TET3 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 at time of analysis: dead or alive. We observed transcriptome-wide differential expression of *TET3* when comparing the primary tumors of triple negative breast cancer patients dead or alive. *TET3* mRNA was present at significantly lower quantities in the tumors of TNBC patients alive. Importantly, *TET3* expression was significantly correlated with recurrence-free survival in basal subtype breast cancer, a molecular subtype sharing significant overlap with triple negative breast cancer. *TET3* 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, *TET3*, tet methylcytosine dioxygenase 3.

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 preliminary 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 TET3 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 TET3 mRNA expression levels with recurrence-free survival (RFS) in $n=417$ 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.

TET3 is differentially expressed in triple negative breast cancer and negatively correlates with survival.

When comparing primary tumors of 87 patients with triple negative breast cancer, alive at time of data collection and 123 TNBC patients, dead at time of data collection, we identified the tet methylcytosine dioxygenase 3, *TET3*, 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, TET3 ranked 199 out of 53617 total transcripts, equivalent to 99.6% differential expression (Chart 1). TET3 differential expression in the tumors of TNBC patients based on survival outcomes was statistically significant (Figure 1; $p=0.00493259$).

TET3 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 TET3 from breast tumors of TNBC patients dead and alive to understand the magnitude and direction of TET3 expression change based on survival outcomes. TET3 was expressed at lower levels in tumors of TNBC patients alive as compared to that of TNBC patients dead (Figure 1). Decreased expression of TET3 in primary breast tumors was statistically significant (Figure 1: $p=0.0068$). TET3 was expressed at 0.23 ± 1.09 arbitrary units (AU) in the primary tumors of TNBC patients alive, while it was expressed at -0.16 ± 0.92 AU in the primary tumors of TNBC patients dead.

TET3 expression correlates with recurrence-free survival in basal subtype human breast cancer.

We referenced a bioinformatics tool containing Kaplan-Meier survival data of human breast cancer patients to understand more thoroughly whether a relationship existed between TET3 primary tumor expression and patient survival in human breast cancer. We found that TET3 expression was negatively and significantly correlated with recurrence-free survival (RFS) in patients with basal or basal-like human breast cancer, a breast cancer molecular subtype which shares significant overlap with triple negative breast cancer (Figure 2; log rank p -value: 0.0034 for recurrence-free survival, hazard ratio: 0.63 (0.46-0.86) (Fig. 2)). TET3 mRNA levels were a negative prognostic indicator in basal subtype breast cancer patients with respect to recurrence-free survival. Median RFS 18 months for basal subtype patients with low tumor expression of TET3 as compared to 24.07 months for basal subtype patients with high tumor expression of TET3.

Thus, we found through unbiased comparative transcriptome analysis that TET3 was among the genes whose expression was most significantly different in the primary tumors of triple negative breast cancer patients alive when compared to that of TNBC patients dead, that TET3 was expressed at significantly lower levels in the primary tumors of TNBC patients alive as compared to TNBC patients dead, but that TET3 expression was positively correlated with recurrence-free survival in the basal or basal-like molecular subtype.

Discussion

It is not understood why women of African descent (black women) are diagnosed with triple negative breast cancer more frequently than white women, and why this disease is more aggressive in black women. TET3 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. We do not yet understand why recurrence-free survival is superior in patients with high primary tumor expression of TET3, despite our finding that TET3 is among the genes whose expression is most different in the tumors of TNBC patients dead and alive, and expressed at significantly lower in the tumors of TNBC patients alive. This may reflect seemingly contrasting roles of TET3 in recurrence and overall survival. 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

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Rank: 199
Probe ID: 16881634
p-value: 0.00493259
t: 2.8114038
B: -3.46
Gene: TET3
Gene name: tet methylcytosine dioxygenase 3

Chart 1: TET3 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: TET3 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 TET3 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 TET3 expression between the primary tumors of TNBC patients dead and alive, a p -value, listed here: $p=0.0068$.

Figure 2: TET3 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 $n=417$ breast cancer patients of basal subtype, based on low or high expression of TET3 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 TET3 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 TET3 expression: 18 months

2 High TET3 expression: 24.07 months

3
4 **Chart 3: Median recurrence-free survival is superior in basal subtype breast cancer patients with high primary tumor expression of TET3.**

5 The median RFS (recurrence-free survival) of $n=417$ basal subtype breast cancer patients, with low
6 primary tumor expression of TET3 and high primary tumor expression of TET3, is listed in this chart.