ADAM6 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 ADAM metallopeptidase domain 6 (pseudogene), encoded by ADAM6 when comparing the primary tumors of triple negative breast cancer patients dead or alive. Importantly, ADAM6 expression was significantly correlated with recurrence-free survival in basal subtype breast cancer, a molecular subtype sharing significant overlap with triple negative breast cancer. ADAM6 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, ADAM6, ADAM metallopeptidase domain 6 (pseudogene).

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 ADAM6 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 ADAM6 mRNA expression levels with recurrence-free survival (RFS) in $n=442$ 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.

ADAM6 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 ADAM metallopeptidase domain 6 (pseudogene), *ADAM6*, 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, ADAM6 ranked 65 out of 53617 total transcripts, equivalent to 99.9% differential expression (Chart 1). ADAM6 differential expression in the tumors of patients based on survival outcomes was statistically significant (Figure 1; $p=0.00167775$).

ADAM6 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 ADAM6 from breast tumors of TNBC patients dead and alive to understand the magnitude and direction of ADAM6 expression change based on survival

1 outcomes. ADAM6 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 ADAM6 in primary breast tumors was
3 statistically significant (Figure 1: $p=0.0008$). ADAM6 was expressed at -0.27 ± 0.84 arbitrary units (AU)
4 in the primary tumors of TNBC patients alive, while it was expressed at 0.17 ± 1.02 AU in the primary
tumors of TNBC patients dead.

5 ADAM6 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 ADAM6 primary tumor
8 expression and patient survival in human breast cancer. We found that ADAM6 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 lower quartile
11 (Figure 2; log rank p -value: 0.077 for recurrence-free survival, hazard ratio: 0.74 (0.53-1.03) (Fig. 2)); this
correlation trended towards statistical significance. ADAM6 mRNA levels were a positive prognostic
indicator in basal subtype breast cancer patients. Median RFS was 17 months for basal subtype patients
with low tumor expression of ADAM6 as compared to 21.82 months for basal subtype patients with high
tumor expression of ADAM6.

12 Thus, we found through unbiased comparative transcriptome analysis that ADAM6 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 ADAM6 was expressed at
15 significantly higher levels in the primary tumors of TNBC patients alive as compared to TNBC patients
16 dead, and that ADAM6 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. Deletion of ADAM6 in mice results in impaired male fertility resulting at least in part due
20 to deficiencies in sperm motility (5). ADAM6 may be of relevance as a biomarker in identifying patients
21 who are more likely to survive, perhaps as part of a panel of genes used for prognostic stratification, and
22 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

1. Purrington, K.S., Knight III, J., Dyson, G., Ali-Fehmi, R., Schwartz, A.G., Boerner, J.L. and Bandyopadhyay, S., 2020. CLCA2 expression is associated with survival among African American women with triple negative breast cancer. *PloS one*, 15(4), p.e0231712.
2. Foulkes, W.D., Smith, I.E. and Reis-Filho, J.S., 2010. Triple-negative breast cancer. *New England journal of medicine*, 363(20), pp.1938-1948.
3. Dietze, E.C., Sistrunk, C., Miranda-Carboni, G., O'regan, R. and Seewaldt, V.L., 2015. Triple-negative breast cancer in African-American women: disparities versus biology. *Nature Reviews Cancer*, 15(4), pp.248-254.
4. Györfy, B., Lanczky, A., Eklund, A.C., Denkert, C., Budczies, J., Li, Q. and Szallasi, Z., 2010. An online survival analysis tool to rapidly assess the effect of 22,277 genes on breast cancer prognosis using microarray data of 1,809 patients. *Breast cancer research and treatment*, 123(3), pp.725-731.
5. Voronina, V.A., Harris, F.M., Schmahl, J., Galligan, C., Oristian, D., Zamfirova, R., Gong, G., Bai, Y., Fury, W., Rajamani, S. and Walls, J.R., 2019. Deletion of Adam6 in *Mus musculus* leads to male subfertility and deficits in sperm ascent into the oviduct. *Biology of reproduction*, 100(3), pp.686-696.

Rank: 65
Probe ID: 16797440
p-value: 0.00167775
t: -3.1420402
B: -3.13
Gene: ADAM6
Gene name: ADAM metalloproteinase domain 6 (pseudogene)

Chart 1: ADAM6 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: ADAM6 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 ADAM6 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 ADAM6 expression between the primary tumors of TNBC patients dead and alive, a p -value, listed here: $p=0.0008$.

Figure 2: ADAM6 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 $n=442$ breast cancer patients of basal subtype, based on low or high expression of ADAM6 in patient primary tumors, in the lower quartile. 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 ADAM6 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 ADAM6 expression: 17 months
High ADAM6 expression: 21.82 months

Chart 2: Median recurrence-free survival is superior in basal subtype breast cancer patients with high primary tumor expression of ADAM6.

The median RFS (recurrence-free survival) of basal subtype breast cancer patients, with low primary tumor expression of ADAM6 and high primary tumor expression of ADAM6 is listed in this chart, in the lower quartile.