Gene Expression Profiles Associated with the Presence of a Fibrotic Focus and the Growth Pattern in Lymph Node–Negative Breast Cancer

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Abstract

Purpose: A fibrotic focus, the scar-like area found in the center of an invasive breast tumor, is a prognostic parameter associated with an expansive growth pattern, hypoxia, and (lymph)angiogenesis. Little is known about the molecular pathways involved.

Experimental Design: Sixty-five patients were selected of whom microarray data of the tumor and H&E slides for histologic analysis were available. The growth pattern and the presence size of a fibrotic focus were assessed. Differences in biological pathways were identified with global testing. The correlations of growth pattern and fibrotic focus with common breast cancer signatures and with clinicopathologic variables and survival were investigated.

Results: Tumors with a large fibrotic focus showed activation of Ras signaling and of the hypoxia-inducible factor-1α pathway. Furthermore, unsupervised hierarchical cluster analysis with hypoxia- and (lymph)angiogenesis-related genes showed that hypoxia-inducible factor-1α, vascular endothelial growth factor A, and carbonic anhydrase 9 were overexpressed. The presence of a fibrotic focus, especially a large fibrotic focus, was associated with the basal-like subtype (P = 0.009), an activated wound-healing signature (P = 0.06), and a poor-prognosis 76-gene signature (P = 0.004). The presence of a fibrotic focus (P = 0.02) and especially of a large fibrotic focus (P = 0.004) was also associated with early development of distant metastasis.

Conclusions: Our results sustain the hypothesis that hypoxia-driven angiogenesis is essential in the biology of a fibrotic focus. Ras and Akt might play a role as downstream modulators. Our data furthermore suggest that vascular endothelial growth factor A does not only drive angiogenesis but also lymphangiogenesis in tumors with a fibrotic focus. Our data also show an association between the presence of a fibrotic focus and infaust molecular signatures.

A fibrotic focus is defined as a scar-like area, consisting of fibroblasts and collagen fibers, which occupies various percentages of the center of an invasive ductal carcinoma of the breast. The fibrotic focus was initially proposed by Hasebe et al. (1) as an indicator of aggressive tumor behavior. The presence of a fibrotic focus is correlated with larger tumor size, increased tumor cell proliferation, higher histologic grade, nuclear accumulation of p53 protein, c-erbB-2 overexpression, and higher pathologic stage (2, 3). Furthermore, the presence of a fibrotic focus has been associated with poor short- and long-term survival (2, 3) and has been confirmed as an independent prognostic factor for patients with breast cancer in both retrospective (4–8) and prospective (9, 10) studies. It was also shown that the relative size of the fibrotic focus (fibrotic focus to tumor ratio) is important for patient outcome (5). The advantages of a fibrotic focus as a practical, easily assessable, and reproducible integrative histologic prognostic variable in breast cancer were recently reviewed (11).

Current understandings assume that hypoxia is the driving force for the formation of a fibrotic focus. Areas of necrosis are more frequently found in breast carcinomas with a fibrotic focus (5). Other studies showed that the presence of a fibrotic focus was correlated with the increased expression of hypoxia-inducible factor-1α (HIF-1α) and carbonic anhydrase 9 (CA9), two endogenous hypoxia markers, both in carcinoma cells and intratumoral fibroblasts (12, 13). The highest CA9 scores were found in fibroblasts of large fibrotic foci occupying more than one third of the tumor area (12). Hypoxia then leads to proliferation and activation of stromal fibroblasts (14) with

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abundant and disorganized deposition of extracellular matrix proteins and to the induction of angiogenesis. The presence of a fibrotic focus has indeed been correlated with increased angiogenesis (4–6, 13, 15). These stromal changes are involved in tumor progression, increased hypoxia, and metastasis (11). The origin of the hypoxia might be related to the growth pattern of the tumor (4). Expansively growing tumors result in hypoxia due to destruction of the normal surrounding parenchyma and formation of new chaotic blood vessels, whereas infiltrative tumors are less hypoxic as these grow along preexisting well-formed blood vessels that are thought to be more efficient in providing oxygen and nutrients to the tumor. Tumors with an expansive growth pattern often show a fibrotic focus, necrosis, expression of CA9 and Hif-1α, and angiogenesis (4, 12, 13).

