PRSS35 is differentially expressed in lymph node metastasis 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 serine protease 35, encoded by PRSS35, 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. PRSS35 mRNA was present at increased quantities in lymph node metastases as compared to primary tumors of the breast. Importantly, expression of PRSS35 in primary tumors of the breast was correlated with patient overall survival, in lymph node positive patients but not in lymph node negative patients. Modulation of PRSS35 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, serine protease 35 , PRSS35, 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 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 serine protease 35, PRSS35, in lymph node 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 $n=71$ primary breast tumors and $n=7$ lymph node metastases from patients with breast cancer; analysis was performed using platform GPL887. 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 PRSS35 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. For Kaplan-Meier survival analysis, we used the Kaplan-Meier plotter online tool⁷ for correlation of PRSS35 mRNA expression levels with overall survival in $n=2032$ breast cancer patients, in $n=230$ lymph node positive breast cancer patients and in $n=180$ lymph node negative 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 metastasis in human breast cancer in an unbiased fashion and for the discovery of novel therapeutic targets.

PRSS35 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 serine protease 35, encoded by PRSS35, 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, PRSS35 ranked 198 out of 18034 total transcripts (Chart 1), equating to 98.9% differential expression. Differential expression of PRSS35 in the lymph node metastases of patients with metastatic breast cancer was statistically significant (Chart 1; $p=2.34e-03$).

To validate transcriptome-wide differential expression of PRSS35 in human breast cancer, we queried a second microarray dataset⁵, here comparing normal breast tissues and primary tumors of the breast. Here, we again identified PRSS35 as a differentially expressed 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, PRSS35 ranked 26850 out of 54675 total transcripts (Chart 2), equating to 50.9% differential expression. Differential expression of PRSS35 in the primary tumors of patients with breast cancer was not statistically significant (Chart 2; $p=1.42e-01$). Thus, transcriptome-wide differential expression of PRSS35 in the tumor tissues of women with breast cancer was conserved across two independent microarray datasets, both in primary and metastatic tumor tissues.

PRSS35 is expressed at higher levels in the lymph node metastases of patients with metastatic breast cancer.

We obtained exact mRNA expression levels for PRSS35, 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 PRSS35 expression in lymph node metastatic tissues. We observed significantly increased expression of PRSS35 in the lymph node metastases of patients with breast cancer as compared to primary tumors of the breast: PRSS35 was expressed at 0.04 ± 0.30 arbitrary units (AU) in primary tumors of the breast, while it was expressed at 0.42 ± 0.30 AU in lymph node metastatic tissues (Figure 1). The difference in PRSS35 mRNA levels between primary tumors of the breast and lymph node metastases was statistically significant (Figure 1; $p=0.001979$).

PRSS35 expression is significantly correlated with survival outcomes in human breast cancer.

We performed Kaplan-Meier survival analysis⁷ in $n=230$ lymph node positive breast cancer patients and $n=180$ lymph node negative breast cancer patients, to evaluate whether PRSS35 tumor expression was correlated with survival outcomes in breast cancer and to evaluate whether lymph node status influenced correlation of PRSS35 tumor expression with survival outcomes in human breast cancer. We observed a correlation between primary tumor expression of PRSS35 and overall survival (OS) in lymph node positive breast cancer (Figure 2). Lymph node positive patients whose primary tumors expressed low levels of PRSS35 possessed median OS of 95.04 months as compared to a median OS of 45.24 months in lymph node positive patients whose tumors expressed high levels of PRSS35. This difference in OS based on PRSS35 tumor expression in lymph node positive patients with breast cancer was statistically significant (Figure 2, Chart 3; logrank p -value: 0.018 (hazard ratio: 1.82 (1.1-2.99))). PRSS35 primary tumor expression was not significantly correlated with overall survival outcomes in lymph node negative patients (Figure 2, Chart 3; logrank p -value: 0.74; hazard ratio: 1.14 (0.52-2.52)).

Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified PRSS35 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 of the breast, we observed significantly increased expression of PRSS35 in lymph node metastases as compared to primary tumors of the breast. Further, we found a significant correlation between PRSS35 expression and patient survival outcomes, as primary tumor expression of PRSS35 was correlated with overall survival in lymph node positive patients but not in lymph node negative patients.

Discussion

We provided evidence here that serine protease 35, PRSS35, is among the genes whose expression

1 is most different in the lymph node metastases of patients with metastatic breast cancer as compared to
2 primary tumors of the breast, that PRSS35 mRNA is present at significantly increased quantities in lymph
3 node metastatic tissues as compared to primary tumors of the breast, and that primary tumor PRSS35
4 expression is significantly correlated with patient survival outcomes in human breast cancer, in lymph
5 node positive patients but not in lymph node negative patients. Evaluation of the effects of genetic
6 depletion of PRSS35 in mouse models of metastatic breast cancer on metastasis to the central nervous
7 system is merited. Modulation of PRSS35 expression may be relevant to the processes by which breast
8 cancer cells exit the breast, enter the vasculature and/or lymphatics, reside in the lymph nodes, evade
9 immune clearance, breach the blood-brain barrier and colonize the brain.
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Rank: 198
Probe ID: 11810
p-value: 2.34e-03
t: -3.1447907
B: -1.5289
Gene: PRSS35
Gene name: serine protease 35

Chart 1: PRSS35 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: 26850
Probe ID: 235874_at
p-value: 1.42e-01
t: 1.48
B: -6.1133718
Gene: PRSS35
Gene name: serine protease 35

Chart 2: PRSS35 is differentially expressed in human breast cancer when comparing primary tumors of the breast to normal breast tissues.

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: PRSS35 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 PRSS35 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 PRSS35 evaluating significance of difference in PRSS35 expression between primary tumors of the breast and lymph node metastases, a *p*-value, is $p=0.001979$.

Figure 2: Significant correlation between PRSS35 primary tumor expression and overall survival in lymph node positive patients but not in lymph node negative patients.

Depicted in these Kaplan-Meier plots is the probability of overall survival in $n=230$ lymph node positive patients (**left**) and $n=180$ lymph node negative patients (**right**), stratified into two groups, based on low or high expression of PRSS35 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 PRSS35 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.

Lymph node positive patients

Low PRSS35 expression: 95.04 months

High PRSS35 expression: 45.24 months

Chart 3: In lymph node positive patients, median overall survival is inferior in patients with high primary tumor expression of PRSS35.

The median OS (overall survival) of patients with low primary tumor expression of PRSS35 and high primary tumor expression of PRSS35 is listed in this chart, for $n=230$ lymph node positive breast cancer patients.