Elevated RARRES1 expression distinguishes basal-like human breast cancer.

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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 retinoic acid receptor responder 1, RARRES1, when comparing tumors of patients with basal-like breast cancer with that of other PAM50 molecular subtypes. RARRES1 mRNA was present at significantly higher quantities in the tumors of patients with basal-like breast cancer. Analysis of patient survival data revealed that RARRES1 primary tumor expression was correlated with recurrence-free survival in luminal A and normal-like patients. Elevated RARRES1 expression appears to distinguish basal-like human breast cancer from the other molecular subtypes.

Keywords: basal subtype, basal-like breast cancer, RARRES1, systems biology of breast cancer, targeted therapeutics in breast cancer, molecular subtype.

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 define basal-like subtype breast cancers, and we present evidence here.

Methods

We utilized datasets GSE74667 (5) and GSE158309 (6) for this global differential gene expression analysis of basal subtype human breast cancer in conjunction with GEO2R. 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. GSE158308 was generated using Affymetrix Human Genome U133A Array technology with $n=52$ tumors from patients with basal-like breast breast and $n=410$ tumors from patients with luminal A, luminal B and HER2+ molecular subtypes; analysis was performed using platform GPL96. 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 RARRES1 gene expression was significantly different between the tumors of patients with basal and non-basal molecular subtype breast cancer using a two-tailed t-test with Welch's correction (PRISM 9.1.1 (225)). For Kaplan-Meier survival analysis, we used the Kaplan-Meier plotter tool (8) for correlation of RARRES1 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

RARRES1 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 23 patients with basal subtype breast cancer and 72 patients with breast cancer of other molecular subtypes (luminal A, luminal B, HER2+ and normal-like) to identify genes whose expression marked the basal-like molecular subtype. We observed transcriptome-wide differential expression of retinoic acid receptor responder 1, RARRES1, when comparing the tumors of patients with basal-like breast cancer with that of patients with luminal A, luminal B, HER2+ and normal-like breast cancers (Table 1). When sorting each of the transcripts whose expression was measured globally, RARRES1 expression ranked 29 out of 31157 total transcripts, equating to 99.9% differential expression; this was statistically significant (p -value: $1.56E-13$).

Analysis of a second published microarray dataset, comparing tumor transcriptome data from a separate cohort of patients (52 patients with basal-like breast cancer and 410 patients with breast cancer of other molecular subtype (luminal A, luminal B, and HER2+) (5) revealed transcriptome-wide differential expression of RARRES1 in patients with basal subtype human breast cancer (Table 2). When sorting each

of the transcripts whose expression was measured globally, RARRES1 expression ranked 897 out of 22283 total transcripts, equating to 96.0% differential expression; this was statistically significant (p -value: $1.85\text{E-}04$). Thus, the data suggested differential expression of RARRES1 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.

RARRES1 expression is significantly higher in basal-like breast cancers.

We obtained exact RARRES1 mRNA expression data for basal-like breast tumors and tumors from other molecular subtypes (luminal A, luminal B, HER2+ and normal-like) to determine magnitude and direction of RARRES1 gene expression change in basal-like breast cancer. RARRES1 was expressed at higher levels in the tumors of patients with basal-like breast cancer as compared to the tumors of patients from other PAM50 molecular subtypes, and this difference was statistically significant (Figure 1; $p < 0.0001$). RARRES1 was expressed at 4.938 ± 1.910 arbitrary units (A.U.) in basal-like breast cancer and 1.772 ± 1.471 A.U. in all other molecular subtypes (Figure 1).

RARRES1 expression correlates with recurrence-free survival in luminal A and normal-like subtype breast cancer patients.

We asked whether primary tumor expression of RARRES1 was correlated with survival outcomes in patients with basal-like breast cancer. We observed a correlation between primary tumor expression of RARRES1 and recurrence-free survival (RFS) in patients with luminal A subtype breast cancer which was statistically significant (Figure 2; p -value: 0.0011; hazard ratio: 1.41 (1.15-1.74)). RARRES1 primary tumor expression was a negative prognostic indicator in patients with luminal A breast cancer. Luminal A patients with low tumor expression of RARRES1 possessed median RFS of 148 months, while luminal A patients with high tumor expression of RARRES1 possessed median RFS of 101.69 months (Table 3).