These hypotheses are mainly based on histochemical and immunohistochemical studies. Although important insights originated from this work, this approach is limited in the number of tumor characteristics and molecules that can be investigated. Therefore, current available data do not allow for an integrated view on the pathways and processes involved with the formation of a fibrotic focus. Modern molecular biology techniques, such as genome-wide expression microarray analysis, enable the analysis of large numbers of genes at the same time and are therefore more adapted for the study of molecular pathways and processes. Because both tumor cells and stromal cells are involved in the formation of a fibrotic focus, the fibrotic focus should be regarded as an integrative histologic marker of hypoxia-driven reactive vascular stroma formation, and further elucidation of the mechanisms involved in its origin and formation might lead to better understanding of the metastatic cascade in breast cancer in general and of tumor-stroma interactions during tumor progression and metastasis in breast cancer in particular. Therefore, the aim of this work was to study gene expression profiles correlated with the presence of a fibrotic focus and growth pattern in lymph node–negative primary breast cancer. In addition, we aimed to assess the pure prognostic value of fibrotic focus and growth pattern without possible confounding effects of therapy and therefore included only patients with lymph node–negative disease not treated with adjuvant systemic therapy.

### Materials and Methods

**Patient selection and survival data.** From the previously published lymph node–negative breast cancer patient population (16), 67 patients were selected. Patient selection was based solely on the availability of both genome-wide gene expression data and formalin-fixed, paraffin-embedded tissue from the primary resection specimen. This was necessary to be able to assess the fibrotic focus and growth pattern of the samples. No other exclusion criteria were used. After revision of the H&E slides, two cases were excluded because no invasive carcinoma was left in the tissue blocks. Clinicopathologic data of the remaining 65 patients are shown in Table 1.

**Assessment of fibrotic focus and growth pattern.** The presence and size of a fibrotic focus and the growth pattern of the primary tumor were assessed on the H&E slides, as previously defined (1, 6, 17). Briefly, a fibrotic focus is defined as a scar-like area, consisting of fibroblasts and collagen fibers, which occupies various percentages of

### Table 1. Clinicopathologic data

<table>
<thead>
<tr>
<th>Fibrotic focus</th>
<th>Growth pattern</th>
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<tbody>
<tr>
<td>Absent</td>
<td>Small</td>
</tr>
<tr>
<td>Age (y) Mean</td>
<td>54.2</td>
</tr>
<tr>
<td>Tumor size (mm) Mean</td>
<td>22</td>
</tr>
<tr>
<td>Histologic type</td>
<td>1.0</td>
</tr>
<tr>
<td>IDA 55</td>
<td>32</td>
</tr>
<tr>
<td>ILA 7</td>
<td>6</td>
</tr>
<tr>
<td>Other 3</td>
<td>3</td>
</tr>
<tr>
<td>Nottingham grade 0.13</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
</tr>
<tr>
<td>3</td>
<td>29</td>
</tr>
<tr>
<td>T status 1</td>
<td>22</td>
</tr>
<tr>
<td>0.17</td>
<td>1</td>
</tr>
<tr>
<td>ER Negative</td>
<td>17</td>
</tr>
<tr>
<td>Positive</td>
<td>48</td>
</tr>
<tr>
<td>PR 0.02</td>
<td>24</td>
</tr>
<tr>
<td>Negative</td>
<td>41</td>
</tr>
<tr>
<td>Positive</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Abbreviations: ILA, infiltrating lobular adenocarcinoma; IDA, invasive ductal adenocarcinoma; ER, estrogen receptor; PR, progesterone receptor; FF, fibrotic focus.
the center of an invasive carcinoma of the breast. If present, the size of the fibrotic focus was assessed as small (one third or less of the tumor area) or large (more than one third of the tumor area; Fig. 1A and B). The growth pattern was assessed as infiltrative, expansive, or mixed. In the infiltrative growth pattern, carcinoma cells infiltrate between preexisting breast parenchymal structures without significant disturbance of the breast architecture (Fig. 1C). In the expansive growth pattern, the tumor forms a well-circumscribed nodule consisting of carcinoma cells and desmoplastic connective tissue. Preexisting breast parenchymal structures are not present inside the tumor but are pushed aside by the expansively growing nodule (Fig. 1D). The growth pattern is mixed infiltrative-expansive when the tumor consists of a central expansive nodule surrounded by carcinoma cells showing an infiltrative growth pattern.

