CD247 is differentially expressed in patients with metastatic breast cancer, in the lymph nodes and in the brain.

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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 metastasis to the lymph nodes and the brain in humans with metastatic breast cancer. We found that the CD3 zeta chain, also known as CD247, 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. CD247 mRNA was present at increased quantities in lymph node metastases as compared to primary tumors of the breast. CD247 was also among the genes most differentially expressed in the brain metastases of patients with metastatic breast cancer. Importantly, expression of CD247 in primary tumors of the breast was significantly correlated with patient overall survival. Modulation of CD247 expression may be relevant to the biology by which tumor cells metastasize from the breast to the brain in humans with metastatic breast cancer. CD247 may function to support tissue infiltration in brain metastatic breast cancer in humans.

Keywords: breast cancer, metastasis, brain metastases, central nervous system metastases, CD3 zeta chain, CD247, 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 lymph node and brain metastatic tumor tissues in patients with metastatic breast cancer, comparing to primary tumors and normal breast tissues, respectively^{4,5}. We discovered transcriptome-wide differential expression of the gene encoding the CD3 zeta chain, CD247, in lymph node and brain metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE10893⁴ and GSE124648⁵ 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; with $n=11$ primary breast tumors and $n=4$ brain metastases from patients with breast cancer; analysis was performed using platform GPL885. GSE124648 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; analysis was performed using platform GPL96. 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 CD247 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 CD247 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 metastasis in human breast cancer in an unbiased fashion and for the discovery of novel therapeutic targets.

CD247 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

CD3 zeta chain, also known as cluster of differentiation 247 (CD247), 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, CD247 ranked 150 out of 18034 total transcripts (Chart 1), equating to 99.2% differential expression. Differential expression of CD247 in the lymph node metastases of patients with metastatic breast cancer was statistically significant (Chart 1; $p=1.56E-03$).

We queried a second microarray dataset to attempt to validate transcriptome-wide differential expression of CD247 in the lymph node metastases of patients with metastatic breast cancer. We again observed CD247 to be among the genes expressed most quantitatively differently in the lymph node metastases of patients with metastatic breast cancer as compared to primary tumors of the breast. When sorting each of the genes expressed in lymph node metastases based on significance of difference as compared to normal breast tissues, CD247 ranked 2823 out of 22283 total transcripts (Chart 2), equating to 87.3% differential expression. Differential expression of CD247 in the lymph node metastases of patients with metastatic breast cancer approached the level of statistical significance (Chart 2; $p=4.35E-04$).

CD247 is differentially expressed in the brain metastases of patients with metastatic breast cancer.

To ascertain whether transcriptome-wide differential expression of CD247 in metastatic breast cancer was specific to the lymph nodes, we again queried microarray data⁴, here comparing primary tumors of the breast to brain metastases. Again, we identified CD247 as a differentially expressed gene in the metastatic tissues of patients with breast cancer (Chart 3). When sorting each of the genes expressed in brain metastases based on significance of difference as compared to primary tumors of the breast, CD247 ranked 2022 out of 17418 total transcripts (Chart 3), equating to 88.4% differential expression. Differential expression of CD247 in the brain metastases of patients with metastatic breast cancer was statistically significant (Chart 3; $p=2.04E-02$). Thus, differential expression of CD247, transcriptome-wide, in the metastatic tissues of women with breast cancer was conserved across two independent microarray datasets, in the lymph nodes and in the brain.

CD247 is expressed at higher levels in the lymph node metastases of patients with metastatic breast cancer.

We obtained exact mRNA expression levels for CD247, 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 CD247 expression in lymph node metastatic tissues. CD247 was expressed at higher levels in the lymph node metastases of patients with breast cancer as compared to primary tumors of the breast, and this difference was statistically significant (Figure 1; $p=0.001876$).

CD247 expression is significantly correlated with survival outcomes in human breast cancer.

