High VEGF-A expression distinguishes basal-like human breast cancer.

Shahan Mamoor, MS¹

¹shahanmamoor@gmail.com

East Islip, NY 11730

Patients diagnosed with basal-like breast cancer face a more aggressive disease course and more dismal prognosis than patients diagnosed with luminal A and luminal B breast cancer molecular subtypes (1-4). We mined published microarray data (5, 6) to understand in an unbiased fashion the most distinguishing transcriptional features of tumors from patients with basal or basal-like subtype breast cancer. We observed transcriptome-wide differential expression of the vascular endothelial growth factor A, VEGFA, when comparing tumors of patients with basal-like breast cancer with that of other PAM50 molecular subtypes. VEGF-A mRNA was present at significantly higher quantities in the tumors of patients with basal-like breast cancer. Analysis of patient survival data revealed that VEGF-A primary tumor expression was correlated with recurrence-free survival, with higher VEGF-A associated with inferior outcomes - in basal-like patients but not in luminal A, luminal B, HER2+, or normal-like patients. High VEGF-A expression appears to distinguish basal-like human breast cancer from the other molecular subtypes.

Keywords: basal subtype, basal-like breast cancer, BLBC, systems biology of breast cancer, targeted therapeutics in breast cancer, breast cancer.

Classification of disease types and disease subtypes enables development of enhanced diagnostic capabilities, prognostic information, as well as an understanding of human disease that can support superior medical, surgical and radiological treatment of complex diseases such as cancer. Molecular subtypes in human breast cancer include luminal A, luminal B, basal or basal-like, and HER2+. Systems-level analyses, such as comparative tumor transcriptome analyses, can inform such approaches. We mined published microarray data (5, 6) to identify the most significant transcriptional differences when comparing human breast cancer molecular subtypes. Integrative microarray dataset analysis has provided data illuminating genes whose expression defines basal-like subtype breast cancers, and we present evidence here.

Methods

We utilized datasets GSE158309 (5) and GSE74667 (6) and for this global differential gene expression analysis of basal subtype human breast cancer in conjunction with GEO2R. GSE158308 was generated using Affymetrix Human Genome U133A Array technology with $n=52$ tumors from patients with basal-like breast cancer and $n=410$ tumors from patients with luminal A, luminal B and HER2+ molecular subtypes; analysis was performed using platform GPL96. The data presented in the graph compare $n=52$ tumors from patients with basal-like breast cancer to $n=182$ tumors from patients with luminal B breast cancer. GSE74667 was generated using Agilent-014850 Whole Human Genome Microarray 4x44K G4112F technology with $n=23$ basal-like breast tumors and $n=72$ breast tumors of other molecular subtypes (luminal A, luminal B, HER2-enriched, and normal-like); analysis was performed using platform GPL6480. The Benjamini and Hochberg method of p -value adjustment was used for ranking of differential expression but raw p -values were used to assess statistical significance of global differential expression. Log-transformation of data was auto-detected, and the NCBI generated category of platform annotation was used. A statistical test was performed to evaluate whether VEGFA gene expression was significantly different between the tumors of patients with basal and non-basal molecular subtype breast cancer (luminal B) using a two-tailed t-test with Welch's correction. For Kaplan-Meier survival analysis, we used the Kaplan-Meier plotter tool (8) for correlation of VEGFA mRNA expression levels with recurrence-free survival in $n=4929$ breast cancer patients, $n=953$ basal subtype breast cancer patients, $n=1809$ luminal A subtype breast cancer patients, $n=1353$ luminal B subtype breast cancer patients, $n=695$ HER2+ subtype breast cancer patients, and $n=119$ normal-like subtype breast cancer patients. .

Results

VEGFA is among the genes whose expression is most different when comparing basal-like breast cancers with breast cancers of other molecular subtypes.

We used published microarray data (5) to compare the whole tumor transcriptomes of 52 patients with basal subtype breast cancer and 410 patients with breast cancer of other molecular subtypes (luminal A, luminal B and HER2+) to identify genes whose expression marked the basal-like molecular subtype. We observed transcriptome-wide differential expression of vascular endothelial growth factor A, VEGF-A, when comparing the tumors of patients with basal-like cancer with that of patients with luminal A, luminal B and HER2+ cancers (Table 1). When sorting each of the transcripts whose expression was measured globally, VEGFA expression ranked 16 out of 22283 total transcripts, equating to 99.9% differential expression; this was statistically significant (p -value: $1.01E-07$).

Analysis of a second published microarray dataset, comparing tumor transcriptome data from a separate cohort of patients 23 patients with basal-like breast cancer and 72 patients with breast cancer of other molecular subtype (luminal A, luminal B, HER2-enriched, and normal-like) (6) again revealed

transcriptome-wide differential expression of VEGF-A in patients with basal subtype human breast cancer (Table 2). When sorting each of the transcripts whose expression was measured globally, VEGFA expression ranked 1213 out of 31157 total transcripts, equating to 96.1% differential expression; this was statistically significant (p -value: $1.89\text{E-}05$). Thus, the data suggested differential expression of VEGF-A was a fundamental feature of basal-like breast cancer in humans rather than an artifact of a single microarray dataset or research group, or a finding derived from cell culture or an animal model that was not relevant to human breast cancer.

VEGF-A expression correlates with recurrence-free survival in basal subtype breast cancer patients.