**Gene expression data analysis.** The gene expression data used in this analysis were obtained from our previously published work and described in detail elsewhere (16). Raw gene expression data <30 were set to 30, and data were centered around the geometric mean per probe set and log2 transformed. Global differences in biological processes and signaling pathways between tumors with different sizes of fibrotic focus and with different growth patterns were studied with Goeman's global testing (18, 19) using biological pathway data provided by BioCarta. Biological processes with both false discovery rate (FDR)-adjusted and comparative $P$ value of $<0.05$ were considered to be significant. When either the FDR-adjusted or the comparative $P$ value was $<0.05$ and the other was $>0.05$ and $<0.1$, we defined it as a trend.

**Expression of hypoxia- and (lymph)angiogenesis-related genes according to fibrotic focus and growth pattern.** To study the expression of hypoxia- and (lymph)angiogenesis-related genes according to the different fibrotic focus categories and growth patterns, a previously published list of 269 genes was used that contains genes that are currently known to be related to and involved in hypoxia response and (lymph)angiogenesis (20). Of this list, 262 unique genes were present on the Affymetrix chips. Unsupervised hierarchical clustering of samples and genes was done to identify patterns of hypoxia- and (lymph)angiogenesis-related gene expression according to different fibrotic focus categories and growth patterns, and differential expression between different groups was analyzed using parametric statistics.

**Association between fibrotic focus, growth pattern, and common breast cancer signatures.** To study the association between fibrotic focus and growth pattern and common breast cancer signatures, the cell-of-origin molecular subtype (21), the wound-healing response signature (22), the stromal signature (23), the invasiveness gene signature (24), and the 76-gene prognostic signature (16) of each sample were assessed. The 76-gene prognostic signature and the cell-of-origin subtype were assessed as previously described (16). Samples were classified according to the other signatures using a centroid-mediated classification algorithm. The genes from each signature were mapped onto the list of Affymetrix probe set IDs using the Affymetrix annotation file (6). This resulted in 252 genes (–452 probe IDs), 408 genes (–775 probe IDs), and 128 genes (–128 probe IDs) of the wound-healing response signature, the stromal signature, and the invasiveness signature that were present on the GeneChips (Affymetrix). These reduced signatures were still able to assign the samples from the original data sets to the correct subgroup. The centroid expression profile of each subgroup was computed as the average expression for each gene based on the samples with the highest correlation in the original data set. If genes were represented by multiple clones, the centroid was calculated by averaging the centroid expression of the representing clones. Then, normalized gene expression data for the signature genes were extracted from our data set. Gene expression data for multiple clones representing a single gene were averaged. Next, data were log2 transformed and median centered on genes and Spearman correlations were calculated between each sample in our series and each centroid. A tumor was assigned to a
subgroup if the correlation coefficient with its centroid was positive and showed a negative correlation with the centroid of the opposite subgroup. If the correlation coefficients with the centroids of both subgroups were both positive or both negative, the sample was assigned to be undetermined.

Statistical analysis. Kaplan-Meier survival plots and log-rank tests were used to assess the differences in time to distant metastasis as a function of fibrotic focus and growth pattern. Hierarchical clustering and analyses of robustness of clustering were done using BRB ArrayTools. All analyses of robustness of clustering were done using BRB ArrayTools.7 All P values are calculated using the χ² test, except where stated otherwise; all P values are two sided.

