The terminal deoxynucleotidyl transferase (TdT, or DNTT) is differentially expressed in brain metastatic tissues of patients with metastatic breast cancer.

Shahan Mamoor, MS¹

¹shahanmamoor@gmail.com

East Islip, NY USA

Metastasis to the brain is a serious complication of breast cancer¹⁻³ with limited treatment options^{4,5}. We mined published microarray data^{6,7} to discover genes associated with brain metastasis in human breast cancer, and identified differential expression of the gene encoding the terminal deoxynucleotidyl transferase TdT, also known as DNTT, when comparing brain metastatic tissues to primary tumors of the breast. DNTT was expressed at significantly lower levels in metastasis to the brain as compared to primary tumors, and primary tumor expression of DNTT significantly correlated with relapse-free survival. We recently reported that the immunoglobulin light chain kappa constant locus, IGKC, was differentially expressed in the brain metastases of patients with metastatic breast cancer⁸. These data indicate that the expression of an immunoglobulin gene segment as well as a critical part of the cellular machinery utilized in expression of rearranged immunoglobulin genes with complete diversity of the immunoglobulin repertoire is differentially expressed and down-regulated in brain metastatic breast cancer, suggesting limiting immunoglobulin repertoire diversity is important for metastasis to the brain.

Keywords: breast cancer, DNTT, TdT, terminal deoxynucleotidyl transferase, brain metastasis, central nervous system metastasis, systems biology of breast cancer, targeted therapeutics in breast cancer.

Patients with metastatic breast cancer are provided limited treatment options^{4,5}. One study reported that the rate of metastasis to the central nervous system in patients treated with trastuzumab, a monoclonal antibody targeting the human epidermal growth factor receptor 2 (HER2), was 34%². The severity of disease and limited treatment options demand an enhanced understanding of the fundamental transcriptional nature of metastasis to the brain in human breast cancer. We mined published microarray data^{6,7} generated using the primary tumors and brain metastatic tissues of patients with human breast cancer to identify genes whose expression was most quantitatively different in metastasis to the brain. These analyses revealed significant differential expression of the DNA nucleotidylexotransferase, DNTT, also known as the terminal deoxynucleotidyl transferase, in brain metastatic tumor tissues of patients with human breast cancer.

Methods

We used datasets GSE43837⁶ and GSE26338⁷ for this differential gene expression analysis of brain metastasis in human breast cancer in conjunction with GEO2R. GSE43837 was generated using Affymetrix Human X3P Array technology with $n=19$ primary breast tumors and $n=19$ brain metastases from patients with breast cancer, analysis performed using platform GPL1352. GSE26338 was generated using Human 1A Microarray G4110A technology with $n=11$ primary breast tumors and $n=3$ brain metastases from patients with breast cancer, analysis performed using platform GPL885. 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 DNTT gene expression was significantly between primary breast tumors and brain metastases in humans with breast cancer using a two-tailed, unpaired t-test with Welch's correction. We used PRISM for all statistical analyses of differential gene expression in human breast cancer (Version 8.4.0)(455). For Kaplan-Meier survival analysis, we used the Kaplan-Meier plotter online tool⁸ for correlation of DNTT mRNA expression levels with relapse (recurrence)-free survival (RFS) in $n=3951$ breast cancer patients.

Results

We performed comparative transcriptome analysis of primary and metastatic tumor tissues from patients with breast cancer using published microarray data^{6,7} to describe the transcriptional landscape of brain metastasis in human breast cancer and for the identification of potential therapeutic targets.

DNTT (TdT) is differentially expressed in the brain metastases of patients with metastatic breast cancer when compared to primary tumors of the breast.

We identified the DNA deoxynucleotidylexotransferase, DNTT, also known as the terminal deoxynucleotidyl transferase, TdT, as among the genes whose expression was most significantly different in the brain metastatic tissues of patients with human breast cancer as compared to primary tumors of the breast. When sorting each of the genes expressed in brain metastases based on significance of change in expression as compared to primary tumors of the breast, DNTT ranked 16 out of 61359 total transcripts (Table 1), equating to 99.97% differential expression. Differential expression of DNTT in metastases to the brain was statistically significant (Table 1; $p=0.00005592$).

We mined a second microarray dataset to validate our observation that DNTT was significantly differentially expressed, at the transcriptome-level, in metastases to the brain in patients with breast cancer. We again identified DNTT as among the genes whose expression was most different in the brain metastatic tissues of patients with human breast cancer as compared to primary tumors of the breast. When sorting each of the genes expressed in brain metastases based on significance of change in expression as compared to primary tumors of the breast, in this dataset, DNTT ranked 1671 out of 22575

total transcripts (Table 2), equating to 92.6% differential expression. Differential expression of DNTT in metastases to the brain was again statistically significant (Table 2; $p=1.67E-02$).

DNTT (TdT) is expressed at significantly lower levels in metastases to the brain as compared to primary tumors of the breast.

We obtained exact mRNA expression values for DNTT from primary tumors of the breast and from brain metastases of patients with breast cancer to evaluate magnitude and direction of change in DNTT mRNA expression in metastasis to the brain. DNTT was expressed at lower levels in metastasis to the brain as compared to primary tumors of the breast (Figure 1), and this difference was statistically significant (Figure 1; $p=0.0004$). We calculated a mean fold change of 0.27 ± 0.32 in DNTT mRNA expression in brain metastases relative to primary tumors of the breast.

