Elevated FOXC1 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 forkhead box C1, FOXC1, when comparing tumors of patients with basal-like breast cancer with that of other PAM50 molecular subtypes. FOXC1 mRNA was present at significantly higher quantities in the tumors of patients with basal-like breast cancer. Analysis of patient survival data revealed that FOXC1 primary tumor expression was correlated with recurrence-free survival in basal-like, luminal A, and luminal B subtype patients. Elevated FOXC1 expression appears to distinguish basal-like human breast cancer from the other molecular subtypes.

Keywords: basal subtype, basal-like breast cancer, FOXC1, 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 FOXC1 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 FOXC1 mRNA expression levels with recurrence-free survival in $n=2032$ breast cancer patients, $n=442$ basal subtype breast cancer patients, $n=631$ luminal A subtype breast cancer patients, $n=566$ luminal B subtype breast cancer patients, $n=358$ HER2+ subtype breast cancer patients, and $n=35$ normal-like subtype breast cancer patients.

Results

FOXC1 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 forkhead box C1, FOXC1, 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, FOXC1 expression ranked 26 out of 31157 total transcripts, equating to 99.9% differential expression; this was statistically significant (p -value: $1.08E-13$).

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 FOXC1 in patients with basal subtype human breast cancer (Table 2). When sorting each of

the transcripts whose expression was measured globally, FOXC1 expression ranked 4174 out of 22283 total transcripts, equating to 81.3% differential expression; this was statistically significant (p -value: $1.04E-02$). Thus, the data suggested differential expression of FOXC1 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.

FOXC1 expression is significantly higher in basal-like breast cancers.

We obtained exact FOXC1 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 FOXC1 gene expression change in basal-like breast cancer. FOXC1 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; $p < 0.0001$). FOXC1 was expressed at 1.529 ± 1.816 arbitrary units (A.U.) in basal-like breast cancer and -1.043 ± 1.049 A.U. in all other molecular subtypes (Figure 1).

FOXC1 expression correlates with recurrence-free survival in basal, luminal A and luminal B subtype breast cancer patients.

We asked whether primary tumor expression of FOXC1 was correlated with survival outcomes in patients with basal-like breast cancer. We observed a correlation between primary tumor expression of FOXC1 and recurrence-free survival (RFS) in patients with basal-like breast cancer which was statistically significant (Figure 2; p -value: 0.01; hazard ratio: 0.67 (0.49-0.91)). FOXC1 primary tumor expression was a positive prognostic indicator in patients with basal-like breast cancer. Patients with low tumor expression of FOXC1 possessed median RFS of 16 months, while patients with high tumor expression of FOXC1 possessed median RFS of 31.56 months (Table 3).

We also observed a correlation between primary tumor expression of FOXC1 and recurrence-free survival (RFS) in patients with luminal A breast cancer which trended towards statistical significance (Figure 2; p -value: 0.079; hazard ratio: 0.73 (0.51-1.04)). FOXC1 primary tumor expression was a positive prognostic indicator in patients with luminal A breast cancer. Patients with low tumor expression of FOXC1 possessed median RFS of 83 months, while patients with high tumor expression of FOXC1 possessed median RFS of 127.2 months (Table 3).

We observed a correlation between primary tumor expression of FOXC1 and recurrence-free survival (RFS) in patients with luminal B breast cancer which was statistically significant (Figure 2; p -value: 0.021; hazard ratio: 0.74 (0.57-0.96)). FOXC1 primary tumor expression was a positive prognostic indicator in patients with luminal B breast cancer. Patients with low tumor expression of FOXC1 possessed median RFS of 97 months, while patients with high tumor expression of FOXC1 possessed median RFS of 148 months (Table 3).

FOXC1 primary tumor expression was not correlated with recurrence-free survival in all patients (irrespective of molecular subtype) (Figure 2; p -value: 0.77; hazard ratio: 0.98 (0.84-1.14)), in HER2+ breast cancer (Figure 2; p -value: 0.86; hazard ratio: 0.97 (0.7-1.34)), or in normal-like breast cancer (Figure 2; p -value: 0.69; hazard ratio: 1.22 (0.46-3.27)).

Discussion

We find here that FOXC1 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 FOXC1

mRNA in BLBC. Kaplan-Meier analysis of patient survival data revealed that FOXC1 expression was correlated with recurrence-free survival in patients with basal-like, luminal A, and luminal B subtype breast cancer. FOXC1 may be one factor that specifies tumor subtype identity in basal-like breast cancer.

References

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Rank: 26
probe ID: A_23_P390504
p-value: 1.08E-13
t: 8.624494
B: 20.71188
Gene: FOXC1
Gene name: forkhead box C1

Table 1: FOXC1 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: 4174
probe ID: 213260_at
p-value: 1.04E-02
t: 2.57238
B: -3.0019208
Gene: FOXC1
Gene name: forkhead box C1

Table 2: In a separate cohort of patients with basal subtype breast cancer, FOXC1 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: FOXC1 expression is significantly higher in basal-like breast cancers.

Depicted is mRNA expression of FOXC1 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: FOXC1 primary tumor expression is correlated with recurrence-free survival in patients with basal-like breast cancer.

Depicted in this Kaplan-Meier plot is the probability of recurrence-free survival for $n=2032$ patients (above, left), $n=442$ basal subtype patients (above, middle), $n=358$ HER2+ subtype patients (above, right), $n=631$ luminal A subtype patients (below, left), $n=566$ luminal B subtype patients (below, middle), and $n=35$ normal-like subtype patients (below, right) stratified into two groups, based on low or high expression of FOXC1 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 FOXC1 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.

Basal subtype

Low FOXC1 expression: 16 months

High FOXC1 expression: 31.56 months

Luminal A subtype

Low FOXC1 expression: 83 months

High FOXC1 expression: 127.2 months

Luminal B subtype

Low FOXC1 expression: 97 months

High FOXC1 expression: 148 months

Table 3: Median RFS is superior in basal, luminal A, and luminal B subtype breast cancer patients with high primary tumor expression of FOXC1.

The median RFS (recurrence-free survival) of $n=442$ basal subtype breast cancer patients, $n=631$ luminal A subtype breast cancer patients and $n=566$ luminal B subtype breast cancer patients, with low primary tumor expression of FOXC1 and high primary tumor expression of FOXC1 is listed in this chart.