Results

Histologic data and survival analysis. The results on the relationships of fibrotic focus and growth pattern with patients and tumor characteristics are presented in Table 1. In 24 of 65 (36.9%) specimens, a fibrotic focus was present, 14 samples with a large fibrotic focus and 10 samples with a small fibrotic focus. Nine (13.9%), 46 (70.8%), and 10 (15.4%) tumors had an infiltrative, mixed, and expansive growth pattern, respectively. The presence (P = 0.007) and relative size (P = 0.005) of the fibrotic focus were associated with the growth pattern: none of the tumors with an infiltrative growth pattern had a fibrotic focus, in contrast to a fibrotic focus in 37.0% and 70% of the tumors with an infiltrative growth pattern, respectively (Table 1). The presence of a fibrotic focus, especially a large fibrotic focus, was associated with progesterone receptor negativity (P = 0.02). Furthermore, the growth pattern was associated with the histologic type (P = 0.03). Patients with a tumor with a fibrotic focus, especially with a large fibrotic focus, had a shorter time to distant metastasis than patients with a tumor without a fibrotic focus (P = 0.02, log-rank test; Fig. 2A). There was no difference in time to distant metastasis between tumors with different growth patterns (Fig. 2B). Multivariate Cox regression analysis did not retain fibrotic focus nor growth pattern as an independent prognostic factor.

Biological pathways associated with the fibrotic focus and growth pattern. To study biological pathways that differ between the fibrotic focus and growth pattern groups, global testing was done. The results are shown in Supplementary Data File 1. The most significant differences were observed when tumors with a large fibrotic focus were compared with the rest. The “Ras signaling pathway” (comparative P = 0.005 and FDR-adjusted P = 0.003), “Hif pathway” (comparative P = 0.004 and FDR-adjusted P = 0.003), “phosphatase and tensin homologue-dependent cell cycle arrest and apoptosis” (comparative P = 0.015 and FDR-adjusted P = 0.004), “chaperones modulate IFN signaling pathway” (comparative P = 0.03 and FDR-adjusted P = 0.01), and “transcription factor cyclic AMP-responsive element binding protein and its extracellular signals” (comparative P = 0.04 and FDR-adjusted P = 0.01) were significantly different between these groups. For “vascular endothelial growth factor (VEGF)-hypoxia and angiogenesis” (comparative P = 0.06 and FDR-adjusted P = 0.01), “Akt signaling” (comparative P = 0.09 and FDR-adjusted P = 0.01), “role of BRCA1, BRCA2, and ATR in cancer susceptibility” (comparative P = 0.06 and FDR-adjusted P = 0.01), and “control of gene expression by vitamin D receptor” (comparative P = 0.07 and FDR-adjusted P = 0.01), a trend was found. No significantly different pathways were seen between tumors with different growth patterns or when tumors with a fibrotic focus were compared with tumors without a fibrotic focus. A more detailed analysis of differential expression of the different components of these pathways showed that the Hif pathway and VEGF-hypoxia and angiogenesis genes are clearly stimulated in tumors with a large fibrotic focus, whereas for the other pathways the expression of some of the genes is increased and of others is decreased (Supplementary Data File 1). Figure 3 shows gene plots for the Hif pathway, VEGF-hypoxia and angiogenesis, Ras, and Akt signaling.

Expression of hypoxia- and (lymph)angiogenesis-related genes according to fibrotic focus and growth pattern. To further explore the differential expression of hypoxia- and (lymph) angiogenesis-related genes in tumors with a fibrotic focus and different growth patterns, a previously published gene list of

