DENND1C 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 following treatment: dead or alive. We observed significant transcriptome-wide differential expression of DENN/MADD domain containing 1C, encoded by DENND1C when comparing the primary tumors of triple negative breast cancer patients dead or alive. Importantly, DENND1C expression was correlated with recurrence-free survival in basal subtype breast cancer, a molecular subtype sharing significant overlap with triple negative breast cancer. DENND1C 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, DENND1C, DENN/MADD domain containing 1C.

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 DENND1C 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 DENND1C 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.

DENND1C 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 DENN/MADD domain containing 1C, *DENND1C*, 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, DENND1C ranked 7 out of 53617 total transcripts, equivalent to 99.99% differential expression (Chart 1). DENND1C differential expression in the tumors of patients based on survival outcomes was statistically significant (Figure 1; $p=0.00016145$).

DENND1C 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 DENND1C from breast tumors of TNBC patients

1 dead and alive to understand the magnitude and direction of DENND1C expression change based on
2 survival outcomes. DENND1C was expressed at higher levels in tumors of TNBC patients alive as
3 compared to that of TNBC patients dead (Figure 1). Increased expression of DENND1C in primary breast
4 tumors was statistically significant (Figure 1: $p < 0.0001$). DENND1C was expressed at -0.27 ± 0.68
5 arbitrary units (AU) in the primary tumors of TNBC patients dead, while it was expressed at 0.26 ± 1.15
6 AU in the primary tumors of TNBC patients alive.

7 DENND1C expression correlates with recurrence-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 DENND1C primary tumor
10 expression and patient survival in human breast cancer. We found that DENND1C expression was
11 correlated with recurrence-free survival in patients with basal or basal-like human breast cancer, a breast
12 cancer molecular subtype which shares significant overlap with triple negative breast cancer (Figure 2; log
13 rank p -value: 0.024 for recurrence-free survival, hazard ratio: 0.78 (0.63-0.97) (Fig. 2)). DENND1C
14 mRNA levels were a positive prognostic indicator in basal subtype breast cancer patients. Median RFS
15 was, on average, markedly shorter for basal subtype patients with low tumor expression of DENND1C as
16 compared to basal subtype patients with high tumor expression of DENND1C.

17 Thus, we found through unbiased comparative transcriptome analysis that DENND1C was among
18 the genes whose expression was most significantly different in the primary tumors of triple negative breast
19 cancer patients alive when compared to that of TNBC patients dead, that DENND1C was expressed at
20 significantly higher levels in the primary tumors of TNBC patients alive as compared to TNBC patients
21 dead, and that DENND1C expression was positively correlated with recurrence-free survival in the basal
22 or basal-like molecular subtype.

23 **Discussion**

24 It is not understood why women of African descent (black women) are diagnosed with triple
25 negative breast cancer more frequently than white women, and why this disease is more aggressive in
26 black women. DENND1C connects Rab35 to the actin cytoskeleton by using its carboxy-terminus to
27 directly bind actin (5). We previously demonstrated that Rab35 was differentially expressed in human
28 lymph node metastatic breast cancer and present at significantly higher quantities in lymph node
metastases as compared to primary tumors of the breast (6). DENND1C 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

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. Marat, A.L., Ioannou, M.S. and McPherson, P.S., 2012. Connecdenn 3/DENND1C binds actin linking Rab35 activation to the actin cytoskeleton. *Molecular biology of the cell*, 23(1), pp.163-175.
6. Mamoor, S., 2021. DENND1C is differentially expressed in the lymph nodes of patients with metastatic breast cancer.

Rank: 7
Probe ID: 16867733
p-value: 0.00016145
t: -3.7727632
B: -2.39
Gene: DENND1C
Gene name: DENN/MADD domain containing 1C

Chart 1: DENND1C 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: DENND1C 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 DENND1C 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 DENND1C expression between the primary tumors of TNBC patients dead and alive, a p -value, listed here: $p < 0.0001$.

Figure 2: DENND1C 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 DENND1C 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 DENND1C 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 DENND1C expression: 18 months
High DENND1C expression: 32.78 months

Chart 2: Median recurrence-free survival is superior in basal subtype breast cancer patients with high primary tumor expression of DENND1C.

The median RFS (recurrence-free survival) of basal subtype breast cancer patients, with low primary tumor expression of DENND1C and high primary tumor expression of DENND1C is listed in this chart.