We also observed a correlation between primary tumor expression of RARRES1 and recurrence-free survival (RFS) in patients with normal-like subtype breast cancer which was statistically significant (Figure 2; p -value: 0.013; hazard ratio: 0.41 (0.2-0.85)). RARRES1 primary tumor expression was a positive prognostic indicator in patients with normal-like breast cancer. Normal-like patients with low tumor expression of RARRES1 possessed, on average, markedly shorter RFS as compared to normal-like patients with high tumor expression of RARRES1 (Table 3).

We also observed a correlation between primary tumor expression of RARRES1 and recurrence-free survival (RFS) in all patients with breast cancer (irrespective of molecular subtype) which was statistically significant (Figure 2; p -value: 0.0067; hazard ratio: 1.15 (1.04-1.27)). RARRES1 primary tumor expression was a negative prognostic indicator in patients with breast cancer. Patients with low tumor expression of RARRES1 possessed median RFS of 228.85 months, while patients with high tumor expression of RARRES1 possessed median RFS of 216.66 months (Table 3).

RARRES1 primary tumor expression was not correlated with recurrence-free survival in basal subtype breast cancer (Figure 2; p -value: 0.45; hazard ratio: 0.92 (0.74-1.14)), in luminal B subtype breast cancer (Figure 2; p -value: 0.56; hazard ratio: 0.95 (0.8-1.13)), or in HER2+ breast cancer (Figure 2; p -value: 0.12; hazard ratio: 0.83 (0.65-1.05)).

Discussion

We find here that RARRES1 is among the genes whose expression is most different when

1 comparing basal-like breast cancers (BLBC) to cancers of other molecular subtypes, with significantly
2 more RARRES1 mRNA in BLBC. Kaplan-Meier analysis of patient survival data revealed that
3 RARRES1 expression was correlated with recurrence-free survival in patients with luminal A and
4 normal-like subtype breast cancer. RARRES1 may be one factor that specifies tumor subtype identity in
5 basal-like breast cancer.
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Rank: 29
probe ID: A_23_P18078
p-value: 1.56E-13
t: 8.551079
B: 20.36003
Gene: RARRES1
Gene name: retinoic acid receptor responder 1

Table 1: RARRES1 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+ and normal-like 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: 897
probe ID: 221872_at
p-value: 1.85E-04
t: 3.769429
B: 0.5467981
Gene: RARRES1
Gene name: retinoic acid receptor responder 1

Table 2: In a separate cohort of patients with basal subtype breast cancer, RARRES1 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, and HER2+.

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: RARRES1 expression is significantly higher in basal-like breast cancers.

Depicted is mRNA expression of RARRES1 in tumors from patients with basal-like breast cancer (left) and from patients with cancer of all other molecular subtypes (luminal A, luminal B, HER2+ and normal-like) (right). 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: RARRES1 primary tumor expression is correlated with recurrence-free survival in patients with luminal A and normal-like breast cancer.

Depicted in this Kaplan-Meier plot is the probability of recurrence-free survival for $n=4929$ patients (above, left), $n=953$ basal subtype patients (above, middle), $n=695$ HER2+ subtype patients (above, right), $n=1809$ luminal A subtype patients (below, left), $n=1353$ luminal B subtype patients (below, middle), and $n=119$ normal-like subtype patients (below, right) stratified into two groups, based on low or high expression of RARRES1 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 RARRES1 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 RARRES1 expression: 148 months
High RARRES1 expression: 101.69 months

Table 3: Median RFS is superior in luminal A subtype breast cancer patients with low primary tumor expression of RARRES1.

The median RFS (recurrence-free survival) of $n=1809$ luminal A subtype breast cancer patients, with low primary tumor expression of RARRES1 and high primary tumor expression of RARRES1 is listed in this chart.