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7 http://linus.nci.nih.gov/~brb/tool.htm
269 hypoxia- and (lymph)angiogenesis-related genes was used. The most robust unsupervised clustering of the 65 samples with these genes resulted in three clusters of samples. The smallest cluster contained 10 samples and was highly enriched in tumors with an expansive growth pattern ($P = 0.001$) and large fibrotic focus ($P = 0.003$): 6 of 10 (60%) and 6 of 10 (60%) tumors had an expansive growth pattern or large fibrotic focus, respectively, and 5 of 10 (50%) tumors had both an expansive growth pattern and a large fibrotic focus. This cluster was also enriched with estrogen receptor–negative tumors (8 of 10 samples in this cluster were estrogen receptor negative compared with 9 of 55 of the other samples; $P < 0.001$; Fig. 4A). Visual inspection of the heat map showed two clusters of 67 and 31 genes that were overexpressed and underexpressed, respectively, in the tumors of the expansive growth pattern–enriched and large fibrotic focus–enriched cluster (Fig. 4B). Among the overexpressed genes were PCNA, Hif-1α, CA9, VEGFA, FGF2, podoplanin, TIMP1, and TIMP2. In the cluster of genes with decreased expression, RARα, CA12, TGFβ3, BCL2, IGF2, ERBB2, and PGK1 were the most remarkable.

![Gene plots of some of the pathways that were differentially expressed between tumors with a large fibrotic focus and tumors with a small fibrotic focus or without a fibrotic focus. These plots represent the influence of individual genes (indicated by their gene symbol) of each pathway to the differential expression in both groups of samples. The horizontal lines in each bar represent how many SDs the gene expression of each gene exceeds the hypothesis that there would be no difference between both groups (z-score). * genes with a $z > 1.96$ ($=P < 0.05$).](image)
genes. When the expression of these genes was compared (univariate t test) between all tumors with a large fibrotic focus versus tumors with a small or no fibrotic focus, PCNA (P = 0.02), Hif-1α (P = 0.03), RARα (P = 0.03), BCL2 (P = 0.01), IGF2 (P = 0.02), and PGK1 (P = 0.02) had a significantly lower expression in tumors with a large fibrotic focus and an expansive growth pattern (P < 0.001). Unsupervised hierarchical cluster analysis of the genes of the hypoxia- and (lymph)angiogenesis-related gene list showed a cluster of genes (37 genes; red square) that is overexpressed and a cluster of genes (67 genes; green square) that is underexpressed in the large fibrotic focus–enriched/ expansive growth pattern–enriched cluster. B, exploded view of both gene clusters. Left, overexpressed genes; right, underexpressed genes.

**Association between fibrotic focus and growth pattern and common breast cancer signatures.** Table 2 shows the association of common breast cancer signatures with the presence and size of a fibrotic focus and with the growth pattern of breast carcinomas. Pair-wise comparisons using Fisher’s exact test showed an overrepresentation of basal-like tumors among tumors with an expansive growth pattern (P = 0.01) and among tumors with a large fibrotic focus (P = 0.009). There was also an association between the presence of an expansive growth pattern and an activated wound-healing response gene signature (P = 0.03) and a trend between the presence of a large fibrotic focus and an activated wound-healing response signature (P = 0.06). Furthermore, all but one tumor with an expansive growth pattern had a positive correlation with the invasiveness gene signature (P = 0.03) and there was a strong correlation between the presence of a fibrotic focus, especially a
large fibrotic focus, and a poor 76-gene prognostic signature ($P = 0.004$).

**Discussion**

This is the first study on gene expression profiles associated with fibrotic focus and growth pattern of breast cancer. A fibrotic focus is defined as a scar-like area that occupies various percentages of the center of an invasive breast carcinoma. The association of the presence of a fibrotic focus, especially a large fibrotic focus, with progesterone receptor negativity and with short time to distant metastasis is in line with previously published data showing that the presence of a fibrotic focus is correlated to other variables of tumor aggressiveness (1–3). Because one of the aims of our study was to assess the pure prognostic value of fibrotic focus and growth pattern, our patients were selected from the lymph node–negative population that had not received systemic adjuvant therapy described by Wang et al. (16). Therefore, our conclusions should be limited to patients with lymph node–negative breast cancer. The fact that the presence of a fibrotic focus was not retained as an independent prognostic factor in the multivariate analysis was probably due to the low number of patients and events because it has been shown before that the presence of a fibrotic focus independently predicts tumor recurrence and tumor death in a larger series of untreated lymph node–negative patients (ref. 4, reviewed in ref. 11).

