TOX3 is a differentially expressed gene in brain metastatic human breast cancer.

Shahan Mamoor, MS¹

¹shahanmamoor@gmail.com

East Islip, NY USA

Metastasis to the brain is a clinical problem in patients with breast cancer¹⁻³. We mined published microarray data^{4,5} to compare primary and metastatic tumor transcriptomes for the discovery of genes associated with brain metastasis in humans with metastatic breast cancer. We found that the thymocyte selection-associated high mobility group box 3, encoded by TOX3, was among the genes whose expression was most different in the brain metastases of patients with metastatic breast cancer as compared to primary tumors of the breast. TOX3 mRNA was present at increased quantities in brain metastatic tissues as compared to primary tumors of the breast. Expression of TOX3 in primary tumors was significantly correlated with patient recurrence-free survival. Modulation of TOX3 expression may be relevant to the biology by which tumor cells metastasize from the breast to the brain in humans with metastatic breast cancer.

Keywords: breast cancer, metastasis, brain metastases, central nervous system metastases, thymocyte selection-associated high mobility group box 3, TOX3, systems biology of breast cancer, targeted therapeutics in breast cancer.

One report described a 34% incidence of central nervous system metastases in patients treated with trastuzumab for breast cancer². More recently, the NEfERT-T clinical trial⁶ which compared administration of either neratinib or trastuzumab in conjunction with paclitaxel demonstrated that in a randomized, controlled setting, in breast cancer patients treated with neratinib, not only was the incidence of central nervous system recurrence significantly lower, the time to central nervous system metastasis was significantly delayed as compared to patients administered trastuzumab⁶. The alarmingly high rate of central nervous system metastasis described, as well as data, both anecdotal² and from a randomized, controlled setting⁶ illustrating that treatment with trastuzumab may be associated with these events demands an enhanced understanding of the transcriptional makeup of brain metastatic tissues to support identification of therapeutic targets, whether they are treatment related or not. We performed a global comparative analysis of primary and metastatic tumors in patients with brain metastatic breast cancer^{4,5}. We discovered differential and increased expression of the gene encoding the thymocyte selection-associated high mobility group box 3, TOX3, in brain metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE10893⁴ and GSE125989⁵ for this global differential gene expression analysis of brain metastatic breast cancer in conjunction with GEO2R. GSE10893 was generated using Agilent-011521 Human 1A Microarray G4110A technology with $n=11$ primary breast tumors and $n=3$ brain metastases from patients with breast cancer; analysis was performed using platform GPL885. GSE125989 was generated using Affymetrix Human Genome U133A 2.0 array technology with $n=16$ primary breast tumors and $n=16$ brain metastases from patients with breast cancer; the samples utilized by investigators for this microarray dataset were paired, matched tumor and metastasis samples; analysis was performed using platform GPL571. 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 TOX3 gene expression was significantly different between primary tumors of the breast and brain metastases in humans with breast cancer using a two-tailed t-test. For Kaplan-Meier survival analysis, we used the Kaplan-Meier plotter online tool for correlation of TOX3 mRNA expression levels with recurrence-free survival in $n=3951$ breast cancer patients.

Results

We performed global comparative transcriptome analysis of metastatic tumor tissues of patients with metastatic breast cancer using published microarray data^{4,5} to describe the transcriptional landscape of brain metastasis in human breast cancer in an unbiased fashion and for the discovery of novel therapeutic targets.

TOX3 is differentially expressed in the brain metastases of patients with brain metastatic breast cancer.

Through blind, systems-level analysis of published microarray data⁴, we identified the thymocyte selection-associated high mobility group box 3, encoded by TOX3, as a differentially expressed gene in the breast metastatic tissues of humans with breast cancer (Table 1). When sorting each of the genes expressed in brain metastases based on significance of difference as compared to primary tumors of the breast in patients with breast cancer, TOX3 ranked 192 out of 17418 total transcripts (Chart 1), equating to 98.9% differential expression. Differential expression of TOX3 in the brain metastases of patients with metastatic breast cancer was statistically significant (Chart 1; $p=8.26e-04$).

We queried a second microarray dataset⁵ to attempt to validate transcriptome-wide differential expression of TOX3 in human brain metastatic breast cancer. Again, we identified TOX3 as a differentially expressed gene in the brain metastatic tissues of patients with breast cancer (Chart 2). When sorting each of the genes expressed in brain metastases based on significance of difference as compared to primary tumors of the breast in patients with breast cancer, in this dataset, TOX3 ranked 1449 out of 22277 total transcripts (Chart 2), equating to 93.5% differential expression. Differential expression of TOX3 in the brain metastases of patients with metastatic breast cancer in this dataset was statistically significant (Chart 2; $p=3.87\text{e-}02$). Thus, differential expression of TOX3, transcriptome-wide, in the brain metastases of women with metastatic breast cancer was conserved across two independent microarray datasets.

