Expression of T-cell receptor gamma, delta, and alpha gene segments 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 (TNBC) patients based on survival at time of analysis: dead or alive. TRAJ27, TRDC, TRDJ1, and TRGV1 emerged as among the most differentially expressed genes, transcriptome-wide, when comparing the primary tumors of triple negative breast cancer patients dead or alive. TRAJ27, TRDC, TRDJ1, and TRGV1 were all present at significantly higher quantities in the tumors of TNBC patients alive. Differential expression of TCR genes TRAJ27, TRDC, TRDJ1, and TRGV1 may be of relevance 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, TRAJ27, TRDC, TRDJ1, TRGV1, TCR, T-cell receptor in breast cancer.

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, demonstrating differential expression of genes encoding segments of the T-cell receptor in patients with triple negative breast cancer.

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 TCR gene expression was significantly different between the primary tumors of TNBC patients that were dead or alive using a two-tailed t-test. Data is graphically represented with standard error of the mean.

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.

TRAJ27 is differentially expressed in triple negative breast cancer and positively 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 TRAJ27 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, TRAJ27 ranked 29 out of 53617 total transcripts, equivalent to 99.9% differential expression (Chart 1). TRAJ27 differential expression in the tumors of patients based on survival outcomes was statistically significant (Figure 1; $p=0.00049078$).

TRAJ27 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 TRAJ27 from breast tumors of TNBC patients dead and alive to understand the magnitude and direction of TRAJ27 expression change based on survival outcomes. TRAJ27 was expressed at higher levels in tumors of TNBC patients alive as compared to that of TNBC patients dead (Figure 1). Increased expression of TRAJ27 in primary breast tumors was statistically significant (Figure 1: $p=0.0002$). TRAJ27 was expressed at -0.25 ± 0.63 arbitrary units (AU) in the primary tumors of TNBC patients alive, while it was expressed at 0.23 ± 1.18 AU in the primary tumors of the TNBC patients dead.

TRDC is differentially expressed in triple negative breast cancer and positively 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 TRDC 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, TRDC ranked 166 out of 53617 total transcripts, equivalent to 99.7% differential expression (Chart 1). TRDC differential expression in the tumors of patients based on survival outcomes was statistically significant (Figure 1; $p=0.00407246$).

TRDC 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 TRDC from breast tumors of TNBC patients dead and alive to understand the magnitude and direction of TRDC expression change based on survival outcomes. TRDC was expressed at higher levels in tumors of TNBC patients alive as compared to that of TNBC patients dead (Figure 1). Increased expression of TRDC in primary breast tumors was statistically significant (Figure 1: $p=0.0006$). TRDC was expressed at -0.23 ± 0.66 arbitrary units (AU) in the primary tumors of TNBC patients alive, while it was expressed at 0.21 ± 1.18 AU in the primary tumors of the TNBC patients dead.

TRDJ1 is differentially expressed in triple negative breast cancer and positively 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 TRDJ1 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, TRDJ1 ranked 37 out of 53617 total transcripts, equivalent to 99.9% differential expression (Chart 1). TRDJ1 differential expression in the tumors of patients based on survival outcomes was statistically significant (Figure 1; $p=0.00078833$).

TRDJ1 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 TRDJ1 from breast tumors of TNBC patients dead and alive to understand the magnitude and direction of TRDJ1 expression change based on survival

outcomes. TRDJ1 was expressed at higher levels in tumors of TNBC patients alive as compared to that of TNBC patients dead (Figure 1). Increased expression of TRDJ1 in primary breast tumors was statistically significant (Figure 1: $p=0.0005$). TRDJ1 was expressed at -0.28 ± 0.88 arbitrary units (AU) in the primary

tumors of TNBC patients alive, while it was expressed at 0.19 ± 1.03 AU in the primary tumors of the TNBC patients dead.

TRGV1 is differentially expressed in triple negative breast cancer and positively 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 TRGV1 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, TRGV1 ranked 145 out of 53617 total transcripts, equivalent to 99.7% differential expression (Chart 1). TRGV1 differential expression in the tumors of patients based on survival outcomes was statistically significant (Figure 1; $p=0.00347012$).

TRGV1 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 TRGV1 from breast tumors of TNBC patients dead and alive to understand the magnitude and direction of TRGV1 expression change based on survival outcomes. TRGV1 was expressed at higher levels in tumors of TNBC patients alive as compared to that of TNBC patients dead (Figure 1). Increased expression of TRGV1 in primary breast tumors was statistically significant (Figure 1: $p=0.0017$). TRGV1 was expressed at -0.19 ± 0.66 arbitrary units (AU) in the primary tumors of TNBC patients alive, while it was expressed at 0.21 ± 1.18 AU in the primary tumors of the TNBC patients dead.

Thus, we found through unbiased comparative transcriptome analysis that TRAJ27, TRDC, TRDJ1, and TRGV1 were 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, and that each of these genes encoding segments of the T-cell receptor was expressed at significantly higher levels in the primary tumors of TNBC patients alive as compared to TNBC patients dead.

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. TRAJ27, TRDC, TRDJ1, and TRGV1 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 gene segments themselves may be important to the biology of triple negative breast cancer etiology or its progression. It is not clear whether our methodology has detected gene expression from tumor-infiltrating T-lymphocytes or perturbed expression of genes otherwise restricted to the T-lymphocyte but arising from the tumor cell, but we have previously demonstrated that perturbed expression of genes otherwise restricted to the B-lymphocyte (e.g., IGKC) in human breast cancer is likely inherent to the tumor cell and not reflective of tumor infiltrating lymphocytes (5). 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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15 tumor.

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27 Rank: 29
28 Probe ID: 16782070
p-value: 0.00049078

t: -3.4857391

B: -2.74

Gene: TRAJ27

Gene name: T-cell receptor alpha joining 27

Chart 1: TRAJ27 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.

TRAJ27

0.4

Figure 1: TRAJ27 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 TRAJ27 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 TRAJ27 expression between the primary tumors of TNBC patients dead and alive, a p -value, denoted by asterisk and listed here: $p=0.0002$.

Rank: 166
Probe ID: 16782003
 p -value: 0.00407246

t: -2.8724947
B: -3.4
Gene: TRDC
Gene name: T-cell receptor delta constant

Chart 2: TRDC 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 2: TRDC 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 TRDC 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 TRDC expression between the primary tumors of TNBC patients dead and alive, a *p*-value, denoted by asterisk and listed here: *p*=0.0006.

Rank: 37
Probe ID: 16781995
p-value: 0.00078833

t: -3.3568603

B: -2.89
Gene: TRDJ1
Gene name: T-cell receptor delta joining

Chart 3: TRDJ1 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 3: TRDJ1 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 TRDJ1 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 TRDJ1 expression between the primary tumors of TNBC patients dead and alive, a *p*-value, denoted by asterisk and listed here: *p*=0.0005.

Rank: 145
Probe ID: 17056857
p-value: 0.00347012

t: -2.9226997

B: -3.35
Gene: TRGV1
Gene name: T-cell receptor gamma variable 1

Chart 4: TRGV1 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 3: TRGV1 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 TRGV1 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 TRGV1 expression between the primary tumors of TNBC patients dead and alive, a *p*-value, denoted by asterisk and listed here: *p*=0.0017.