We performed Kaplan-Meier survival analysis⁸ in $n=1402$ breast cancer patients in total, to evaluate whether CD247 tumor expression was correlated with survival outcomes in breast

cancer. We observed a statistically significant correlation between primary tumor expression of CD247 and overall survival (OS) in patients with breast cancer (Figure 2). Patients whose primary tumors expressed low levels of CD247 possessed median OS of 78 months, while patients whose tumors expressed high levels of CD247 possessed median OS of 138 months. This difference in OS based on CD247 tumor expression in patients with breast cancer was statistically significant (Figure 2, Chart 3; logrank p -value: 0.0044; hazard ratio: 0.73 (0.59-0.91)).

Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified the CD3 zeta chain, encoded by CD247, as among the genes whose expression was most different, transcriptome-wide, in the lymph node and brain metastases of patients with breast cancer, when compared to primary tumors of the breast and to normal breast tissues, respectively; we observed significantly increased expression of CD247 in lymph node metastases as compared to primary tumors of the breast. Further, we found a significant correlation between CD247 expression and patient survival outcomes, as overall survival was significantly greater in patients whose primary tumors expressed higher levels of CD247 as compared to patients whose primary tumors expressed lower levels of CD247.

Discussion

We provided evidence here that the zeta chain of CD3, also known as CD247, is among the genes whose expression is most different in the lymph node and brain metastases of patients with metastatic breast cancer as compared to primary tumors of the breast, that CD247 mRNA is present at significantly increased quantities in lymph node metastatic tissues as compared to primary tumors of the breast, and that primary tumor CD247 expression is significantly correlated with patient survival outcomes in human breast cancer. Evaluation of the effects of genetic depletion of CD247 in mouse models of metastatic breast cancer on metastasis to the central nervous system is merited. While we found that higher primary tumor expression of CD247 was correlated with enhanced patient survival, patient survival outcomes based on primary tumor expression do not necessarily correlate with phenotypic consequence of inhibition. Moreover, the survival data we presented here is based on expression of CD247 in primary tumors, not the lymph node metastatic tissues, in which we found significantly increased expression of CD247. One study has described the importance of CD247 in facilitating T-cell infiltration into the kidneys⁸. We propose that in human metastatic breast cancer, up-regulation of CD247 by circulating tumor cells that reside in the lymph nodes functions similarly to support infiltration, but of the brain. Modulation of CD247 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. Inhibition of CD247 represents a potential therapeutic approach to interfering with brain infiltration of metastatic tumor cells in patients with lymph node metastatic (“regional”) breast cancer.

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Rank: 150
Probe ID: 21020
p-value: 1.56E-03
t: -3.2762599
B: -1.1891
Gene: CD247
Gene name: CD3 zeta chain

Chart 1: CD247 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: 2823
Probe ID: 210031_at
p-value: 4.35E-04
t: -3.74206
B: -0.674687
Gene: CD247
Gene name: CD3 zeta chain

Chart 2: CD247 is differentially expressed in lymph node metastatic breast cancer when comparing lymph node metastases 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.

Rank: 2022
probe ID: 21020
p-value: 2.04E-02
t: 2.58
B: -3.350036
Gene: CD247
Gene name: CD3 zeta chain

Chart 3: CD247 is differentially expressed in brain metastatic breast cancer when comparing brain metastases to primary tumors of the breast.

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.

CD247

Figure 1: CD247 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 CD247 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 CD247 expression between primary tumors of the breast and lymph node metastases, a p -value, is $p=0.001876$.

Figure 2: Significant correlation between CD247 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 CD247 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 CD247 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 CD247 expression: 78 months
High CD247 expression: 138 months

Chart 3: Median overall survival is inferior in patients with low primary tumor expression of CD247.

The median OS (overall survival) of patients with low primary tumor expression of CD247 and high primary tumor expression of CD247 is listed in this chart, for $n=1402$ breast cancer patients.