ARNTL expression associates with survival in triple negative breast cancer.

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We mined published microarray data (1) to understand the most significant gene expression differences in the tumors of triple negative breast cancer patients based on survival at time of analysis: dead or alive. We observed significant transcriptome-wide differential expression of aryl hydrocarbon receptor nuclear translocator-like, encoded by ARNTL when comparing the primary tumors of triple negative breast cancer patients dead or alive. Importantly, ARNTL expression was significantly correlated with recurrence-free survival in basal subtype breast cancer, a molecular subtype sharing significant overlap with triple negative breast cancer. ARNTL may be of relevance as a biomarker or as a molecule of interest in understanding the etiology or progression of triple negative breast cancer.

Keywords: triple negative breast cancer, TNBC, systems biology of breast cancer, targeted therapeutics in breast cancer, ARNTL, aryl hydrocarbon receptor nuclear translocator-like.

Triple negative breast cancer is defined by lack of expression of the receptors for the hormones progesterone and estrogen (PR negative and ER negative), as well as for the human epidermal growth factor receptor 2 (HER2 negative) (2, 3). Though not completely identical, triple negative breast cancer shares significant overlap with the basal or basal-like molecular subtype of human breast cancer (3). TNBC is more frequently diagnosed in women of African descent (black women) for reasons not understood, and their disease course is similarly more aggressive than in white women with triple negative breast cancer (2, 3). To understand the transcriptional biology of triple negative breast cancer in an unbiased fashion and at the systems level, we mined published microarray data paired with patient survival data (1), performing comparative transcriptome analysis based on survival at time of data collection: dead or alive. We present here a finding from the results of this analysis.

Methods

We used dataset GSE142102 (1) for this differential gene expression analysis in conjunction with GEO2R. GSE142102 was generated using Affymetrix Human Gene 2.1 ST Array technology; in this analysis, we used data from $n=87$ tumors from TNBC patients that were dead at time of analysis and $n=123$ tumors from TNBC patients that were alive at time of analysis. 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 ARNTL expression was significantly different between the primary tumors of TNBC patients that were dead or alive using a two-tailed t-test. For Kaplan-Meier survival analysis, we used the Kaplan-Meier plotter tool (4) for correlation of ARNTL mRNA expression levels with recurrence-free survival (RFS) in $n=953$ patients with basal subtype breast cancer.

Results

We performed comparative transcriptome analysis using published microarray data (1) to understand in an unbiased fashion the most significant gene expression differences in the primary tumors of patients with triple negative breast cancer based on survival outcomes.

ARNTL is differentially expressed in triple negative breast cancer and negatively correlates with survival.

When comparing primary tumors of 87 live patients with triple negative breast cancer with that of 123 dead TNBC patients we identified aryl hydrocarbon receptor nuclear translocator-like, *ARNTL*, as among the most significant transcriptional differences based on survival in human triple negative breast cancer. When ranking each of the transcripts whose expression was measured by microarray based on significance of difference in expression between the tumors of patients dead and alive, ARNTL ranked 27 out of 53617 total transcripts, equivalent to 99.9% differential expression (Chart 1). ARNTL differential expression in the tumors of patients based on survival outcomes was statistically significant (Figure 1; $p=0.00044439$).

ARNTL is expressed at significantly higher levels in the breast tumors of TNBC patients when comparing based on survival.

We obtained exact mRNA expression levels for ARNTL from breast tumors of TNBC patients dead and alive to understand the magnitude and direction of ARNTL expression change based on survival

1 outcomes. ARNTL was expressed at higher levels in tumors of TNBC patients alive as compared to that
2 of TNBC patients dead (Figure 1). Increased expression of ARNTL in primary breast tumors was
3 statistically significant (Figure 1: $p=0.0003$). ARNTL was expressed at -0.26 ± 0.88 arbitrary units (AU)
4 in the primary tumors of TNBC patients alive, while it was expressed at 0.23 ± 1.05 AU in the primary
tumors of TNBC patients dead.

5 ARNTL expression correlates with recurrence-free survival in basal subtype human breast cancer.

6 We referenced a bioinformatics tool containing Kaplan-Meier survival data of human breast cancer
7 patients to understand more thoroughly whether a relationship existed between ARNTL primary tumor
8 expression and patient survival in human breast cancer. We found that ARNTL expression was correlated
9 with recurrence-free survival in patients with basal or basal-like human breast cancer, a breast cancer
10 molecular subtype which shares significant overlap with triple negative breast cancer, in the upper tertile;
11 this correlation trended towards statistical significance (Figure 2; log rank p -value: 0.057 for
recurrence-free survival, hazard ratio: 0.8 (0.63-1.01) (Fig. 2)). ARNTL mRNA levels were a positive
prognostic indicator in basal subtype breast cancer patients. Median RFS was 19.4 months for basal
subtype patients with low tumor expression of ARNTL as compared to 32.78 months for basal subtype
patients with high tumor expression of ARNTL.

12 Thus, we found through unbiased comparative transcriptome analysis that ARNTL was among the
13 genes whose expression was most significantly different in the primary tumors of triple negative breast
14 cancer patients alive when compared to that of TNBC patients dead, that ARNTL was expressed at
15 significantly higher levels in the primary tumors of TNBC patients alive as compared to TNBC patients
16 dead, and that ARNTL expression was positively correlated with recurrence-free survival in the basal or
basal-like molecular subtype.

16 **Discussion**

17 It is not understood why women of African descent (black women) are diagnosed with triple
18 negative breast cancer more frequently than white women, and why this disease is more aggressive in
19 black women. ARNTL may be of relevance as a biomarker in identifying patients who are more likely to
20 survive, perhaps as part of a panel of genes used for prognostic stratification, and the molecule itself may
21 be important to the biology of triple negative breast cancer etiology or more likely its progression. Blind
systems-level analyses, like this, can help shed light on the fundamental molecular characteristics of a
disease whose racial propensities demand further explanation.

References

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Rank: 27
Probe ID: 16722217
p-value: 0.00044439
t: -3.5122176
B: -2.71
Gene: ARNTL
Gene name: aryl hydrocarbon receptor nuclear translocator-like

Chart 1: ARNTL is differentially expressed at the transcriptome-level when comparing the primary tumors of TNBC patients dead and alive.

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: ARNTL is expressed at significantly higher levels in the primary tumors of TNBC patients alive as compared to the primary tumors of TNBC patients dead.

The mRNA expression level of ARNTL in the primary tumors of TNBC patients dead (left) and in primary tumors of TNBC patients alive (right) is graphically depicted with the result of a statistical test evaluating significance of difference in ARNTL expression between the primary tumors of TNBC patients dead and alive, a *p*-value, listed here: *p*=0.0003.

Figure 2: ARNTL expression correlates with recurrence-free survival in basal subtype human breast cancer.

Depicted in this Kaplan-Meier plot is the probability of recurrence-free survival (RFS) for $n=953$ breast cancer patients of basal subtype, based on low or high expression of ARNTL 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 ARNTL 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 ARNTL expression: 19.4 months
High ARNTL expression: 32.78 months

Chart 2: Median recurrence-free survival is superior in basal subtype breast cancer patients with high primary tumor expression of ARNTL.

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