From a biological point of view, there are no arguments to assume that the biology of a fibrotic focus is different in breast tumors with and without lymph node metastases: the association of the presence of a fibrotic focus with the presence of necrosis, expression of hypoxia markers, and increased (lymph)angiogenesis has been shown in both lymph node–negative and lymph node–positive study populations (4, 12, 13). Current hypotheses suggest that intratumoral hypoxia leads to a wound-healing–like process with activation of fibroblasts, deposition of extracellular matrix, and induction of angiogenesis (11). The results of the global tests indeed show that the Hif-1α pathway is highly active in tumors with a large fibrotic focus. Hif-1α, a key regulator in the cellular response to hypoxia, is stabilized under hypoxic conditions and serves to propagate a cascade of pathways. VEGFA and CA9 are important Hif-1α target molecules, involved in angiogenesis and microenvironmental pH regulation, respectively (25). For “VEGF hypoxia and angiogenesis,” a trend was found in the global test and the cluster of hypoxia- and (lymph)angiogenesis-related genes, highly expressed in tumors with a large fibrotic focus and with an expansive growth pattern, contained Hif-1α, CA9, and VEGFA. The results of the pathway analysis are validated by the fact that in immunohistochemical studies endogenous markers for hypoxia, such as CA9 and Hif-1α, have been previously found to be increased in tumors with a fibrotic focus not only in tumor cells but also in fibroblasts (12, 13). However, the current data enable a more integrated view on the pathways that are involved. For instance, the differential expression of genes involved in “Ras and Akt signaling” might also be related to increased hypoxic signaling. In an in vitro angiogenesis model, it has been shown that the Ras pathway plays a critical role in VEGF-induced angiogenesis (26) and oncogenic Ras transformation and hypoxia synergistically modulate VEGF expression in different cell types (27, 28). The mechanism acts through Ras increasing Hif-1α protein expression via a phosphatidylinositol 3′-kinase/Akt–mediated step (28, 29). Stimulation of T-47D breast cancer cells with VEGF also induced phosphorylation of phosphatidylinositol 3′-kinase, Akt, and forkhead receptor L1 (30). On the other hand, differences in Ras and Akt signaling might also be related to the differential expression of phosphatase and tensin homologue–dependent cell cycle arrest and apoptosis (31, 32). Further insight in the role of each of these pathways and their interaction in the biology of the fibrotic focus might lead to better insight in the mechanisms of breast cancer aggressiveness.

<table>
<thead>
<tr>
<th>Table 2. Association of fibrotic focus and growth pattern with common breast cancer signatures</th>
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<tbody>
<tr>
<td><strong>Fibrotic focus</strong></td>
</tr>
<tr>
<td>Cell-of-origin subtype</td>
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<tr>
<td>Basal</td>
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<td>ERBB2</td>
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<tr>
<td>LumA</td>
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<tr>
<td>LumB</td>
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<tr>
<td>Normal</td>
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<td>Activated</td>
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<td>Stromal signature</td>
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<tr>
<td>DTF</td>
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<tr>
<td>Invasiveness gene signature</td>
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<td>Positive</td>
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<tr>
<td>Good</td>
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<tr>
<td>Poor</td>
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| **Growth pattern** | Infiltrative | Mixed | Expansive | **P** |
|---------------------|-------------|--------|-----------|

Abbreviations: SFT, solitary fibrous tumor; DTF, desmoid-type fibromatosis.
and to new therapeutic targets. The unsupervised hierarchical cluster analysis also showed a cluster of genes with decreased expression in tumors with a large fibrotic focus. Several genes in this cluster are associated with inhibition of tumor progression and angiogenesis. For example, **PGK1** inhibits tumor angiogenesis by increasing plasma levels of angiotatin (33). Decreased **PGK1** is necessary for the induction of angiogenic cytokines at metastatic prostate cancer sites (34). Other downregulated genes in tumors with a large fibrotic focus are **RARα**, **CA12**, and **IGF2**, all of which have been associated with less aggressive breast cancer (35–38).

