMed: 18160686]
6. Wedam SB, Low JA, Yang SX, et al. Antiangiogenic and antitumor effects of bevacizumab in patients with inflammatory and locally advanced breast cancer. *J Clin Oncol* 2006;24:769–77. [PubMed: 16391297]
7. Greene, FLPD.; Fleming, ID., et al. *Ajcc cancer staging handbook*. Vol. 6. New York, NY: Springer; 2002.
8. Therasse P, Arbuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors. European organization for research and treatment of cancer, national cancer institute of the united states, national cancer institute of canada. *J Natl Cancer Inst* 2000;92:205–16. [PubMed: 10655437]
9. Tan AR, Yang X, Hewitt SM, et al. Evaluation of biologic end points and pharmacokinetics in patients with metastatic breast cancer after treatment with erlotinib, an epidermal growth factor receptor tyrosine kinase inhibitor. *J Clin Oncol* 2004;22:3080–90. [PubMed: 15284258]
10. Yang SX, Simon RM, Tan AR, et al. Gene expression patterns and profile changes pre- and post-erlotinib treatment in patients with metastatic breast cancer. *Clin Cancer Res* 2005;11:6226–32. [PubMed: 16144925]
11. Pavlidis P, Qin J, Arango V, et al. Using the gene ontology for microarray data mining: A comparison of methods and application to age effects in human prefrontal cortex. *Neurochem Res* 2004;29:1213–22. [PubMed: 15176478]
12. Ball SG, Shuttleworth CA, Kielty CM. Vascular endothelial growth factor can signal through platelet-derived growth factor receptors. *J Cell Biol* 2007;177:489–500. [PubMed: 17470632]
13. Inoue S, Hartman A, Branch CD, et al. Mda-7 in combination with bevacizumab treatment produces a synergistic and complete inhibitory effect on lung tumor xenograft. *Mol Ther* 2007;15:287–94. [PubMed: 17235306]
14. Weidner N, Folkman J, Pozza F, et al. Tumor angiogenesis: A new significant and independent prognostic indicator in early-stage breast carcinoma. *J Natl Cancer Inst* 1992;84:1875–87. [PubMed: 1281237]
15. Weidner N, Semple JP, Welch WR, et al. Tumor angiogenesis and metastasis--correlation in invasive breast carcinoma. *N Engl J Med* 1991;324:1–8. [PubMed: 1701519]
16. Wehman AM, Staub W, Baier H. The anaphase-promoting complex is required in both dividing and quiescent cells during zebrafish development. *Dev Biol* 2007;303:144–56. [PubMed: 17141209]
17. Lyseng-Williamson KA, Fenton C. Docetaxel: A review of its use in metastatic breast cancer. *Drugs* 2005;65:2513–31. [PubMed: 16296875]
18. Hannemann J, Oosterkamp HM, Bosch CA, et al. Changes in gene expression associated with response to neoadjuvant chemotherapy in breast cancer. *J Clin Oncol* 2005;23:3331–42. [PubMed: 15908647]
19. Chang JC, Wooten EC, Tsimelzon A, et al. Gene expression profiling for the prediction of therapeutic response to docetaxel in patients with breast cancer. *Lancet* 2003;362:362–9. [PubMed: 12907009]
20. Jubb AM, Hurwitz HI, Bai W, et al. Impact of vascular endothelial growth factor-a expression, thrombospondin-2 expression, and microvessel density on the treatment effect of bevacizumab in metastatic colorectal cancer. *J Clin Oncol* 2006;24:217–27. [PubMed: 16365183]
21. Goedert M, Wischik CM, Crowther RA, et al. Cloning and sequencing of the cDNA encoding a core protein of the paired helical filament of alzheimer disease: Identification as the microtubule-associated protein tau. *Proc Natl Acad Sci U S A* 1988;85:4051–5. [PubMed: 3131773]

Fig.1.

Expression of CD31, PDGFR- β and tumor VEGF-A in representative patient tumor biopsies. There were high levels of CD31 (A) and VEGF-A (C), and PDGFR- β (E) in tumor biopsy sections from a patient with PR compared to low levels of CD31 (B) and tumor VEGF-A (D) in those from a patient with PD or low level of PDGFR- β (F) in the section from a patient with SD. PR, partial response; PD, progressive disease; SD, stable disease.

