The VEGF-Hypoxia Signature Is Upregulated in Basal-like Breast Tumors from Women of African Ancestry and Associated with Poor Outcomes in Breast Cancer

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ABSTRACT

Purpose: Black women experience the highest breast cancer mortality rate compared with women of other racial/ethnic groups. To gain a deeper understanding of breast cancer heterogeneity across diverse populations, we examined a VEGF-hypoxia gene expression signature in breast tumors from women of diverse ancestry.

Experimental Design: We developed a NanoString nCounter gene expression panel and applied it to breast tumors from Nigeria (n = 182) and the University of Chicago (Chicago, IL; n = 161). We also analyzed RNA sequencing data from Nigeria (n = 84) and The Cancer Genome Atlas (TCGA) datasets (n = 863). Patient prognosis was analyzed using multiple datasets.

Results: The VEGF-hypoxia signature was highest in the basal-like subtype compared with other subtypes, with greater expression in Black women compared with White women. In TCGA dataset, necrotic breast tumors had higher scores for the VEGF-hypoxia signature compared with non-necrosis tumors (P < 0.001), with the highest proportion in the basal-like subtype. Furthermore, necrotic breast tumors have higher scores for the proliferation signature, suggesting an interaction between the VEGF-hypoxia signature, proliferation, and necrosis. T-cell gene expression signatures also correlated with the VEGF-hypoxia signature when testing all tumors in TCGA dataset. Finally, we found a significant association of the VEGF-hypoxia profile with poor outcomes when using all patients in the METABRIC (P < 0.0001) and SCAN-B datasets (P = 0.002).

Conclusions: These data provide further evidence for breast cancer heterogeneity across diverse populations and molecular subtypes. Interventions selectively targeting VEGF-hypoxia and the immune microenvironment have the potential to improve overall survival in aggressive breast cancers that disproportionately impact Black women in the African Diaspora.

Introduction

Angiogenesis, the process of new blood vessel formation, plays a central role in tumor development, invasion, and metastasis in breast cancer (1, 2). The key mediator of angiogenesis, VEGF, is produced early in breast cancer. Notably, hypoxia is the basic initiating factor of tumor angiogenesis, which leads to the increase of VEGF, angiopoietin, and hypoxia-inducible factor (HIF-1) in hypoxic cells (3–6). HIF1α, as a key hypoxia transcription factor, is associated with progression of triple-negative breast cancer (TNBC) from the perspectives of angiogenesis, glycosylation, invasion and metastasis, as well as cancer stem cell enrichment (7, 8). Intriguingly, TNBC has a more pronounced signature of hypoxia than that of other breast cancer subtypes (9, 10), and HIF1α-induced VEGF mRNA levels were significantly higher in TNBC than in other breast cancer subtypes (11).

TNBC is a heterogeneous clinical category and includes the basal-like molecular subtype of breast cancer. It is by far the deadliest of all breast cancer subtypes because established molecular targets for pharmacologic therapy are not present. These biologic characteristics, along with socioeconomic factors, make TNBC a serious threat to high-risk women, especially in resource-limited settings. Thus, breast cancer represents a significant example of health disparity in the United States and globally. Unfortunately, a paucity of data from diverse populations potentially widens the knowledge gap that contributes to global disparities in breast cancer outcomes (12–14).

Recent studies demonstrate that Black women are more likely to develop TNBC or basal-like breast cancer in particular (15). We have shown that indigenous West Africans had an even higher proportion of basal-like breast cancers than do African Americans (AA; ref. 16). Specifically, while the reported proportion of TNBC was 21% in European Americans (EA) and 32% in AA, we reported 55% in Africans living in Nigeria and Senegal. This finding has been confirmed by other studies, with the highest frequency of TNBC reported in West Africa (17). Using whole-exome and whole-genome sequencing, we further identified higher proportions of tumors with dysregulated homologous recombination DNA repair pathways in Nigerian breast cancers (18, 19), while other studies have reported that tumor-associated immunologic profiles were distinct in patients of West Africa (20).

We previously observed that the majority of TNBC tumors were basal-like or unclassified subtypes in West African women when using IHC-based subtype definitions, and the unclassified tumors can be
Translational Relevance
Clinical utilization of gene expression profiles has expanded in the predictive and prognostic oncology settings. This study identified differential expression of a VEGF-hypoxia signature between Black women and White women, providing potential insights into breast cancer heterogeneity in diverse populations. Furthermore, our discovery of increased VEGF-hypoxia signature scores in Black women with basal-like breast cancers and its association with necrosis, proliferation, and immune infiltration might have significant clinical relevance. The findings may contribute to the development of novel combination therapies that target tumors and their microenvironments to optimize treatment of breast cancer.