The presence of podoplanin, a glomerulocyte protein used as a lymph vessel–specific marker, in the highly expressed cluster is probably due to increased lymphangiogenesis in these tumors. It has been shown that not only increased angiogenesis but also increased lymphangiogenesis is found in tumors with a fibrotic focus (39, 40). Although **VEGF-C** and **VEGF-D** are thought to be the most important lymphangiogenic factors (41, 42), no differential expression of **VEGF-C** in tumors with a (large) fibrotic focus was observed and **VEGF-D** was even decreased. However, **VEGFA** is not only an angiogenic but also a lymphangiogenic factor (43) and **VEGFR2**, the most important receptor for **VEGFA** in angiogenesis, is also expressed by lymph vessels (44, 45). Therefore, the increased expression of **VEGFA-hypoxia** and angiogenesis genes in tumors with a large fibrotic focus suggests that **VEGFA** might play a role in both angiogenesis and lymphangiogenesis in a fibrotic focus. This is furthermore supported by the fact that, in tumors with a fibrotic focus, tortuous, newly formed lymph vessels are often found in vascular hotspots at the border of the fibrotic focus together with newly formed blood vessels. Another factor involved might be **FGF2**, which is also highly expressed in tumors with a large fibrotic focus. **FGF2** has also been shown to stimulate both angiogenesis and lymphangiogenesis (reviewed in ref. 46).

The presence of a fibrotic focus is a histologic indicator of tumor aggressiveness (1–3). The association of an expansive growth pattern and the presence of a fibrotic focus with gene expression signatures associated with poor prognosis, such as the basal-like cell-of-origin subtype, an activated wound-healing response signature, the invasiveness gene signature, and a poor 76-gene prognostic signature, shows that histopathologic and molecular markers of tumor aggressiveness and poor prognosis are associated. Others have also shown that the basal subtype (47) or the expression of basal cytokeratins (48) was associated with the presence of geographic necrosis, an expansive growth pattern, and a central scar, but did not investigate other common breast cancer signatures. Although the presence of a fibrotic focus has been associated with overexpression of the **ERBB2** protein (1), association with the absence of a fibrotic focus and the **ERBB2** cell-of-origin subtype was observed. In our data set, only one tumor with a fibrotic focus belonged to the **ERBB2**-overexpressing subtype. This suggests that **ERBB2** expression is not a characteristic of tumors with a fibrotic focus in lymph node–negative breast cancer. The most remarkable association with common breast cancer signatures was the fact that all but one tumor with a large fibrotic focus had a poor 76-gene prognostic signature. The value of genome-wide gene expression analysis and prognostic signatures in predicting the prognosis of patients with breast cancer has been described by different groups (16, 49).

Although gene expression assays are costly and time consuming, the assessment of a fibrotic focus can easily be introduced in routine surgical pathology practice (11). Therefore, assessment of the fibrotic focus and gene expression analysis might be additive for breast cancer and further studies of the association between both could lead to an integrated use in clinical practice.

In conclusion, the association of the fibrotic focus, especially of a large fibrotic focus, with other variables of tumor aggressiveness and with a worse prognosis in patients with lymph node–negative breast cancer was confirmed. The results further strengthen the hypothesis that hypoxia plays an important role in the biology of the fibrotic focus. Hif-1α is the key regulator that increases other molecules, such as **VEGFA** and **CA9**. Ras and Akt signaling might also be involved. Furthermore, Hif-1α and VEGFA seem to be driving not only angiogenesis but also lymphangiogenesis in these tumors. The presence of a fibrotic focus was also correlated to the basal-like subtype and to other gene expression signatures associated with a worse prognosis of patients with breast cancer. Our data therefore show that a fibrotic focus, an easily identifiable histopathologic marker, is associated with a specific biology and with tumor aggressiveness in lymph node–negative breast cancer.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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