ACADL expression associates with survival in triple negative breast cancer.

Shahan Mamoor, MS¹

¹shahanmamoor@gmail.com

East Islip, NY USA

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 acyl-CoA dehydrogenase, long chain, encoded by ACADL when comparing the primary tumors of triple negative breast cancer patients dead or alive. Importantly, ACADL expression was significantly correlated with distant metastasis-free survival in basal subtype breast cancer, a molecular subtype sharing significant overlap with triple negative breast cancer. ACADL 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, ACADL, acyl-CoA dehydrogenase, long chain.

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 ACADL 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 ACADL mRNA expression levels with distant metastasis-free survival (DMFS) in $n=571$ 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.

ACADL 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 the acyl-CoA dehydrogenase, long chain, *ACADL*, 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, ACADL ranked 56 out of 53617 total transcripts, equivalent to 99.9% differential expression (Chart 1). ACADL differential expression in the tumors of patients based on survival outcomes was statistically significant (Figure 1; $p=0.00142325$).

ACADL 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 ACADL from breast tumors of TNBC patients

1 dead and alive to understand the magnitude and direction of ACADL expression change based on survival
2 outcomes. ACADL was expressed at lower levels in tumors of TNBC patients alive as compared to that
3 of TNBC patients dead (Figure 1). Decreased expression of ACADL in primary breast tumors was
4 statistically significant (Figure 1: $p=0.0021$). ACADL was expressed at 0.23 ± 1.11 arbitrary units (AU)
in the primary tumors of TNBC patients alive, while it was expressed at -0.21 ± 0.86 AU in the primary
tumors of TNBC patients dead.

5 ACADL expression correlates with distant metastasis-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 ACADL primary tumor
8 expression and patient survival in human breast cancer. We found that ACADL expression was correlated
9 with distant metastasis-free survival in patients with basal or basal-like human breast cancer, a breast
10 cancer molecular subtype which shares significant overlap with triple negative breast cancer; this
11 correlation trended towards statistical significance (Figure 2; log rank p -value: 0.08 for distant
12 metastasis-free survival, hazard ratio: 1.3 (0.97-1.75) (Fig. 2)). ACADL mRNA levels were a negative
prognostic indicator in basal subtype breast cancer patients. Median DMFS was 43.44 months for basal
subtype patients with low tumor expression of ACADL as compared to 28.75 months for basal subtype
patients with high tumor expression of ACADL.

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

17 **Discussion**

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

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Rank: 56
Probe ID: 16907812
p-value: 0.00142325
t: 3.1898948
B: -3.08
Gene: ACADL
Gene name: acyl-CoA dehydrogenase, long chain

Chart 1: ACADL 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: ACADL 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 ACADL 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 ACADL expression between the primary tumors of TNBC patients dead and alive, a p -value, listed here: $p=0.0021$.

Figure 2: ACADL 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 $n=571$ breast cancer patients of basal subtype, based on low or high expression of ACADL 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 ACADL 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 ACADL expression: 43.44 months

High ACADL expression: 28.75 months

Chart 2: Median distant metastasis-free survival is superior in basal subtype breast cancer patients with low primary tumor expression of ACADL.

The median DMFS (distant metastasis-free survival) of basal subtype breast cancer patients, with low primary tumor expression of ACADL and high primary tumor expression of ACADL is listed in this chart.