Further divided into two distinct clusters according to VEGF expression (16). The VEGF-positive subgroup (VEGF-A and VEGF-C positive) had significantly higher histologic grade, higher mitotic index, and greater microvessel density than the VEGF-negative tumors (negative for both VEGF-A and VEGF-C). Furthermore, high expression of a 13-gene signature called the VEGF-hypoxia profile was associated with distant metastases and poor outcomes in breast cancer (21). The VEGF-hypoxia profile is the mean expression of 13 genes (VEGF, ANGPTL4, ADM, GAL, SLC16A3, NDRG1, RAGD, FABP5, UCHL1, PLOD, DDI74, PNP, and FLVCR2), which includes genes/proteins that regulate angiogenesis, lymphogenesis, and a lactic acid cellular efflux pump. At least eight out of 13 genes contained HIF1α binding sites, were HIF1α-regulated, and their expression was correlated with HIF1α IHC positivity. In this study, we examine the inter-relatedness of this VEGF-hypoxia profile and other prognostic features in women of African and European ancestry and identify relationships that could advance the field of cancer health disparities and accelerate progress in precision oncology.

Materials and Methods
Patient and specimen selection
All the studies included in the NanoString nCounter array and RNA sequencing (RNA-seq) have been approved by the Institutional Review Boards of the University of Chicago and the University College Hospital in Ibadan (UCHI), Nigeria. Archival formalin-fixed paraffin-embedded (FFPE) materials were collected from Department of Pathology at UCHI and studied anonymously to develop the NanoString assay. Prospectively enrolled participants provided written informed consent to allow for use of their tissue samples for research. All the methods were carried out in accordance with recognized ethical guidelines and regulations of the University of Chicago (Chicago, IL). This project is affiliated with the Nigerian Breast Cancer Study (18, 19, 22, 23), and tumor samples containing >60% tumor cellularity were selected for the study. For each patient, two paraffin-embedded tumor blocks from the surgical specimen, or the original biopsy if no surgical tumor block was available, were obtained from the pathology departments of the hospitals.

NanoString nCounter assay
RNA was extracted from FFPE breast tumors using a High Pure RNA Paraffin Kit (Sigma, SKU 3270289001). Positive and negative assay controls, developed by the External RNA Control Consortium (24), were included to ensure that the test samples met predefined quality thresholds. Positive controls consisted of six probes that hybridize to synthetic RNA targets present in the hybridization reaction, whereas the negative controls had eight probes with DNA sequences not homologous to those of any known organism. Extracted RNA samples were hybridized to capture and reporter probes for the measured genes. These multiplexed hybridizations were carried out in a single tube for 15–21 hours at 65°C using 100 ng RNA. Subsequently, the target–probe complexes were processed on the nCounter Analysis system according to the manufacturer’s protocols.

NanoString data preprocessing and analysis
The PAM50 subtype classifier was developed using a distinct NanoString CodeSet, and thus gene specific platform bias was assessed with principal component analysis. The median expression of each gene was estimated from the North American subset of the current cohort, assuming 50% estrogen receptor–positive samples, as with the primary assay. These correction factors were applied during execution of the PAM50 classifier to mitigate technical bias in the resulting subtype assignments. The background of a sample was estimated from negative controls, and a background threshold was set to two SDs above the mean of the negative controls. Gene expression estimates falling below the background threshold were set to the threshold value. Expression data were then normalized to the geometric mean of the endogenous housekeeper genes. Samples with high normalization factors were excluded from further analysis. The NanoString datasets are available through Gene Expression Omnibus (GEO; GSE229005).

RNA-seq data analysis
The Cancer Genome Atlas (TCGA) breast invasive carcinoma (BRCA) RNA-seq data were downloaded from the Broad Institute TCGA GDAC Firehose (https://gdac.broadinstitute.org/), and the clinical data were downloaded from the Genomic Data Commons Data portal (https://portal.gdc.cancer.gov/projects/TCGA-BRCA). Genomic ancestry has been previously ascertained for these patients using multiple approaches with full concordance (18, 25, 26). To combine TCGA BRCA RNA-seq data and Nigerian RNA-seq data for downstream analysis, we applied Distance-Weighted Discrimination (27) followed by column standardization, to normalize the Nigerian set to TCGA cohort as the reference. For the identification of breast cancer subtypes, we calculated PAM50 subtypes as described in Fernandez-Martinez and colleagues (28), which adjusts for ER/PR/HER2 status and then makes subtype calls. We also applied a claudin-low subtype predictor according to Prat and colleagues (29). A total of six intrinsic subtypes (basal-like, claudin-low, luminal A, luminal B, HER2-enriched, and normal-like) were obtained and subjected to further analyses.

