PDZD4 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 PDZ-domain containing 4, encoded by PDZD4 when comparing the primary tumors of triple negative breast cancer patients dead or alive. Importantly, PDZD4 expression was correlated with overall survival in patients with breast cancer. PDZD4 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, PDZD4, PDZ-domain containing 4.

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 PDZD4 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 PDZD4 mRNA expression levels with overall survival (OS) in $n=1090$ patients with 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.

PDZD4 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 PDZ-domain containing 4, *PDZD4*, 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, PDZD4 ranked 239 out of 53617 total transcripts, equivalent to 99.6% differential expression (Chart 1). PDZD4 differential expression in the tumors of patients based on survival outcomes was statistically significant (Figure 1; $p=0.00576335$).

PDZD4 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 PDZD4 from breast tumors of TNBC patients dead and alive to understand the magnitude and direction of PDZD4 expression change based on survival outcomes. PDZD4 was expressed at lower levels in tumors of TNBC patients alive as compared to that of

1 TNBC patients dead (Figure 1). Decreased expression of PDZD4 in primary breast tumors was statistically
2 significant (Figure 1: $p=0.0053$). PDZD4 was expressed at 0.18 ± 1.03 arbitrary units (AU) in the primary
3 tumors of TNBC patients dead, while it was expressed at -0.21 ± 0.89 AU in the primary tumors of TNBC
4 patients alive.

5 PDZD4 expression correlates with overall survival in 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 PDZD4 primary tumor
8 expression and patient survival in human breast cancer. We found that PDZD4 expression was correlated
9 with overall survival in patients with breast cancer (Figure 2; log rank p -value: 0.024 for overall survival,
10 hazard ratio: 0.69 (0.5-0.95) (Fig. 2)). PDZD4 mRNA levels were a positive prognostic indicator in
11 patients with breast cancer. Median OS was 129.1 months for patients with low tumor expression of
12 PDZD4 as compared to 142.23 months for patients with high tumor expression of PDZD4.

13 Thus, we found through unbiased comparative transcriptome analysis that PDZD4 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 PDZD4 was expressed at
16 significantly lower levels in the primary tumors of TNBC patients alive as compared to TNBC patients
17 dead, and that PDZD4 expression was positively correlated with overall survival in patients with breast
18 cancer.

19 **Discussion**

20 It is not understood why women of African descent (black women) are diagnosed with triple
21 negative breast cancer more frequently than white women, and why this disease is more aggressive in
22 black women. PDZD4 may be of relevance as a biomarker in identifying patients who are more likely to
23 survive, perhaps as part of a panel of genes used for prognostic stratification, and the molecule itself may
24 be important to the biology of triple negative breast cancer etiology or more likely its progression. Blind
25 systems-level analyses, like this, can help shed light on the fundamental molecular characteristics of a
26 disease whose racial propensities demand further explanation.
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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. Nagy, Á., Munkácsy, G. and Györfy, B., 2021. Pancancer survival analysis of cancer hallmark genes. *Scientific reports*, 11(1), pp.1-10.

Rank: 239
Probe ID: 17115254
p-value: 0.00576335
t: 2.7609505
B: -3.51
Gene: PDZD4
Gene name: PDZ-domain containing 4

Chart 1: PDZD4 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: PDZD4 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 PDZD4 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 PDZD4 expression between the primary tumors of TNBC patients dead and alive, a *p*-value, listed here: *p*=0.0053.

Figure 2: PDZD4 expression correlates with overall survival in human breast cancer.

Depicted in this Kaplan-Meier plot is the probability of overall survival (OS) for $n=1090$ breast cancer patients, based on low or high expression of PDZD4 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 PDZD4 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.

1 Low PDZD4 expression: 129.1 months
2 High PDZD4 expression: 142.23 months

3
4 **Chart 2: Median overall survival is superior in breast cancer patients with high primary tumor expression of PDZD4.**

5 The median OS (overall survival) of breast cancer patients, with low primary tumor expression of PDZD4
6 and high primary tumor expression of PDZD4 is listed in this chart.