TSPY is differentially expressed in lymph node metastases in human breast cancer.

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Metastasis to the brain is a clinical problem in patients with breast cancer¹⁻³. Between the breast and the brain reside the secondary lymphoid organ, the lymph nodes. We mined published microarray data^{4,5} to compare primary and metastatic tumor transcriptomes for the discovery of genes associated with metastasis to the lymph nodes in humans with metastatic breast cancer. We found that the testis-specific protein on Y chromosome, TSPY, was among the genes whose expression was most different in the lymph node metastases of patients with metastatic breast cancer as compared to primary tumors of the breast. Analysis of a separate microarray dataset revealed that TSPY was also differentially expressed in brain metastatic tissues. TSPY mRNA was present at increased quantities in lymph node metastases as compared to primary tumors of the breast. Modulation of TSPY expression may be relevant to the biology by which tumor cells metastasize from the breast to the lymph nodes and the brain in humans with metastatic breast cancer.

Keywords: breast cancer, metastasis, brain metastasis, central nervous system metastasis, lymph node metastasis, testis-specific protein on Y chromosome TSPY, 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 trastuzumab¹⁰. The alarmingly high rate of central nervous system metastasis described, as well as data, both anecdotal² and from a randomized, controlled setting¹⁰ illustrating that treatment with trastuzumab may be associated with these events demands an enhanced understanding of the transcriptional makeup of brain and lymph node metastatic tissues to support identification of therapeutic targets, whether they are treatment related or not. The lymph nodes reside between the breast and the brain. We performed a global comparative analysis of lymph node metastatic tumor tissues in patients with metastatic breast cancer, compared to primary tumors and normal breast tissues^{4,5}. We discovered transcriptome-wide differential expression of the gene encoding the testis-specific protein on Y chromosome, TSPY, in lymph node metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE10893⁴ and GSE100534⁵ 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 $n=71$ primary breast tumors and $n=7$ lymph node metastases from patients with breast cancer; analysis was performed using platform GPL887. GSE100534 was generated using Affymetrix Human Gene 1.0 ST Array with $n=16$ primary breast tumors and $n=3$ brain metastases from patients metastatic breast cancer; analysis was performed using platform GPL6244. 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 TSPY gene expression was significantly different between primary tumors of the breast and lymph node metastases in humans with breast cancer using a two-tailed t-test.

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

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

Through blind, systems-level analysis of published microarray data⁴, we identified the testis-specific protein on Y chromosome, encoded by TSPY, as a differentially expressed gene in the lymph node metastatic tissues of humans with breast cancer (Table 1). When sorting each of the genes expressed in lymph node metastases based on significance of difference as compared to primary tumors of the breast in patients with breast cancer, TSPY ranked 69 out of 18034 total transcripts (Chart 1), equating to 99.6% differential expression. Differential expression of TSPY in the lymph node metastases of patients with metastatic breast cancer was statistically significant (Chart 1; $p=4.40e-04$).

To attempt to validate transcriptome-wide differential expression of TSPY in metastatic tissues of patients with metastatic breast cancer, we queried a second microarray dataset⁵, here comparing primary tumors from patients with breast cancer to brain metastases. Again, we identified TSPY as a differentially expressed gene in human brain metastatic breast cancer, here by a probe detecting TSPY10///TSPY4///TSPY3///TSPY2///TSPY1 (Chart 2) When sorting each of the genes expressed in brain metastases based on significance of difference as compared to normal breast tissues, TSPY10///TSPY4///TSPY3///TSPY2///TSPY1 ranked 8418 out of 41093 total transcripts (Chart 2), equating to 79.5% differential expression. Differential expression of TSPY10///TSPY4///TSPY3///TSPY2///TSPY1 in the brain metastases of patients with metastatic breast cancer approached statistical significance (Chart 2; $p=5.77e-02$). Thus, differential expression of TSPY, transcriptome-wide, in the metastatic tissues of women with metastatic breast cancer was conserved across two independent microarray datasets. This suggests that TSPY differential expression in human metastatic breast cancer is a *bona fide* biological process.

TSPY is expressed at higher levels in the lymph node metastases of patients with metastatic breast cancer.

We obtained exact mRNA expression levels for TSPY, in primary tumors of the breast and in lymph node metastases of patients with metastatic breast cancer to determine direction and statistical significance of change in TSPY expression in lymph node metastatic tissues. We observed significantly increased expression of TSPY in the lymph node metastases of patients with breast cancer as compared to primary tumors of the breast: TSPY was expressed at -0.87 ± 0.17 arbitrary units (AU) in primary tumors of the breast, while it was expressed at -0.17 ± 1.05 AU in lymph node metastatic tissues (Figure 1). The difference in TSPY mRNA levels between primary tumors of the breast and lymph node metastases was statistically significant (Figure 1; $p=0.000474$).

Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified TSPY as among the genes whose expression was most different, transcriptome-wide, in the lymph node metastases of patients with breast cancer, when compared to primary tumors; we observed significantly increased expression of TSPY in lymph node metastases as compared to primary tumors of the breast.

Discussion

We provided evidence here that the testis-specific protein on Y chromosome, TSPY, is among the genes whose expression is most different in the lymph node metastases of patients with metastatic breast cancer as compared to primary tumors of the breast, that TSPY is also differentially expressed in brain metastatic tissues in human breast cancer, that TSPY mRNA is present at significantly increased quantities in lymph node metastatic tissues as compared to primary tumors of the breast. Evaluation of the effects of genetic depletion of TSPY in mouse models of metastatic breast cancer on metastasis to the central nervous system is merited. Modulation of TSPY expression may be relevant to the processes by which breast cancer 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: 69
Probe ID: 4287
p-value: 4.40e-04
t: -3.6691594
B: -0.1143
Gene: TSPY
Gene name: testis-specific protein on Y chromosome

Chart 1: TSPY is differentially expressed in lymph node metastatic breast cancer when comparing lymph node 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: 8418
Probe ID: 8176517
p-value: 5.77e-02
t: 2.0133625
B: -4.585747
Gene: TSPY10///TSPY4///TSPY3///TSPY2///TSPY1
Gene name: testis-specific protein on Y chromosome 10/4/3/2/1

Chart 2: TSPY 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.

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

The mRNA expression level of TSPY in primary tumors of the breast (left) and in lymph node metastases of women with metastatic breast cancer (right) is graphically depicted; the result of a statistical test evaluating significance of difference in TSPY expression between primary tumors of the breast and lymph node metastases, a p -value, is $p=0.000474$.