Kaplan–Meier survival and HR analyses
Three datasets [METABRIC (n = 1,992), SCAN-B (n = 2,770), and CALGB40603 datasets (n = 330)] were utilized for the analyses. For each dataset, each gene module score was calculated on the normalized gene expression matrix. In the Kaplan–Meier plot, the VEGF gene signature was split in tertiles. Overall survival days were censored at 5,000 days. The log-rank test was performed to test the significance among survival curves. In the HR plot, the univariate Cox regression model was used to test the association between the gene module score and the overall survival, and a P value < 0.05 was used to declare significance.

Gene expression, proliferation signature, and necrosis status analyses
Using the fully combined dataset, we calculated a gene signature score for each sample and each signature using a list of public curated gene signatures including 194 immune signatures. These gene signatures were...
partially summarized previously (30, 31). A gene signature score was computed as the median expression of all genes in a given signature. The VEGF-hypoxia signature contained 13 genes and was derived from Hu and colleagues (21). The proliferation signatures were obtained from a list of publications and gene set enrichment analysis signatures published in the Molecular Signature Database (30, 32–36), and the necrosis status was derived from Heng and colleagues (37).

Statistical analysis
All analysis was done using R (http://www.r-project.org) v4.0.3. One-way ANOVA tests were performed using R/ggpubr package. The pairwise t test was done using t.test() function in R.

Data availability statements
The NanoString datasets are publicly available in GEO at GSE229005. There are no restrictions on availability of data. The data that support the findings of this study are available from the corresponding author upon request.

Results
The basal-like subtype is predominant among patients with breast cancer in Nigeria
To determine the importance of the VEGF-hypoxia signature in breast cancer in women of African ancestry, we first designed a NanoString nCounter codeset, comprised of 110 genes (namely, PAM110) including the PAM50 genes (n = 50; ref. 33), VEGF-hypoxia signature genes (n = 13; ref. 21), claudin-low signature genes (n = 30; ref. 29), along with housekeeping (n = 5) and other genes of interest in breast cancers (n = 12; Supplementary Table S1). A total of 343 tumor samples were subjected to the PAM110 assay, collected from Nigeria (n = 182) and in the United States, specifically the University of Chicago (n = 161; n = 90 UChicago-AA, and n = 71 UChicago-EA; Table 1). Furthermore, to overcome the limited sample size of UChicago patients in the first approach (Table 1), we analyzed RNA-seq data from a small sample set of Nigerian patients (n = 84) combined with 863 breast tumors from TCGA-AA (n = 169) and TCGA-EA (n = 694).

Table 1. PAM50 subtypes of patients with breast cancer.

<table>
<thead>
<tr>
<th>Subtype</th>
<th>NanoString nCounter</th>
<th>RNA-seq</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nigerian n = 182</td>
<td>UChicago-AA n = 90</td>
</tr>
<tr>
<td>Basal-like</td>
<td>73 (40.1)</td>
<td>52 (35.6)</td>
</tr>
<tr>
<td>HER2</td>
<td>39 (21.4)</td>
<td>3 (3.3)</td>
</tr>
<tr>
<td>Luminal A</td>
<td>36 (19.8)</td>
<td>36 (40.0)</td>
</tr>
<tr>
<td>Luminal B</td>
<td>26 (14.3)</td>
<td>11 (12.2)</td>
</tr>
<tr>
<td>Normal-like</td>
<td>8 (4.4)</td>
<td>8 (8.9)</td>
</tr>
</tbody>
</table>

Characteristics of the RNA-seq study participants were reported previously (18). PAM50 analyses of breast cancer subtypes revealed that, among four subtypes of breast cancer (basal-like, HER2-enriched, luminal A, and luminal B), the basal-like breast cancer was most prevalent in Nigerian patients (40.1% and 44.0% for nCounter and RNA-seq sets, respectively), followed by AA (35.6% and 36.1%, respectively) and EA patients (23.9% and 15.9%, respectively; Fig. 1A and B). The data indicate varying proportions of breast cancer subtypes across ethnicities, with the basal-like subtype predominating in Nigerian patients.

