Differential expression of atonal bHLH transcription factor 8 in cancers of the breast.

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Breast cancer affects women at relatively high frequency¹. We mined published microarray datasets^{2,3} to determine in an unbiased fashion and at the systems level genes most differentially expressed in the primary tumors of patients with breast cancer. We report here significant differential expression of the gene encoding atonal bHLH transcription factor 8, ATOH8, when comparing primary tumors of the breast to the tissue of origin, the normal breast. ATOH8 mRNA was present at significantly lower quantities in tumors of the breast as compared to normal breast tissue. Analysis of human survival data revealed that expression of ATOH8 in primary tumors of the breast was correlated with recurrence-free survival in patients with luminal B and HER2+ subtype cancer, demonstrating a relationship between primary tumor expression of a differentially expressed gene and patient survival outcomes influenced by PAM50 molecular subtype. ATOH8 may be of relevance to initiation, maintenance or progression of cancers of the female breast.

Keywords: breast cancer, ATOH8, atonal bHLH transcription factor 8, systems biology of breast cancer, targeted therapeutics in breast cancer.

Invasive breast cancer is diagnosed in over a quarter of a million women in the United States each year¹ and in 2018, breast cancer was the leading cause of cancer death in women worldwide⁴. While patients with localized breast cancer are provided a 99% 5-year survival rate, patients with regional breast cancer, cancer that has spread to lymph nodes or nearby structures, are provided an 86% 5-year survival rate^{5,6}. Patients with metastasis to distant sites, like the brain, are provided a 27% 5-year survival rate^{5,6}. Understanding how primary tumors are most transcriptionally different from the tissue from which they originate, the breast, can facilitate development of novel diagnostic and therapeutics to promote early detection and enhanced treatment, and contribute to efforts to prevent progression to metastatic stages. We mined published microarray data^{2,3} to understand at the transcriptome level and in an unbiased fashion genes most differentially expressed in primary tumors of the breast as compared to normal breast tissue. Atonal bHLH transcription factor 8 emerged as among the most differentially expressed genes in cancer of the female breast.

Methods

We utilized datasets GSE42568² and GSE109169³ for this global differential gene expression analysis of female breast cancer. GSE42568 was generated using Affymetrix Human Genome U133 Plus 2.0 array technology with $n=17$ normal breast tissue biopsies and $n=104$ primary breast tumor biopsies from patients with breast cancer; analysis was performed using platform GPL570; the majority of patients whose tumors were analyzed were age 50 or older. GSE109169 was generated using Affymetrix Human Exon 1.0 ST Array technology with $n=25$ normal breast tissue and $n=25$ tumors of the breast; analysis was performed using platform GPL5175. The tissues whose expression was profiled in this dataset are paired tissues (25 tumors matching 25 breast tissues from 25 patients). 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 ATOH8 expression was significantly different between primary breast tumors and normal breast tissue using a two-tailed t-test. For Kaplan-Meier survival analysis, we used the Kaplan-Meier plotter online tool⁷ for correlation of ATOH8 mRNA expression levels with recurrence-free survival (RFS) in $n=442$ patients with basal-like subtype cancer, $n=631$ patients with luminal A subtype cancer, $n=566$ patients with luminal B subtype cancer, $n=358$ patients with HER2+ cancer, and $n=35$ patients with normal-like subtype cancer.

Results

We performed discovery of genes associated with breast cancer in females by mining two independently published microarray datasets^{2,3}.

ATOH8 is differentially expressed in primary tumors of the breast.

Studying the global gene expression profiles of 104 breast cancers from patients aged 31 to 89 revealed that the gene encoding atonal bHLH transcription factor 8, ATOH8, was among the genes most differentially expressed in tumors of the breast in human breast cancer (Chart 1). When sorting each of the genes expressed in tumors of the breast based on significance of difference as compared to normal breast tissue, ATOH8 ranked 136 out of 54675 total transcripts, equating to 99.8% differential expression (Chart 1). Differential expression of ATOH8 in female breast cancer was statistically significant (Chart 1; $p=3.17E-29$).

Analysis of a second microarray dataset³, here studying global gene expression patterns in the tumors of 25 patients with early-onset breast cancer, again revealed significant differential expression of

1 ATOH8 in human breast cancer (Chart 2). When sorting each of the genes expressed in the tumors of
2 patients with breast cancer based on significance of difference as compared to normal breast tissue,
3 ATOH8 ranked 1105 out of 19076 total transcripts, equating to 94.2% differential expression (Chart 2).
4 Differential expression of ATOH8 in the tumors of patients with breast cancer was statistically significant
(Chart 2; $p=1.14E-08$). These data suggested that differential expression of ATOH8 was not an artifact of
5 a single microarray dataset, nor was it strictly associated with early-onset breast cancer, rather a general
6 feature of cancers of the breast.

6 **ATOH8 is expressed at significantly lower levels in breast tumors as compared to the breast.**

7 We obtained exact mRNA expression levels for ATOH8 from the breast and from breast tumors to
8 understand the magnitude and direction of ATOH8 expression change. ATOH8 was expressed at lower
9 levels in tumors of the breast as compared to normal breast tissue (Figure 1). Decreased expression of
10 ATOH8 in primary breast tumors was statistically significant (Figure 1: $p<0.0001$). ATOH8 was
11 expressed at 5.40 ± 0.79 arbitrary units (AU) in normal breast tissue, while it was expressed at 3.27 ± 0.50
12 AU in tumors of the breast. We calculated a mean fold change of 0.61 in ATOH8 mRNA levels when
13 comparing primary tumors of the breast to normal breast tissues.

