Low BHLHE40 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 basic helix-loop-helix family member e40, BHLHE40, when comparing tumors of patients with basal-like breast cancer with that of other PAM50 molecular subtypes. BHLHE40 mRNA was present at significantly lower quantities in the tumors of patients with basal-like breast cancer. Analysis of patient survival data revealed that BHLHE40 primary tumor expression was correlated with overall survival in human breast cancer. Low BHLHE40 expression appears to distinguish basal-like human breast cancer from the other molecular subtypes.

Keywords: basal subtype, basal-like breast cancer, BHLHE40, 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, acquisition of 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 GSE87049 (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. GSE87049 was generated using Affymetrix Human Gene 1.0 ST Array [transcript (gene) version] technology with $n=14$ basal-like breast tumors and $n=109$ breast tumors of other molecular subtypes (luminal A, luminal B, HER2-enriched, and normal-like); analysis was performed using platform GPL6244. 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 BHLHE40 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 BHLHE40 mRNA expression levels with overall survival in $n=1879$ breast cancer patients, $n=431$ basal subtype breast cancer patients, $n=596$ luminal A subtype breast cancer patients, $n=439$ luminal B subtype breast cancer patients, $n=362$ HER2+ subtype breast cancer patients, and $n=51$ normal-like subtype breast cancer patients.

Results

BHLHE40 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 basic helix-loop-helix family member e40, BHLHE40, 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, BHLHE40 expression ranked 239 out of 31157 total transcripts, equating to 99.2% differential expression; this was statistically significant (p -value: $8.46E-09$).

Analysis of a second published microarray dataset, comparing tumor transcriptome data from a separate cohort of patients (14 patients with basal-like breast cancer and 109 patients with breast cancer of other molecular subtype (luminal A, luminal B, HER2+ and normal-like) (5) revealed transcriptome-wide differential expression of BHLHE40 in patients with basal subtype human breast cancer (Table 2). When sorting each of the transcripts whose expression was measured globally, BHLHE40 expression ranked 256

out of 33297 total transcripts, equating to 99.2% differential expression; this was statistically significant (p -value: $1.85\text{E-}11$). Thus, the data suggested differential expression of BHLHE40 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.

BHLHE40 expression is significantly lower in basal-like breast cancers.

We obtained exact BHLHE40 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 BHLHE40 gene expression change in basal-like breast cancer. BHLHE40 was expressed at lower 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; $p < 0.0001$). BHLHE40 was expressed at -0.212 ± 0.444 arbitrary units (A.U.) in basal-like breast cancer and 0.595 ± 0.546 A.U. in all other molecular subtypes (Figure 1).

BHLHE40 expression correlates with overall survival in human breast cancer.

We asked whether primary tumor expression of BHLHE40 was correlated with survival outcomes in patients with breast cancer when analyzing outcomes based on PAM50 molecular subtype. We observed a correlation between primary tumor expression of BHLHE40 and overall survival (OS) in patients with breast cancer, irrespective of molecular subtype, which was statistically significant (Figure 2; p -value: 0.036; hazard ratio: 0.82 (0.68-0.99)). BHLHE40 primary tumor expression was a positive prognostic indicator in patients with breast cancer. Patients with low tumor expression of BHLHE40 possessed median OS of 80.64 months, while patients with high tumor expression of BHLHE40 possessed median OS of 115 months (Table 3).

BHLHE40 primary tumor expression was not correlated with overall survival in basal subtype breast cancer (Figure 2; p -value: 0.036; hazard ratio: 0.82 (0.68-0.99)), in luminal A subtype breast cancer (Figure 2; p -value: 0.85; hazard ratio: 1.04 (0.68-1.59)), in luminal B subtype breast cancer (Figure 2; p -value: 0.65; hazard ratio: 1.08 (0.76-1.54)), in HER2+ breast cancer (Figure 2; p -value: 0.23; hazard ratio: 0.79 (0.54-1.16)), or in normal-like breast cancer (Figure 2; p -value: 0.27; hazard ratio: 1.75 (0.64-4.83)).

Discussion

We find here that BHLHE40 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 BHLHE40 mRNA in BLBC. Kaplan-Meier analysis of patient survival data revealed that BHLHE40 expression was correlated with overall survival in patients with breast cancer. BHLHE40 may be one factor that specifies tumor subtype identity in basal-like breast cancer.

References

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Rank: 239
probe ID: A_24_P268676
p-value: 8.46E-09
t: -6.29473
B: 9.88818
Gene: BHLHE40
Gene name: basic helix-loop-helix family member e40

Table 1: BHLHE40 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: 256
probe ID: 8077441
 p -value: 1.85E-11
 t : -7.3916546
 B : 15.7187
Gene: BHLHE40
Gene name: basic helix-loop-helix family member e40

Table 2: In a separate cohort of patients with basal subtype breast cancer, BHLHE40 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+ 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: BHLHE40 expression is significantly lower in basal-like breast cancers.

Depicted is mRNA expression of BHLHE40 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: BHLHE40 primary tumor expression is correlated with overall survival in patients with breast cancer.

Depicted in this Kaplan-Meier plot is the probability of overall survival for $n=1879$ patients (above, left), $n=431$ basal subtype patients (above, middle), $n=362$ HER2+ subtype patients (above, right), $n=596$ luminal A subtype patients (below, left), $n=439$ luminal B subtype patients (below, middle), and $n=51$ normal-like subtype patients (below, right) stratified into two groups, based on low or high expression of BHLHE40 in patient primary tumors. The log rank p -value denoting statistical significance of difference in overall 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 BHLHE40 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.

All patients

Low BHLHE40 expression: 80.64 months

High BHLHE40 expression: 115 months

Table 3: Median OS is superior in breast cancer patients with high primary tumor expression of BHLHE40.

The median OS (overall survival) of $n=1879$ breast cancer patients, with low primary tumor expression of BHLHE40 and high primary tumor expression of BHLHE40 is listed in this chart.