Figure 1.
PAM50 analyses of breast cancer subtypes in Nigerians, AA, and EA. Four subtypes of breast cancer (basal-like, HER2-enriched, luminal A, and luminal B) were analyzed using NanoString nCounter assays (A) and RNA-seq (B), showing varying proportions (%) of breast cancer subtypes across ethnicities, with the basal-like subtype being predominant in Nigerian patients.
Increased VEGF-hypoxia expression in basal-like breast cancer and Black women

We next analyzed the expression levels of VEGF-hypoxia genes by breast cancer subtype in both nCounter assay and RNA-seq datasets (Fig. 2A and B). The VEGF-hypoxia profile had the highest score in basal-like breast cancer, followed by the HER2-enriched and luminal subtypes, in each cohort and all cohorts combined. When we compared the VEGF-hypoxia signature by ethnic groups, Nigerian women exhibited significantly higher VEGF-hypoxia signatures compared with women of European ancestry (Fig. 3A and C). Nigerians showed the highest level of VEGF-hypoxia signature in the nCounter assay set ($P < 2.2e^{-16}$; Fig. 3A), while Nigerians and TCGA-AA showed similar levels in the RNA-seq dataset (Fig. 3B). Further analyses of ethnic differences in the basal-like subtype revealed a consistently higher score for the VEGF-hypoxia signature in Nigerians compared with UChicago patients in the nCounter assay set (Fig. 3C). In the RNA-seq dataset, the VEGF-hypoxia signature remained high in TCGA-AA compared with TCGA-EA, although it was not statistically significantly higher in Nigerians, in part because of the limited sample size of Nigerian patients ($n = 37$; Fig. 3D). Collectively, the data indicated increased expression of VEGF-hypoxia genes in women of African ancestry.

Higher expression of VEGF-hypoxia signature in necrotic breast tumors

To determine whether the VEGF-hypoxia profile is associated with pathologic features of breast tumors, we investigated histopathologic characteristics of TCGA-breast tumors using morphologic features identified previously (37, 38). The VEGF-hypoxia profile was significantly higher in necrotic (necrosis-present) breast tumors compared with non-necrotic (necrosis-absent) tumors, both in AA and EA women ($P = 2.9e^{-8}$ and $P = 2.2e^{-16}$, respectively; Fig. 4A). When we analyzed sample proportions by subtypes, we observed a predominant proportion of the basal-like subtype in necrotic tumors, especially in AA women (Fig. 4B). Further analyses of the basal-like subtype only samples revealed consistently higher VEGF-hypoxia profile scores in necrotic tumors compared with non-necrotic tumors ($P = 0.049$ and $P = 0.019$ in TCGA-AA and TCGA-EA, respectively; Fig. 4C). Moreover, our analyses of tumor proliferation using different expression modules revealed higher proliferation scores in necrotic tumors compared with non-necrotic tumors ($P < 0.0001$; Fig. 5A). Collectively, these findings indicate an association of the VEGF-hypoxia profile with breast tumor necrosis and proliferation, and support the likelihood of this signature contributing to aggressive features of basal-like breast cancer.
Increased T-cell infiltration in VEGF-hypoxia high tumors

We next determined whether the VEGF-hypoxia signature is correlated with immune infiltration in breast tumors (Fig. 5B). For this analysis, we divided TCGA samples into three groups according to the VEGF-hypoxia score (VEGF-hypoxia-high, -medium, and -low groups) and plotted them against each immune signature (30, 39). Many T-cell immune module scores were significantly higher in VEGF-hypoxia signature-high tumors, including memory naïve CD4, follicular helper CD4 (TFH), and CD8-positive T-cell modules, as well as gamma and delta T-cell modules (Fig. 5B; Supplementary Fig. S1). The VEGF-hypoxia profile was also positively correlated with IgG, plasma cells and CD274 (PDL-1) immune modules, as well as the MKI67 proliferation signature (Supplementary Figs. S2 and S3). In contrast, breast tumor subtype marker genes (ESR1 and ERBB2) were negatively correlated with the VEGF-hypoxia signature (Supplementary Fig. S3). When we analyzed the correlation in the basal-like subtype only, we did not observe any significant association between the immune signatures and the VEGF signature within this subtype (Supplementary Fig. S4). Collectively, our data indicated that the VEGF-hypoxia module was positively correlated with T-cell immune modules when all subtypes were combined, but not specifically within the basal-like subtype.