Fig. 2.

The change in CD31 expression after BV treatment alone in patients with IBC and LABC. Shown was the relative change in CD31 expression in the tumor vasculature in 15 patients that paired biopsies were available for analysis. Pt, patient; BV, bevacizumab.

Table 1
Baseline angiogenic/tumor markers and clinical response

Marker	No. patients	Median (range)	<i>P</i> *
CD31			0.0004
PR	12	33.5 (16.6 – 48.8)	
SD/PD	6	13.2 (6.2 – 18.2)	
PDGFR- β			0.013
PR	13	5.9 (0.3 – 36.6)	
SD/PD	5	0.6 (0.06 – 1.43)	
VEGF-A			0.043
PR	14	2.65 (0.06 – 39.4)	
SD/PD	6	0.25 (0 – 2.4)	
pVEGFR2(Y996)			0.60
PR	14	4.26 (0.4 – 46.55)	
SD/PD	6	9.47 (0.08 – 52.16)	
pVEGFR2(Y951)			0.90
PR	14	21.78 (1.36 – 49.68)	
SD/PD	6	29.36 (0.77 – 76.76)	
MVD			0.55
PR	14	276.9 (1.0 – 441.6)	
SD/PD	6	159.45 (93.2 – 383.0)	
Ki67			0.44
PR	14	27.1 (5.5 – 47.2)	
SD/PD	6	29.5 (6.6 – 54.2)	
Apoptosis			0.79
PR	14	1.45 (0 – 12.8)	
SD/PD	6	1.55 (0.6 – 6.0)	

All *P* values were two tailed, determined by Wilcoxon rank sum test.

Table 2
GO categories which discriminate the responders from non-responders

GO category	GO Term*	GO description	No. genes [†]	LS permutation P	KS permutation P
0005581	CC	Collagen	25	1e-05	0.0001194
0044420	CC	Extracellular matrix part	47	1e-05	9.66e-05
0006817	BP	Phosphate transport	39	0.0003932	0.0016308
0005201	MF	Extracellular matrix structural constituent	39	0.0006586	1e-05
0030934	CC	Anchoring collagen	5	0.0009974	0.0078597
0015698	BP	Inorganic anion transport	50	0.0010409	0.0044008
0005819	CC	Spindle	11	0.0014625	0.0074051
0006820	BP	Anion transport	60	0.0015095	0.0098402
0005021	MF	Vascular endothelial growth factor receptor activity	5	0.0017757	0.0183713
0005001	MF	Transmembrane receptor protein tyrosine phosphatase activity	9	0.001975	0.0776652
0019198	MF	Transmembrane receptor protein phosphatase activity	9	0.001975	0.0776652
0007185	MF	Transmembrane receptor protein tyrosine phosphatase signaling pathway	5	0.0025524	0.1774075
0031072	MF	Heat shock protein binding	18	0.0025617	0.0271924
0007167	BP	Enzyme linked receptor protein signaling pathway	54	0.0033393	0.1507051
0015662	MF	ATPase activity\, coupled to transmembrane movement of ions\, phosphorylative mechanism	11	0.003359	0.0396246
0050767	BP	Regulation of neurogenesis	6	0.003614	0.0041765
0005813	CC	Centrosome	15	0.0046869	0.0427838
0051094	BP	Positive regulation of development	6	0.0060322	0.0041765
0030693	MF	Caspase activity	8	0.0066096	0.001746
0042623	MF	ATPase activity\, coupled	74	0.0239713	0.0031284
0006928	BP	Cell motility	80	0.0376735	0.0048878
0040011	BP	Locomotion	80	0.0376735	0.0048878
0051674	BP	Localization of cell	80	0.0376735	0.0048878
0016887	MF	ATPase activity	84	0.0419617	0.0036955
0007389	BP	Pattern specification	6	0.1218883	0.0033214

GO category	GO Term [*]	GO description	No. genes [†]	LS permutation <i>P</i>	KS permutation <i>P</i>
0000226	BP	Microtubule cytoskeleton organization and biogenesis	14	0.1229871	0.0011392

^{*} CC: Cellular Component; MF: Molecular Function; BP: Biological Process.

[†] All the genes from the 26 GO categories are listed in Supplementary Table 1.