Dystonin is differentially expressed in metastatic breast cancer, both in metastases to the brain and to the lymph nodes.

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 to discover genes associated with brain metastasis in patients with metastatic breast cancer. We found that dystonin, encoded by *DST*, was among the genes whose expression was most different in the brain and lymph node metastases of patients with metastatic breast cancer as compared to normal breast tissues. *DST* mRNA was present at significantly reduced quantities in brain metastatic tissues as compared to normal breast tissues. Down-regulation of *DST* may be an important event for metastasis of primary tumor-derived cancer cells to the brain in humans with metastatic breast cancer, and irrespective of its importance, appears to be a transcriptional event generally associated with metastasis to the brain. The fact that we have identified dystonin as a component of the transcriptional fingerprint of both lymph node and brain metastases at the transcriptome-level and in a blinded fashion alludes to a common clonal origin for metastases that reside in the lymph nodes and successfully colonize the brain in human metastatic breast cancer.

Keywords: breast cancer, metastasis, brain metastases, central nervous system metastases, *DST*, dystonin, systems biology of breast cancer, targeted therapeutics in breast cancer.

One report described a 34% incidence of central nervous system metastases in trastuzumab-treated patients with breast cancer². This alarmingly high frequency of CNS metastasis 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 significant differential and decreased expression of the dystonin, encoded by *DST*, in brain metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE52604⁴ and GSE124648⁵ and for this global differential gene expression analysis of brain metastatic breast cancer in conjunction with GEO2R. GSE52604 was generated using Whole Human Genome Microarray 4x44K G4112F technology with $n=10$ normal breast tissues and $n=35$ brain metastases from patients with breast cancer, analysis performed using platform GPL6480. GSE124648, a microarray dataset, was generated using Affymetrix Human Genome U133A Array technology with $n=10$ normal breast tissues and $n=44$ lymph node metastases from patients with breast cancer; platform GPL96 was used for this 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 *DST* gene expression was significantly between normal breast tissues 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).

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 and lymph node metastasis in human breast cancer in an unbiased fashion and for the discovery of novel therapeutic targets.

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

Identification of differentially expressed genes across datasets in cancer can be difficult and more challenging than when doing so in benign organ tissue and sorted cell populations due to higher levels of heterogeneity in tumors, and differing methods of tumor sampling between laboratories. Thus, we asked how gene expression was most significantly different between brain metastases from patients with breast cancer and normal breast tissue as opposed to primary tumors of the breast, by comparing global gene expression profiles of 10 normal breast tissues to 35 brain metastases⁴. Using this strategy, we identified dystonin, *DST*, as among the genes whose expression was most significantly different in the brain metastases of patients with brain metastatic breast cancer⁴ (Table 1). When sorting each of the genes expressed in brain metastases based on significance of difference in expression between brain metastases and normal breast tissues, *DST* ranked 27 out of 41093 total transcripts (Table 1), equating to 99.9% differential expression. Differential expression of *DST* in brain metastases from patients with brain metastatic breast cancer in this dataset was statistically significant (Table 1; $p=9.88E-17$).

DST is differentially expressed in the lymph node metastases of patients with metastatic breast cancer.

Next, we queried a second microarray dataset⁵ generated using lymph node metastases and normal breast tissues from patients with breast cancer to determine whether we could validate differential expression of DST in metastatic tissues of humans with breast cancer, and whether differential expression of DST was specific to brain metastases or could be observed in metastases to other sites in patients with metastatic breast cancer. When sorting each of the genes expressed in lymph node metastases based on significance of difference as compared to normal breast tissues in patients with breast cancer, DST ranked 1 out of 22283 total transcripts (Table 2), equating to 99.996% differential expression. Differential expression of DST in the lymph node metastases of patients with metastatic breast cancer was statistically significant (Table 2; $p=3.32E-33$).

DST is expressed at significantly lower levels in the brain metastases of patients with brain metastatic breast cancer.

We obtained exact messenger RNA (mRNA) expression levels for DST, in normal breast tissues and in brain metastasis of patients with brain metastatic breast cancer to determine direction and statistical significance of change in DST expression in brain metastatic tissues. DST mRNA was present at lower quantities in the brain metastases of patients with breast cancer as compared to normal breast tissues, and this difference was statistically significant (Figure 1; $p<0.0001$).

Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified DST as among the genes whose expression was most different in the brain metastases of patients with breast cancer both when compared to the normal breast; DST expression, as compared to the benign breast, was significantly lower levels in brain metastases.

Discussion

We provided evidence here that dystonin, encoded by DST, is among the genes whose expression is most different in the brain metastases of patients with brain metastatic brain cancer, and that DST is expressed at significantly lower levels in brain metastases as compared normal breast tissues. Dystonin has architectural functions in the cytoskeleton of neuronal cells, linked filamentous actin (F-actin) with intermediate filaments, and in dendrites, the cell bodies of neurons, and in axons; the dorsal root ganglion of DST mutant mice, dystonin musculorum, display disorganization of the cytoskeleton in the neurites⁶. Thus, structural changes in neuronal the cytoskeleton resulting from dystonin down-regulation may be an important event during progression of primary tumors of the breast to metastasis in the lymph nodes and brain⁶. Moreover, shared transcriptional perturbation of dystonin in both the brain and lymph node metastases of patients with metastatic breast cancer suggests a common origin for circulating tumor cells that reside in the lymph nodes, successfully evade immune clearance in the lymphatics, and colonize the central nervous system.

References

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Rank	ID	p-value	t	B	Gene	Gene name
27	A_23_P59388	9.88E-17	-12.6497938	27.710505	DST	dystonin

Table 1: DST is differentially expressed in brain metastases in patients with metastatic breast cancer when compared to normal breast tissues.

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.

Rank	ID	p-value	t	B	FC	Gene	Gene name
1	216918_s_at	3.32E-33	26.528165	63.975107	0.55 ± 0.5	DST	dystonin

Table 2: DST is differentially expressed in lymph node metastases of patients with metastatic breast cancer when compared to normal breast tissue.

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: DST is expressed at significantly lower levels in the brain metastases of patients with brain metastatic breast cancer as compared to normal breast tissues.

The mRNA expression level of DST in normal breast tissues (left) and in the brain metastases of brain metastatic breast cancer patients (right) is graphically represented here with mean DST levels marked and the result of a statistical test evaluating difference in mRNA expression level between brain metastases and normal tissues of the breast, a *p*-value, listed above.

Figure 2: DST is expressed at significantly lower levels in the lymph node metastases of patients with brain metastatic breast cancer as compared to normal breast tissues.

The mRNA expression level of DST in normal breast tissues (left) and in the lymph node metastases of metastatic breast cancer patients (right) is graphically represented here with mean DST levels marked and the result of a statistical test evaluating difference in mRNA expression level between lymph node metastases and normal tissues of the breast, a *p*-value, listed above.