TTC5 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 tetratricopeptide repeat domain 5, encoded by TTC5 when comparing the primary tumors of triple negative breast cancer patients dead or alive. Importantly, TTC5 expression was correlated with recurrence-free survival in patients with breast cancer. TTC5 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, TTC5, tetratricopeptide repeat domain 5.

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 *TTC5* 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 *TTC5* mRNA expression levels with overall survival (RFS) 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.

TTC5 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 tetratricopeptide repeat domain 5, *TTC5*, 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, *TTC5* ranked 177 out of 53617 total transcripts, equivalent to 99.7% differential expression (Chart 1). *TTC5* differential expression in the tumors of patients based on survival outcomes was statistically significant (Figure 1; $p=0.00444413$).

TTC5 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 *TTC5* from breast tumors of TNBC patients dead and alive to understand the magnitude and direction of *TTC5* expression change based on survival outcomes. *TTC5* was expressed at higher levels in tumors of TNBC patients alive as compared to that of

1 TNBC patients dead (Figure 1). Increased expression of TTC5 in primary breast tumors was statistically
2 significant (Figure 1: $p=0.0036$). TTC5 was expressed at -0.22 ± 0.91 arbitrary units (AU) in the primary
3 tumors of TNBC patients dead, while it was expressed at 0.18 ± 1.04 AU in the primary tumors of TNBC
4 patients alive.

5 TTC5 expression correlates with recurrence-free 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 TTC5 primary tumor
8 expression and patient survival in human breast cancer. We found that TTC5 expression was correlated
9 with recurrence-free survival in patients with breast cancer (Figure 2; log rank p -value: 0.015 for
10 recurrence-free survival, hazard ratio: 0.59 (0.38-0.91) (Fig. 2)). TTC5 mRNA levels were a positive
11 prognostic indicator in breast cancer patients. Median RFS was 100.47 months for patients with low
12 tumor expression of TTC5 as compared to 128.73 months for patients with high tumor expression of
13 TTC5.

14 Thus, we found through unbiased comparative transcriptome analysis that TTC5 was among the
15 genes whose expression was most significantly different in the primary tumors of triple negative breast
16 cancer patients alive when compared to that of TNBC patients dead, that TTC5 was expressed at
17 significantly higher levels in the primary tumors of TNBC patients alive as compared to TNBC patients
18 dead, and that TTC5 expression was positively correlated with recurrence-free survival in patients with
19 breast cancer.

20 **Discussion**

21 It is not understood why women of African descent (black women) are diagnosed with triple
22 negative breast cancer more frequently than white women, and why this disease is more aggressive in
23 black women. TTC5 may be of relevance as a biomarker in identifying patients who are more likely to
24 survive, perhaps as part of a panel of genes used for prognostic stratification, and the molecule itself may
25 be important to the biology of triple negative breast cancer etiology or more likely its progression. Blind
26 systems-level analyses, like this, can help shed light on the fundamental molecular characteristics of a
27 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 Györffy, B., 2021. Pancancer survival analysis of cancer hallmark genes. *Scientific reports*, 11(1), pp.1-10.

Rank: 177
Probe ID: 16789899
p-value: 0.00444413
t: -2.8447866
B: -3.43
Gene: TTC5
Gene name: tetratricopeptide repeat domain 5

Chart 1: TTC5 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: TTC5 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 TTC5 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 TTC5 expression between the primary tumors of TNBC patients dead and alive, a *p*-value, listed here: *p*=0.0036.

Figure 2: TTC5 expression correlates with recurrence-free survival in human breast cancer.

Depicted in this Kaplan-Meier plot is the probability of recurrence-free survival (RFS) for $n=1090$ breast cancer patients, based on low or high expression of TTC5 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 TTC5 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 TTC5 expression: 100.47 months

High TTC5 expression: 128.73 months

Chart 2: Median recurrence-free survival is superior in breast cancer patients with high primary tumor expression of TTC5.

The median RFS (recurrence-free survival) of breast cancer patients, with low primary tumor expression of TTC5 and high primary tumor expression of TTC5 is listed in this chart.