VPS37B 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 vacuolar protein sorting 37 homolog B, encoded by VPS37B, 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. VPS37B mRNA was present at increased quantities in brain metastatic tissues as compared to primary tumors of the breast. Importantly, expression of VPS37B in primary tumors was significantly correlated with patient overall survival. Modulation of VPS37B 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, vacuolar protein sorting 37 homolog B , VPS37B, 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 vacuolar protein sorting 37 homolog B, VPS37B, in brain metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE10893⁴ and GSE42568⁵ 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. GSE42568 was generated using Affymetrix Human Genome U133 Plus 2.0 array technology with $n=17$ normal breast tissue biopsies and $n=104$ primary breast tumor biopsies from patients with breast cancer; analysis was performed using platform GPL570. 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 VPS37B 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 tool⁷ for correlation of VPS37B mRNA expression levels with overall survival in $n=1402$ breast cancer patients.

Results

We performed global comparative transcriptome analysis of metastatic and primary 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.

VPS37B 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 vacuolar protein sorting 37 homolog B, encoded by VPS37B, 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, VPS37B ranked 168 out of 17418 total transcripts (Chart 1), equating to 99.0% differential expression. Differential expression of VPS37B in the brain metastases of patients with metastatic breast cancer was statistically significant (Chart 1; $p=4.14\text{E-}04$).

To validate transcriptome-wide differential expression of VPS37B in human breast cancer, we queried a second microarray dataset⁵, here comparing normal breast tissues and primary tumors of the breast. Again, we identified VPS37B 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 normal breast tissues, VPS37B ranked 3663 out of 54675 total transcripts (Chart 2), equating to 93.3% differential expression. Differential expression of VPS37B in the primary tumors of patients with breast cancer approached the level of statistical significance (Chart 2; $p=8.56\text{E-}09$). Thus, differential expression of VPS37B, transcriptome-wide, in the tumor tissues of women with breast cancer was conserved across two independent microarray datasets, both in primary and metastatic tumor tissues.

VPS37B is expressed at higher levels in the brain metastases of patients with metastatic breast cancer.

We obtained exact mRNA expression levels for VPS37B, 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 VPS37B expression in brain metastatic tissues. VPS37B 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; $p=0.000492$).

VPS37B expression is significantly correlated with survival outcomes in human breast cancer.

We performed Kaplan-Meier survival analysis⁷ in 1402 breast cancer patients in total, to evaluate whether VPS37B tumor expression was correlated with survival outcomes in breast cancer. We observed a statistically significant correlation between primary tumor expression of VPS37B and overall survival (OS) in patients with breast cancer (Figure 2). Patients whose primary tumors expressed low levels of VPS37B possessed median OS of 118.03 months, while patients whose tumors expressed high levels of VPS37B possessed median OS of 81.87 months. This difference in OS based on VPS37B tumor expression in patients with breast cancer was statistically significant (Figure 2, Chart 3; logrank p -value: 0.43; hazard ratio: 1.25 (1.01-1.54)).

Thus, by mining published microarray data^{4,5} in an unbiased fashion, we identified vacuolar protein sorting 37 homolog B encoded by VPS37B, as among the genes whose expression was most different, transcriptome-wide, in the brain metastases and primary tumors of patients with breast cancer; we observed significantly increased expression of VPS37B in brain metastases as compared to primary tumors of the breast. Further, we found a significant correlation between VPS37B expression and patient survival outcomes, as overall survival was significantly greater in patients whose primary tumors expressed lower levels of VPS37B as compared to patients whose primary tumors expressed higher levels of VPS37B.

Discussion

We provided evidence here that vacuolar protein sorting 37 homolog B , encoded by VPS37B, is among the genes whose expression is most different in the brain metastases of patients with brain metastatic breast cancer, that VPS37B mRNA is present at significantly increased quantities in brain metastatic tissues as compared to primary tumors of the breast, that VPS37B is also differentially expressed in primary tumors of patients with breast cancer and that primary tumor VPS37B expression is significantly correlated with patient survival outcomes in human breast cancer. Evaluation of the effects of genetic depletion of VPS37B in mouse models of metastatic breast cancer on metastasis to the central nervous system is merited. Modulation of VPS37B 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: 168
Probe ID: 11720
p-value: 4.14E-04
t: -4.45
B: 0.221078
Gene: VPS37B
Gene name: vacuolar protein sorting 37 homolog B

Chart 1: VPS37B 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: 3663
probe ID: 221704_s_at
p-value: 8.56E-09
t: -6.19
B: 9.4732571
Gene: VPS37B
Gene name: vacuolar protein sorting 37 homolog B

Chart 2: VPS37B is differentially expressed in human breast cancer when comparing primary tumors of the breast to normal breast tissues.

The rank of global differential expression, 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: VPS37B 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 VPS37B in primary tumors of the breast (left) and in brain metastases of women with metastatic breast cancer (right) is graphically depicted; the result of a statistical test evaluating significance of difference in VPS37B expression between primary tumors of the breast and brain metastases is $p=0.000492$.

Figure 2: Significant correlation between VPS37B primary tumor expression and overall survival in patients with breast cancer.

Depicted in this Kaplan-Meier plot is the probability of overall survival for $n=1402$ total patients stratified into two groups, based on low or high expression of VPS37B in patient primary tumors. The log rank p -value denoting statistical significance of difference in overall 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 VPS37B 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 VPS37B expression: 118.03 months

High VPS37B expression: 81.87 months

Chart 3: Median overall survival is inferior in patients with high primary tumor expression of VPS37B.

The median OS (overall survival) of patients with low primary tumor expression of VPS37B and high primary tumor expression of VPS37B is listed in this chart, for $n=1402$ breast cancer patients.