An association of the VEGF-hypoxia profile with prognosis

Finally, we tested for associations of the VEGF-hypoxia profile with patient outcomes using multiple datasets (METABRIC, SCAN-B, and CALGB40603). METABRIC (Molecular Taxonomy of Breast Cancer International Consortium) and SCAN-B (Sweden Cancerome Analysis Network—Breast) are the multicenter consortiums with the majority of patients with breast cancer coming from United Kingdom/Canada (40) and Sweden (41), respectively. CALGB (Cancer and Leukemia Group B) 40603 represents patients with clinical stage II to III breast cancer with TNBC (42). When we analyzed the VEGF-hypoxia signature across all breast cancer subtypes, we found that high expression of the VEGF signature was significantly associated with poor outcomes in both METABRIC (n = 1,992, P < 0.0001) and SCAN-B datasets (n = 2,770, P = 0.002; Fig. 6A). In comparison, when testing only patients with TNBC, the VEGF signature is not prognostic in all datasets analyzed [METABRIC (n = 320), SCAN-B (n = 115), and CALGB40603 (n = 330)] (Fig. 6B). The data support potential importance for the VEGF-hypoxia signature’s association with clinical outcomes.
outcomes in patients with all subtypes of breast cancer, but not specifically in patients with TNBC.

Discussion

In this study, we tested the hypothesis that gene expression profiles in breast tumors differ between women of African and European heritage, providing insights into the heterogeneity of breast cancer across diverse populations. In particular, we found that the VEGF-hypoxia signature was enriched in basal-like breast cancer compared with other subtypes of breast cancer (Fig. 2A and B) and was significantly higher in women of African descent compared with those of European descent (Fig. 3A and D). Furthermore, histologic analyses revealed a significant association of the VEGF-hypoxia profile with breast tumor necrosis (Fig. 4A and C) and proliferation (Fig. 5A). This profile was also positively associated with immune cell modules (Fig. 5B) in all subtypes of breast cancer, but not specifically in the basal-like breast cancer. Finally, we found a significant association of the VEGF-hypoxia profile with poor outcomes in all patients with breast cancer, but not

Figure 4.
Elevated VEGF-hypoxia signature in necrotic breast tumors. The VEGF-hypoxia signature score was compared between necrotic and non-necrotic breast tumors using TCGA samples in all subtype samples combined (A and B) or in basal-like subtype only samples (C). The VEGF-hypoxia signature score is higher in necrosis-present (necrotic) samples, regardless of racial group (A). There were more basal-like subtypes in the necrotic samples, and the proportion of basal-like subtypes was higher in TCGA-AA necrotic samples than in TCGA-EA necrotic samples (B). Further analysis of only basal-like subtypes shows that this subtype had higher VEGF-hypoxia profile scores in necrotic samples, regardless of racial group (C).
within patients with TNBC (Fig. 6). This study identified high expression of the VEGF-hypoxia signature in women of African ancestry, providing insights into the etiology of breast cancer heterogeneity in diverse populations.

It is notable that the VEGF-hypoxia signature is positively associated with tumor necrosis, which is an important feature of aggressive cancers related to increased tumor grade/size, estrogen receptor-negative status, and macrophage infiltration (43–45). In endometrial cancer, tumor necrosis is an important hallmark of aggressive cancer and is associated with hypoxia, angiogenesis, and inflammation responses (46). Degraded tumor cells in necrotic tissue release proinflammatory cytokines that cause accumulation of inflammatory cells within necrotic foci (47). Angiogenic factors may also be secreted by tumor-associated macrophages in such areas (48), stimulating angiogenesis and cancer progression (49). Accordingly, necrosis has been associated with tumor progression and increased resistance to radiation and chemotherapy (50, 51). These data, combined with our new findings here, indicate the importance of the VEGF-hypoxia signature in association with tumor necrosis and progression, as well as possible therapeutic responses.

Interestingly, recent findings indicate no significant differences in immunologic features between AA and EA breast tumors represented in TCGA data (52, 53). In comparison, we found that the VEGF-hypoxia signature was significantly different between women of African and European descent. We also observed that the VEGF-hypoxia signature is positively correlated with the immune phenotype when analyzed using all subtypes of breast cancer, but not within the basal-like subtype of TCGA dataset (Supplementary Fig. S4). Similarly, the signature is significantly associated with poor outcomes when using all patients with breast cancer, but not specifically for patients...
with TNBC (Fig. 6B). These data provide further evidence for the heterogeneity of breast cancer across diverse populations and molecular subtypes with a potential to improve overall survival in breast cancer.

