ATP7B is a differentially expressed gene in brain metastatic human breast cancer.

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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 ATPase copper transporting beta, also known as Wilson disease protein, encoded by ATP7B, 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. ATP7B mRNA was present at increased quantities in brain metastatic tissues as compared to primary tumors of the breast. Expression of ATP7B in primary tumors was significantly correlated with patient overall survival. Modulation of ATP7B 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, ATPase copper transporting beta, ATP7B, 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 the ATPase copper transporting beta, ATP7B, in brain metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE10893⁴ and GSE125989⁵ 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. GSE125989 was generated using Affymetrix Human Genome U133A 2.0 array technology with $n=16$ primary breast tumors and $n=16$ brain metastases from patients with breast cancer; the samples utilized by investigators for this microarray dataset were paired, matched tumor and metastasis samples; analysis was performed using platform GPL571. 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 ATP7B 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 tool for correlation of ATP7B mRNA expression levels with overall survival in $n=1402$ 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 brain metastasis in human breast cancer in an unbiased fashion and for the discovery of novel therapeutic targets.

ATP7B 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 gene

1 encoding the ATPase copper transporting beta, encoded by ATP7B, as a differentially
2 expressed gene in the breast metastatic tissues of humans with breast cancer (Table 1). When
3 sorting each of the genes expressed in brain metastases based on significance of difference as
4 compared to primary tumors of the breast in patients with breast cancer, ATP7B ranked 39 out of
5 17418 total transcripts (Chart 1), equating to 99.8% differential expression. Differential
expression of ATP7B in the brain metastases of patients with metastatic breast cancer was
statistically significant (Chart 1; $p=5.69E-05$).

6 We queried a second microarray dataset⁵ to attempt to validate transcriptome-wide
7 differential expression of ATP7B in human brain metastatic breast cancer. Again, we identified
8 ATP7B as a differentially expressed gene in the brain metastatic tissues of patients with breast
9 cancer (Chart 2). When sorting each of the genes expressed in brain metastases based on
10 significance of difference as compared to primary tumors of the breast in patients with breast
11 cancer, in this dataset, ATP7B ranked 7167 out of 22277 total transcripts (Chart 2), equating to
12 67.8% differential expression. Differential expression of ATP7B in the brain metastases of
patients with metastatic breast cancer in this dataset was not deemed statistically significant
(Chart 2; $p=2.78E-01$). Thus, differential expression of ATP7B, transcriptome-wide, in the brain
metastases of women with metastatic breast cancer was conserved across two independent
microarray datasets.

13 **ATP7B is expressed at higher levels in the brain metastases of patients with metastatic** 14 **breast cancer.**

15 We obtained exact mRNA expression levels for ATP7B, in primary tumors of the breast
16 and in brain metastasis of patients with brain metastatic breast cancer to determine direction and
17 statistical significance of change in ATP7B expression in brain metastatic tissues. ATP7B was
18 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;
 $p=0.000126$).

19 **ATP7B expression is significantly correlated with survival outcomes in human breast** 20 **cancer.**

21 We performed Kaplan-Meier survival analysis⁷ to evaluate correlation in 1402 breast
22 cancer patients in total, to evaluate whether ATP7B tumor expression was correlated with
23 survival outcomes in breast cancer. We observed a statistically significant correlation between
24 primary tumor expression of ATP7B and overall survival (OS) (Figure 2). Patients whose
25 primary tumors expressed low levels of ATP7B possessed median OS of 79.2 months, while
patients whose tumors expressed high levels of ATP7B possessed median OS of 130 months.
This difference in OS based on ATP7B tumor expression in breast cancer was statistically
significant (Figure 2, Chart 3; logrank p -value: 0.01; hazard ratio: 0.75 (0.61-0.94)).

26 Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we
27 identified the ATPase copper transporting beta, ATP7B, as among the genes whose expression
28 was most different, transcriptome-wide, in the brain metastases of patients with breast cancer

when compared to primary tumors of the breast; we observed significantly increased expression of ATP7B in brain metastases as compared to primary tumors of the breast. Further, we found a significant correlation between ATP7B expression and patient survival outcomes, as overall survival was significantly higher in patients whose primary tumors expressed higher levels of ATP7B as compared to patients whose primary tumors expressed lower levels of ATP7B.

Discussion

We provided evidence here that the ATPase copper transporting beta, also known as Wilson disease protein, encoded by ATP7B, is among the genes whose expression is most different in the brain metastases of patients with brain metastatic breast cancer, and that primary tumor ATP7B expression is significantly correlated with patient survival outcomes in human breast cancer. Evaluation of the effects of genetic depletion of ATP7B in mouse models of metastatic breast cancer on metastasis to the central nervous system is merited. ATP7B is found in the trans-golgi network, uses ATP to regulate copper localization and thus copper concentration in the cell, and is expressed in the brain⁸. The phenotypic consequence of modulating ATP7B on copper concentration in the cytosol and ensuing result of changes in copper concentration in circulating tumor cells may be relevant as a target of study in humans with metastatic breast cancer.

References

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Rank: 39
Probe ID: 6289
p-value: 5.69E-05
t: -5.44
B: 2.039841
Gene: ATP7B
Gene name: ATPase copper transporting beta

Chart 1: ATP7B 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: 7167
Probe ID: 204624_at
p-value: 2.78E-01
t: -1.10096
B: -5.4249
Gene: ATP7B
Gene name: ATPase copper transporting beta

Chart 2: ATP7B 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 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: ATP7B 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 ATP7B in primary tumors of the breast (left) and in brain metastases from women diagnosed with breast cancer (right) is graphically depicted; the result of a statistical test evaluating significance of difference in ATP7B expression between primary tumors and brain metastases is $p=0.000126$.

Figure 2: Significant correlation between ATP7B 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 ATP7B 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 ATP7B 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 ATP7B expression: 79.2 months

High ATP7B expression: 130 months

Chart 3: Median overall survival is inferior in patients with low primary tumor expression of ATP7B.

The median OS (overall survival) of patients with low primary tumor expression of ATP7B and high primary tumor expression of ATP7B is listed in this chart.