TMEM71 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 transmembrane protein 71, encoded by TMEM71 when comparing the primary tumors of triple negative breast cancer patients dead or alive. Importantly, TMEM71 expression was correlated with overall survival in patients with human breast cancer. TMEM71 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, TMEM71, transmembrane protein 71.

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 TMEM71 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 TMEM71 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.

TMEM71 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 transmembrane protein 71, *TMEM71*, 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, TMEM71 ranked 34 out of 53617 total transcripts, equivalent to 99.9% differential expression (Chart 1). TMEM71 differential expression in the tumors of patients based on survival outcomes was statistically significant (Figure 1; $p=0.00070833$).

TMEM71 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 TMEM71 from breast tumors of TNBC patients dead and alive to understand the magnitude and direction of TMEM71 expression change based on

1 survival outcomes. TMEM71 was expressed at higher levels in tumors of TNBC patients alive as
2 compared to that of TNBC patients dead (Figure 1). Increased expression of TMEM71 in primary breast
3 tumors was statistically significant (Figure 1: $p=0.0005$). TMEM71 was expressed at -0.27 ± 0.82
4 arbitrary units (AU) in the primary tumors of TNBC patients dead, while it was expressed at 0.20 ± 1.11
5 AU in the primary tumors of TNBC patients alive.

6 TMEM71 expression correlates with overall survival in human breast cancer.

7 We referenced a bioinformatics tool containing Kaplan-Meier survival data of human breast cancer
8 patients to understand more thoroughly whether a relationship existed between TMEM71 primary tumor
9 expression and patient survival in human breast cancer. We found that TMEM71 expression was
10 correlated with overall survival in patients with breast cancer (Figure 2; log rank p -value: 0.0041 for
11 overall survival, hazard ratio: 0.62 (0.45-0.86) (Fig. 2)). TMEM71 mRNA levels were a positive
12 prognostic indicator in patients with breast cancer. Median OS was 115.73 months for patients with low
13 tumor expression of TMEM71 as compared to 142.23 months for patients with high tumor expression of
14 TMEM71.

15 Thus, we found through unbiased comparative transcriptome analysis that TMEM71 was among
16 the genes whose expression was most significantly different in the primary tumors of triple negative breast
17 cancer patients alive when compared to that of TNBC patients dead, that TMEM71 was expressed at
18 significantly higher levels in the primary tumors of TNBC patients alive as compared to TNBC patients
19 dead, and that TMEM71 expression was positively correlated with overall survival in patients with breast
20 cancer.

21 **Discussion**

22 It is not understood why women of African descent (black women) are diagnosed with triple
23 negative breast cancer more frequently than white women, and why this disease is more aggressive in
24 black women. TMEM71 may be of relevance as a biomarker in identifying patients who are more likely
25 to survive, perhaps as part of a panel of genes used for prognostic stratification, and the molecule itself
26 may be important to the biology of triple negative breast cancer etiology or more likely its progression.
27 Blind systems-level analyses, like this, can help shed light on the fundamental molecular characteristics of
28 a disease whose racial propensities demand further explanation.

References

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Rank: 34
Probe ID: 17081347
p-value: 0.00070833
t: -3.3863368
B: -2.86
Gene: TMEM71
Gene name: transmembrane protein 71

Chart 1: TMEM71 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: TMEM71 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 TMEM71 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 TMEM71 expression between the primary tumors of TNBC patients dead and alive, a p -value, listed here: $p=0.0005$.

Figure 2: TMEM71 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 TMEM71 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 TMEM71 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 TMEM71 expression: 115.73 months

2 High TMEM71 expression: 142.23 months

3
4 **Chart 2: Median overall survival is superior in breast cancer patients with positive primary tumor expression of TMEM71.**

5 The median OS (overall survival) of breast cancer patients, with low primary tumor expression of
6 TMEM71 and high primary tumor expression of TMEM71 is listed in this chart.