We asked whether primary tumor expression of VEGF-A was correlated with survival outcomes in patients with basal-like breast cancer. We observed a statistically significant correlation between primary tumor expression of VEGFA and recurrence-free survival (RFS) in patients with basal-like breast cancer (Figure 2; p -value: 0.053; hazard ratio: 1.23 (1-1.53)). VEGF-A primary tumor expression was a positive prognostic indicator with respect to recurrence-free survival in patients with basal-like breast cancer. Patients with low tumor expression of VEGF-A possessed median RFS of 29 months, while patients with high tumor expression of VEGF-A possessed median RFS of 19.81 months.

VEGF-A primary tumor expression was not correlated with recurrence-free survival in all patients (Figure 2; p -value: 0.63; hazard ratio: 1.03 (0.93-1.13)), in luminal A subtype breast cancer (Figure 2; p -value: 0.14; hazard ratio: 0.85 (0.69-1.05)), in luminal B subtype breast cancer (Figure 2; p -value: 0.32; hazard ratio: 0.92 (0.77-1.09)), in HER2+ breast cancer (Figure 2; p -value: 0.99; hazard ratio: 1 (0.79-1.27)), or in normal-like breast cancer (Figure 2; p -value: 1; hazard ratio: 1 (0.51-1.97)).

Discussion

We find here that VEGFA is among the genes whose expression is most different when comparing basal-like breast cancers (BLBC) to cancers of other molecular subtypes, with significantly more VEGF-A mRNA in BLBC. Kaplan-Meier analysis of patient survival data revealed that VEGFA expression was correlated with recurrence-free survival in human patients with basal-like breast cancer, with higher expression of VEGF-A associated with inferior outcomes. VEGF-A may be one factor that specifies tumor subtype identity in basal-like breast cancer, but it is more likely a factor whose expression defines the subtype rather than specifies it. One study demonstrated that VEGF-A blockade using bevacizumab impeded tumor growth in basal-like breast cancer xenografts but not in luminal-like breast cancer xenografts (8).

Importantly, these data are one piece of evidence that argues that the Food and Drug Administration decision to withdraw its approval of bevacizumab (Avastin), a monoclonal antibody specifically designed to target VEGF-A, for use in metastatic breast cancer is inherently flawed, as it came to this decision based on an assessment that the safety profile of this drug (risks) could not justify its use in metastatic breast cancer (benefits) (9-10), and any such assessment likely was not nuanced enough to appreciate a potential specific benefit for VEGF-A blockade in basal-like breast cancer: it did not include discussion of information presented here that demonstrates that VEGF-A expression appears to be a part of the molecular identity of basal-like cancers, that VEGF-A expression is correlated with patient survival outcomes in basal-like breast cancer but not in other subtypes, and that high VEGF-A primary tumor expression is associated with significantly inferior recurrence-free survival in basal-like breast cancer patients.

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Rank: 16
probe ID: 210513_s_at
p-value: 1.01E-07
t: 5.410847
B: 7.4184536
Gene: VEGFA
Gene name: vascular endothelial growth factor A

Table 1: VEGFA is among the genes whose expression is most different, across the transcriptome, when comparing basal-like breast cancers with luminal A, luminal B, and HER2+ breast cancers.

The rank of global differential expression, probe ID, the *p*-value with respect to differential expression transcriptome-wide, *t*, a moderated *t*-statistic, and *B*, the log-odds of differential expression between the groups compared are listed in this chart.

Rank: 1213
probe ID: A_23_P81805
p-value: 1.89E-05
t: 4.494974
B: 2.54644
Gene: VEGFA
Gene name: vascular endothelial growth factor A

Table 2: In a separate cohort of patients with basal subtype breast cancer, VEGFA is again among the genes most differentially expressed transcriptome-wide when compared to the tumors of patients with breast cancer of other molecular subtypes: luminal A, luminal B, HER2+ and normal-like.

The rank of global differential expression, probe/transcript ID, the *p*-value with respect to differential expression transcriptome-wide, t, a moderated t-statistic, B, the log-odds of differential expression between the groups compared, the gene and gene name are listed in this chart.

Figure 1: VEGF-A expression is significantly higher in basal-like breast cancers.

Depicted is mRNA expression of VEGFA in tumors from patients with basal-like breast cancer (left) and from patients with cancer of another molecular subtype (right): luminal B. The horizontal bar corresponds to the mean value, and the result of a statistical test evaluating the significance of difference in expression between the two groups compared, a *p*-value, is listed above.

Figure 2: VEGFA primary tumor expression is correlated with recurrence-free survival, in basal-like breast cancer, but not in luminal A, luminal B, HER2+, or normal-like breast cancer patients, nor in all patients.

Depicted in this Kaplan-Meier plot is the probability of recurrence-free survival for $n=4929$ patients (all patients, top right), $n=953$ basal subtype patients (top middle), $n=1809$ luminal A subtype patients (top right), $n=1353$ luminal B subtype patients (below right), $n=695$ HER2+ subtype patients (below middle), and $n=119$ normal-like subtype patients (below right) stratified into two groups, based on low or high expression of VEGFA in patient primary tumors. The log rank p -value denoting statistical significance of difference in recurrence-free survival when comparing the two groups, as well as hazard ratio for this comparison is listed above. Listed below is the number of patients at risk (number of patients alive) per interval, after stratification based on VEGFA expression; in the first interval, number at risk is number of patients alive; in each subsequent interval, number at risk is the number at risk less those who have expired or are censored.

Low VEGFA expression: 29 months
High VEGFA expression: 19.81 months

Table 3: Median RFS is superior in basal subtype breast cancer patients with low primary tumor expression of VEGFA.

The median RFS (recurrence-free survival) of $n=953$ basal subtype breast cancer patients, with low primary tumor expression of VEGFA and high primary tumor expression of VEGFA is listed in this chart.