DNTT (TdT) expression associates with survival outcomes in breast cancer.

We performed Kaplan-Meier survival analysis to evaluate correlation between DNTT mRNA expression levels with survival outcomes in patients with breast cancer. In 3951 breast cancer patients, we found a statistically significant correlation between DNTT expression and relapse-free survival (RFS) (Figure 2; log rank p -value: $4.2e-06$ for relapse-free survival, hazard ratio: 0.77 (0.69 - 0.86) (Fig. 2)). Lower DNTT mRNA levels in primary tumors of the breast, like which we observed in metastases to the brain, was a negative prognostic indicator in patients with breast cancer. Median RFS was 39.26 months for patients with low tumor expression of DNTT while median RFS was 61.2 months for patients with high tumor expression of DNTT (Table 3).

Thus, through analysis of published microarray datasets, we found that DNTT, also known as TdT was among the genes whose expression was most significantly different in the brain metastases of patients with metastatic breast cancer as compared to primary tumors of the breast, that DNTT expression was significantly lower in brain metastatic tissues as compared to primary tumors of the breast, and that low primary tumor expression of DNTT significantly correlated with worse relapse-free survival as compared to patients whose tumors expressed high levels of DNTT.

Discussion

During rearrangement of immunoglobulin and T-cell receptor genes in B- and T-lymphocytes, respectively, nucleotides missing at junctional regions of gene segments that are fused together during the process of rearrangement are inserted into the nascent gene segment by the function of the terminal nucleotidyl transferase, TdT, or DNTT¹⁰. Thus, N-regions, portions of rearranged immunoglobulin and TCR genes in which N-nucleotides are inserted require TdT catalytic function, and mice deficient in TdT are lacking in N-regions, both in immunoglobulin chains in B-lymphocytes and in antigen receptors in T-lymphocytes¹⁰⁻¹², resulting in loss of diversity in the antigen repertoire. In T-cell progenitors, TdT also interacts with Ku, a protein involved repair of DNA double-strand breaks and important for V(D)J recombination¹³. N-nucleotide usage is more predominantly associated with immunoglobulin heavy chains, due to temporally-restricted expression patterns of TdT in B-lymphocytes; in a tumor cell aberrantly expressing light chain and TdT, we assume this would not be the case.

We provided evidence here that the terminal deoxynucleotidyl transferase, TdT (DNTT) is among the genes most differentially expressed in brain metastases from patients with metastatic breast cancer, that TdT is significantly down-regulated when comparing brain metastatic tissues to primary tumors of the breast, and that primary tumor TdT expression significantly correlates with patient overall survival. We are now using systems-level transcriptome analysis to gain insights as to why immunoglobulin gene expression is active in human breast cancer, and why TdT and IGK/IGKC are among the genes most specifically and most significantly down-regulated during transition from primary tumor to metastasis of the brain. Limiting tumor-intrinsic immunoglobulin repertoire diversity may be important for metastasis to the brain in human breast cancer.

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Rank	ID	p-value	t	B	FC	Gene	Gene name
16	Hs2.407439.1.A1_3p_at	0.00005592	-4.4732575	1.47032	0.27 ± 0.32	DNTT	DNA nucleotidylexo transferase

Table 1: Differential expression of DNTT/TdT in brain metastatic tissues as compared to primary tumors in patients with breast cancer.

Rank of differential expression, probe ID, *p*-value with respect to differential expression, *t*, a moderated *t*-statistic, *B*, the log-odds of differential expression between the two groups compared, fold change (FC) of DNTT expression between the brain metastases of patients with breast cancer and primary tumors of the breast, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
1671	19737	1.67E-02	-2.70	-3.17264	DNTT	deoxynucleotidyltransferase, terminal

Table 2: Differential expression of DNTT/TdT in brain metastatic tissues as compared to primary tumors in patients with breast cancer.

Rank of differential expression, probe ID, *p*-value with respect to differential expression, *t*, a moderated *t*-statistic, *B*, the log-odds of differential expression between the two groups compared, gene and gene name are listed in this chart.

Figure 1: DNTT/TdT is expressed at significantly lower levels in brain metastases as compared to primary tumors of the breast in metastatic breast cancer.

The mRNA expression level of DNTT in primary tumors of the breast (left) and in the brain metastases of brain metastatic breast cancer patients (right) is graphically represented here with mean DNTT levels marked and the result of a statistical test evaluating difference in mRNA expression level between brain metastases and primary tumors of the breast, a *p*-value, listed above. The Y-axis is set to natural logarithm (ln).

Figure 2: Expression of DNTT/TdT in primary tumors of the breast significantly correlates with relapse-free survival in patients with breast cancer.

Depicted in this Kaplan-Meier plot is the probability of relapse-free survival (RFS) for $n=3951$ total patients stratified into two groups, based on low or high expression of DNTT in patient primary tumors. The log rank p -value denoting statistical significance of difference in relapse-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 DNTT 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 expression cohort (months)	High expression cohort (months)
39.26	61.2

Table 3: Median relapse-free survival is greater in breast cancer patients whose primary tumors express high levels of DNTT/TdT.

The median relapse-free survival of $n=3951$ breast cancer patients based on stratification into low or high tumor expression of DNTT is listed in this chart.