On the basis of our data and others, we postulate that the VEGF/hypoxia signature might not directly relate to immune phenotypes and patient prognosis in TNBC. It is more likely to contribute to new vessel formation, migration, and metastasis, which may attract more immune cells into angiogenesis sites. Furthermore, considering that degraded tumor cells in necrotic tissue release proinflammatory cytokines that cause accumulation of inflammatory cells within necrotic foci (47), tumor necrosis driven by tumor overgrowth and hypoxia may release damage-associated molecular patterns in response to cell death and attract tumor-associated macrophages in such areas, which perhaps stimulates immune infiltration into tumors. Further studies are warranted to dissect the detailed interactions between the VEGF-hypoxia signature and tumor immune microenvironment.

Notably, other studies have reported that a hypoxia signature predicted pathologic complete response to bevacizumab (54). Particularly, a single gene marker of NDRG1 showed a significant predictive value for pCR using digital image analysis of the tissue microarray samples in univariate logistic regression. Out of the 13 genes of the VEGF-hypoxia profile genes, NDRG1 is one of the eight genes which contain HIF1α binding sites and are regulated by HIF1α (55). Further studies are warranted to investigate the regulation of NDRG1 expression by hypoxia and a possible role for NDRG1 as a biomarker of bevacizumab sensitivity in aggressive breast cancer, especially in women of African ancestry.

There are limitations associated with this work. Our sample sizes were limited in the NanoString nCounter group, particularly for the HER2-enriched subtype of breast cancer in UChicago patients. We overcame this limitation by including a larger TCGA cohort in the RNA-seq group. Although we were not able to determine the concordance directly in this study, we believe there is a general concordance between nCounter and RNA-seq sets. This is based on our previous work that clearly demonstrated a high concordance (96% of the cases) between NanoString and RNA-seq platforms on PAM50 calls (56). It is worth noting that there were only Nigerian patients representing African populations in our study. We acknowledge the diversity and heterogeneity in African populations. Our findings may not be generalizable to other African countries or regions; hence we used TCGA samples for the replication cohort. Another detail of our study is that normal-like expression subtype breast tumors were not included in the subtype analyses because we believe the normal-like subtype does not represent a real biological subtype but instead represents samples that are predominantly normal cells; thus, we only included biologically meaningful subtypes of breast cancers in the analyses.

Despite the limitations discussed above, we believe that this is the largest study of VEGF-hypoxia signature in women of African ancestry. Starting with an unscreened population of Nigerian women...
presenting with symptomatic breast cancer, we revealed differential VEGF-hypoxia expression profiles between women of African and European descent. As the clinical utilization of gene expression profiles expands in the predictive and prognostic oncology settings, increasing availability and analysis of gene expression profiles from diverse populations should be an essential priority for biomarker informed clinical trials. Our discovery of increased VEGF-hypoxia signature scores in Black women might open up new possibilities for multilayered therapies for the control of breast cancer progression in women of African descent. It is significant that HIF1α inhibitors, VEGF inhibitors, PARP inhibitors, and/or immune checkpoint blockade are currently being investigated in clinical trials. Considering the importance of tumor necrosis and the tumor immune microenvironment in tumor progression, our findings on the association of VEGF-hypoxia signaling pathways with necrosis, proliferation, and immune infiltration may have clinical relevance, particularly for the development of targeted therapies and immunotherapies, and should be further explored.

Authors’ Disclosures

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Authors’ Contributions

Y.I. Han: Conceptualization, data curation, visualization, writing—original draft, writing—review and editing. S. Liu: Conceptualization, data curation, formal analysis, validation, visualization, methodology, writing—review and editing. A. Hardeman: Conceptualization, data curation, investigation, methodology. P.S. Rajagopal: Data curation, formal analysis, funding acquisition, methodology. J. Mueller: Data curation, methodology. G. Khrantsov: Data curation, methodology. A. Sanni: Formal analysis. M. Ajani: Data curation, methodology. W. Clayton: Data curation, methodology. T.F. Yoshimatsu: Writing—review and editing. Y. Zheng: Writing—review and editing. J. Parker: Formal analysis, methodology, writing—review and editing. C.M. Perou: Conceptualization, data curation, supervision, funding acquisition, investigation, writing—review and editing. O.I. Olopade: Conceptualization, data curation, supervision, funding acquisition, investigation, writing—review and editing.

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Note

Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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Han et al.