TOX3 is expressed at higher levels in the brain metastases of patients with metastatic breast cancer.

We obtained exact mRNA expression levels for TOX3, in primary tumors of the breast and in brain metastasis of patients with brain metastatic breast cancer to determine direction and statistical significance of change in TOX3 expression in brain metastatic tissues. TOX3 was expressed at higher levels in the brain metastases of patients with breast cancer as compared to primary tumors of the breast, and this difference was statistically significant (Figure 1; $p=0.001619$).

TOX3 expression is significantly correlated with survival outcomes in human breast cancer.

We performed Kaplan-Meier survival analysis⁷ to evaluate correlation in 3951 breast cancer patients in total, to evaluate whether TOX3 tumor expression was correlated with survival outcomes in breast cancer. We observed a statistically significant correlation between primary tumor expression of TOX3 and recurrence-free survival (RFS) in the upper survival quartile (Figure 2). Patients whose primary tumors expressed low levels of TOX3 possessed median RFS of 216.66 months, while patients whose tumors expressed high levels of TOX3 possessed median RFS of 164.3 months. This difference in OS based on TOX3 tumor expression in breast cancer was statistically significant (Figure 2, Chart 3; logrank p -value: $2.9\text{e-}5$; hazard ratio: 1.29 (1.14-1.45)).

Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified the thymocyte selection-associated high mobility group box 3, TOX3, as among the genes whose expression was most different, transcriptome-wide, in the brain metastases of patients with breast cancer when compared to primary tumors of the breast; we observed significantly increased expression of TOX3 in brain metastases as compared to primary tumors of the breast. Further, we found a significant correlation between TOX3 expression and patient survival outcomes, as recurrence-free survival was significantly higher in patients whose primary tumors expressed lower levels of TOX3 as compared to patients whose primary tumors expressed higher levels of TOX3.

Discussion

We provided evidence here that the thymocyte selection-associated high mobility group box 3, encoded by TOX3, is among the genes whose expression is most different in the brain metastases of patients with brain metastatic breast cancer, and that primary tumor TOX3 expression is significantly correlated with patient survival outcomes in human breast cancer. Evaluation of the effects of genetic depletion of TOX3 in mouse models of metastatic breast cancer on metastasis to the central nervous system is merited. Changes in TOX3 expression may be relevant to the processes by which tumor cells exit the breast, enter the vasculature and/or lymphatics, reside in the lymph nodes, evade immune clearance, breach the blood-brain barrier and colonize the brain.

References

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Rank: 192
Probe ID: 3951
p-value: 8.26e-04
t: -4.18
B: -0.3904
Gene: TOX3
Gene name: thymocyte selection-associated high mobility group box 3

Chart 1: TOX3 is differentially expressed in brain metastatic breast cancer when comparing brain metastases to primary tumors of the breast.

The rank of global differential expression, the probe/transcript ID, the *p*-value with respect to differential expression transcriptome-wide, *t*, a moderated *t*-statistic, *B*, the log-odds of differential expression between the groups compared, the gene and gene name are listed in this chart.

Rank: 1449
Probe ID: 214774_x_at
p-value: 3.87e-02
t: -2.1424118
B: -3.918
Gene: TOX3
Gene name: thymocyte selection-associated high mobility group box 3

Chart 2: TOX3 is differentially expressed in brain metastatic breast cancer when comparing brain metastases to primary tumors of the breast.

The rank of global differential expression, the p-value with respect to differential expression transcriptome-wide, t, a moderated t-statistic, B, the log-odds of differential expression between the groups compared, the gene and gene name are listed in this chart.

Figure 1: TOX3 is expressed at significantly higher levels in the brain metastases of patients with metastatic breast cancer when compared to primary tumors of the breast.

The mRNA expression level of TOX3 in primary tumors of the breast (left) and in brain metastases from women diagnosed with breast cancer (right) is graphically depicted; the result of a statistical test evaluating significance of difference in TOX3 expression between primary tumors and brain metastases is $p=0.001619$.

Figure 2: Significant correlation between TOX3 primary tumor expression and recurrence-free survival in patients with breast cancer.

Depicted in this Kaplan-Meier plot is the probability of recurrence-free survival for $n=3951$ total patients stratified into two groups, based on low or high expression of TOX3 in patient primary tumors, in the upper survival quartile. 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 TOX3 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 TOX3 expression: 216.66 months

2 High TOX3 expression: 164.3 months

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4 **Chart 3: Median recurrence-free survival is inferior in patients with low primary tumor expression of TOX3.**

5 The median RFS (recurrence-free survival) of patients with low primary tumor expression of TOX3 and
6 high primary tumor expression of TOX3 is listed in this chart, in the upper survival quartile.