11 **ATOH8 expression correlates with survival outcomes in luminal B and HER2+ subtype human 12 breast cancer.**

13 We performed Kaplan-Meier survival analysis to study relationships between tumor ATOH8
14 mRNA expression levels and survival outcomes in patients with breast cancer. We observed a correlation
15 between ATOH8 expression and recurrence-free survival (RFS) in patients with luminal B subtype breast
16 cancer which was statistically significant (Figure 2; log rank p -value: 0.011 for recurrence-free survival,
17 hazard ratio: 0.71 (0.55-0.93) (Fig. 2)). ATOH8 mRNA levels were a positive prognostic indicator in
18 luminal B subtype breast cancer patients. Median RFS was 32 months for luminal B patients with low
19 tumor expression of ATOH8 while median RFS was 37.52 months for luminal B patients with high tumor
20 expression of ATOH8 (Chart 3).

21 We also observed a correlation between ATOH8 expression and recurrence-free survival (RFS) in
22 patients with HER2+ subtype breast cancer which was statistically significant (Figure 2; log rank p -value:
23 0.028 for recurrence-free survival, hazard ratio: 0.69 (0.5-0.96) (Fig. 2)). ATOH8 mRNA levels were a
24 positive prognostic indicator in HER2+ subtype breast cancer patients. Median RFS was 98.4 months for
25 HER2+ patients with low tumor expression of ATOH8 while median RFS was 171.43 months for HER2+
26 patients with high tumor expression of ATOH8 (Chart 3).

27 ATOH8 primary tumor expression was not correlated with recurrence-free survival in basal-like
28 breast cancer (Figure 2; log rank p -value: 0.17 for RFS, hazard ratio: 0.81 (0.59-1.1) (Fig. 2)), luminal A
subtype breast cancer (Figure 2; log rank p -value: 0.43 for RFS, hazard ratio: 0.87 (0.61-1.23) (Fig. 2)), or
in patients with normal-like breast cancer (Figure 2; log rank p -value: 0.44 for RFS, hazard ratio: 1.47
(0.55-3.95) (Fig. 2)).

24 Thus, through comparative transcriptome analysis of primary tumors of the breast and normal
25 breast tissue, we found that differential expression and down-regulation of ATOH8 was among the most
26 significant transcriptional features in primary tumors from patients with breast cancer. ATOH8 expression
27 in primary tumors of the breast was correlated with recurrence-free survival in patients with luminal B and
28 HER2+ subtype disease, with mRNA levels of ATOH8 a positive prognostic indicator for luminal B and
HER2+ breast cancer patients.

Discussion

Invasive breast cancer is a medical problem with a 27% 5-year survival rate for women whose disease has spread to distant sites^{5,6}. To facilitate understanding of the basic transcriptional differences between primary tumors of the breast and the tissues from which these tumors originate, normal breast tissues, we performed comparative transcriptome analysis using two independently published microarray datasets^{2,3}, providing evidence here that differential expression of atonal bHLH transcription factor 8, encoded by ATOH8, is a defining transcriptional feature of human breast cancer: in patients diagnosed after age 50, and in early onset-breast cancer. ATOH8 was expressed at significantly lower levels in primary tumors from patients with breast cancer as compared to normal breast tissue. Importantly, in patients with luminal B and HER2+ subtype breast cancers, expression of ATOH8 was correlated with recurrence-free survival. Atonal bHLH transcription factor 8 and the molecular processes to which it pertains to may be relevant to the initiation or progression of human breast cancer.

References

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Rank: 136
Probe ID: 228890_at
p-value: 3.17E-29
t: 1.50E+01
B: 55.9217604
Gene: ATOH8
Gene name: atonal bHLH transcription factor 8

Chart 1: ATOH8 is differentially expressed in the primary tumors of patients with breast cancer.

Rank of differential expression, probe ID, p-value of global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the groups compared, gene and gene name are listed in this chart.

Rank: 1105
Probe ID: 2491788
p-value: 1.14E-08
t: 6.8
B: 9.580245
Gene: ATOH8
Gene name: atonal bHLH transcription factor 8

Chart 2: ATOH8 is differentially expressed in the primary tumors of patients with early-onset human breast cancer.

Rank of differential expression, probe ID, p-value of global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the groups compared, gene and gene name are listed in this chart.

Figure 1: ATOH8 is expressed at significantly lower levels in primary breast tumors as compared to normal breast tissue.

The mRNA expression level of ATOH8 in normal breast tissue (left) and in primary tumors of the breast (right) is graphically depicted with the result of a statistical test evaluating significance of difference in ATOH8 expression between normal breast tissue and primary tumors of the breast, a *p*-value, listed above.

Figure 2: ATOH8 expression correlates with recurrence-free survival in patients with luminal B and HER2+ subtype breast cancer, but not in patients with basal, luminal A, or normal-like subtype cancer.

Depicted in this Kaplan-Meier plot is the probability of recurrence-free survival (RFS) for $n=442$ patients with basal-like breast cancer (upper left), $n=358$ patients with HER2+ breast cancer (upper right), $n=631$ patients with luminal A breast cancer (middle left), $n=566$ patients with luminal B breast cancer (middle right), and $n=35$ patients with normal-like breast cancer (below) stratified into two groups, based on low or high expression of ATOH8 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 ATOH8 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.

Luminal B patients

Low ATOH8 expression: 32 months

High ATOH8 expression: 37.52 months

HER2+ patients

Low ATOH8 expression: 98.4 months

High ATOH8 expression: 171.43 months

Chart 3: In luminal B and HER2+ subtype breast cancer, median recurrence-free survival is superior in patients with high primary tumor expression of ATOH8.

The recurrence-free survival of $n=566$ luminal B subtype and $n=358$ HER2+ breast cancer patients based on stratification into low or high tumor expression of ATOH8 is listed in this chart.