CTLA4 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 significant transcriptome-wide differential expression of the T-cell inhibitory co-receptor for the B7 ligand, CTLA4, when comparing the primary tumors of triple negative breast cancer patients dead or alive. Importantly, CTLA4 expression was significantly correlated with overall survival in basal subtype breast cancer, a molecular subtype sharing significant overlap with triple negative breast cancer. CTLA4 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, CTLA4, cytotoxic T-lymphocyte associated protein 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 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 CTLA4 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 CTLA4 mRNA expression levels with overall survival (OS) in $n=278$ 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.

CTLA4 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 cytotoxic T-lymphocyte associated protein 4, *CTLA4*, 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, CTLA4 ranked 74 out of 53617 total transcripts, equivalent to 99.9% differential expression (Chart 1). CTLA4 differential expression in the tumors of TNBC patients based on survival outcomes was statistically significant (Figure 1; $p=0.00183945$).

CTLA4 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 CTLA4 from breast tumors of TNBC patients dead and alive to understand the magnitude and direction of CTLA4 expression change based on survival outcomes. CTLA4 was expressed at lower levels in tumors of TNBC patients alive as compared to that of TNBC patients dead (Figure 1). Decreased expression of CTLA4 in primary breast tumors was statistically significant (Figure 1: $p=0.0018$). CTLA4 was expressed at -0.26 ± 0.93 arbitrary units (AU) in the primary tumors of TNBC patients alive, while it was expressed at 0.18 ± 1.05 AU in the primary tumors of TNBC patients dead.

CTLA4 expression correlates with overall 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 CTLA4 primary tumor expression and patient survival in human breast cancer. We found that CTLA4 expression was negatively and significantly correlated with overall survival 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: 0.0041 for overall survival, hazard ratio: 0.42 (0.26-0.69) (Fig. 2)). CTLA4 mRNA levels were a positive prognostic indicator in basal subtype breast cancer patients. Median OS was, on average, markedly greater for basal subtype patients with low tumor expression of CTLA4 as compared to basal subtype patients with high tumor expression of CTLA4.

Thus, we found through unbiased comparative transcriptome analysis that CTLA4 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 CTLA4 was expressed at significantly lower levels in the primary tumors of TNBC patients alive as compared to TNBC patients dead, and that CTLA4 expression was positively correlated with overall 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. CTLA4 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. 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: 74
Probe ID: 16889819
p-value: 0.00183945
t: -3.1150011
B: -3.16
Gene: CTLA4
Gene name: cytotoxic T-lymphocyte associated protein 4

Chart 1: CTLA4 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: CTLA4 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 CTLA4 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 CTLA4 expression between the primary tumors of TNBC patients dead and alive, a *p*-value, listed here: *p*=0.0018.

Figure 2: CTLA4 expression correlates with overall survival in basal subtype human breast cancer.

Depicted in this Kaplan-Meier plot is the probability of overall survival (OS) for $n=278$ breast cancer patients of basal subtype, based on low or high expression of CTLA4 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 CTLA4 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.