LRRC8A 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 following treatment: dead or alive. We observed significant transcriptome-wide differential expression of leucine rich repeat containing 8 family, member A, encoded by LRRC8A when comparing the primary tumors of triple negative breast cancer patients dead or alive. Importantly, LRRC8A expression was correlated with distant metastasis-free survival in basal subtype breast cancer, a molecular subtype sharing significant overlap with triple negative breast cancer. LRRC8A 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, LRRC8A, leucine rich repeat containing 8 family, member A.

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 LRRC8A 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 LRRC8A mRNA expression levels with distant metastasis-free survival (DMFS) in $n=283$ 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.

LRRC8A 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 leucine rich repeat containing 8 family, member A, *LRRC8A*, 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, LRRC8A ranked 196 out of 53617 total transcripts, equivalent to 99.6% differential expression (Chart 1). LRRC8A differential expression in the tumors of patients based on survival outcomes was statistically significant (Figure 1; $p=0.00486315$).

LRRC8A is expressed at significantly lower levels in the breast tumors of TNBC patients when comparing based on survival.

We obtained exact mRNA expression levels for LRRC8A from breast tumors of TNBC patients

1 dead and alive to understand the magnitude and direction of LRRC8A expression change based on
2 survival outcomes. LRRC8A was expressed at lower levels in tumors of TNBC patients alive as
3 compared to that of TNBC patients dead (Figure 1). Decreased expression of LRRC8A in primary breast
4 tumors was statistically significant (Figure 1: $p=0.0049$). LRRC8A was expressed at 0.23 ± 1.00 arbitrary
5 units (AU) in the primary tumors of TNBC patients alive, while it was expressed at -0.17 ± 0.97 AU in the
6 primary tumors of TNBC patients dead.

7 LRRC8A expression correlates with distant metastasis-free survival in basal subtype human breast cancer.

8 We referenced a bioinformatics tool containing Kaplan-Meier survival data of human breast cancer
9 patients to understand more thoroughly whether a relationship existed between LRRC8A primary tumor
10 expression and patient survival in human breast cancer. We found that LRRC8A expression was
11 correlated with distant metastasis-free survival in patients with basal or basal-like human breast cancer, a
12 breast cancer molecular subtype which shares significant overlap with triple negative breast cancer, in the
13 lower tertile (Figure 2; log rank p -value: 0.018 for distant metastasis-free survival, hazard ratio: 1.93
14 (1.11-3.37) (Fig. 2)). LRRC8A mRNA levels were a negative prognostic indicator in basal subtype breast
15 cancer patients. Median DMFS was, on average, markedly greater for basal subtype patients with low
16 tumor expression of LRRC8A as compared to basal subtype patients with high tumor expression of
17 LRRC8A.

18 Thus, we found through unbiased comparative transcriptome analysis that LRRC8A was among the
19 genes whose expression was most significantly different in the primary tumors of triple negative breast
20 cancer patients alive when compared to that of TNBC patients dead, that LRRC8A was expressed at
21 significantly lower levels in the primary tumors of TNBC patients alive as compared to TNBC patients
22 dead, and that LRRC8A expression was negatively correlated with distant metastasis-free survival in the
23 basal or basal-like molecular subtype.

24 **Discussion**

25 It is not understood why women of African descent (black women) are diagnosed with triple
26 negative breast cancer more frequently than white women, and why this disease is more aggressive in
27 black women. LRRC8A is a leucine-rich repeat-containing protein expressed on the surface of
28 thymocytes; ligation of LRRC8A by a ligand expressed by thymic epithelial cells results in activation of
the AKT signaling pathway through PI3 kinase (5). LRRC8A may be of relevance as a biomarker in
identifying patients who are more likely to survive, perhaps as part of a panel of genes used for prognostic
stratification, and the molecule itself may 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: 196
Probe ID: 17089868
p-value: 0.00486315
t: 2.8159622
B: -3.46
Gene: LRRC8A
Gene name: leucine rich repeat containing 8 family, member A

Chart 1: LRRC8A 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: LRRC8A is expressed at significantly lower levels in the primary tumors of TNBC patients alive as compared to the primary tumors of TNBC patients dead.

The mRNA expression level of LRRC8A 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 LRRC8A expression between the primary tumors of TNBC patients dead and alive, a p -value, listed here: $p=0.0049$.

Figure 2: LRRC8A expression correlates with distant metastasis-free survival in basal subtype human breast cancer.

Depicted in this Kaplan-Meier plot is the probability of distant metastasis-free survival (DMFS) for $n=283$ breast cancer patients of basal subtype, based on low or high expression of LRRC8A in patient primary tumors, in the lower tertile. 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 LRRC8A 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.