DENND1C is differentially expressed in the lymph nodes of patients with metastatic 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 DENN domain-containing protein 1C, DENND1C, 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. DENND1C mRNA was present at increased quantities in lymph node metastases as compared to primary tumors of the breast. Importantly, expression of DENND1C in primary tumors of the breast was correlated with patient overall survival, in lymph node negative patients but not in lymph node positive patients. Modulation of DENND1C 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, the DENN domain-containing protein 1C , DENND1C, 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 the DENN domain-containing protein 1C, DENND1C, in lymph node 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. 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 DENND1C 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 DENND1C mRNA expression levels with overall survival in $n=1879$ 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.

DENND1C 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 DENN domain-containing protein 1C, encoded by DENND1C, 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, DENND1C ranked 68 out of 18034 total transcripts (Chart 1), equating to 99.6% differential expression. Differential expression of DENND1C in the lymph node metastases of patients with metastatic breast cancer was statistically significant (Chart 1; $p=4.29e-04$).

We queried a second microarray dataset⁵ to attempt to validate transcriptome-wide differential expression of DENND1C in the lymph node metastases of patients with metastatic breast cancer. We again observed DENND1C 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, DENND1C ranked 3784 out of 22283 total transcripts (Chart 2), equating to 83.0% differential expression. Differential expression of DENND1C in the lymph node metastases of patients with metastatic breast cancer approached the level of statistical significance (Chart 2; $p=1.83\text{e-}03$).

DENND1C is expressed at higher levels in the lymph node metastases of patients with metastatic breast cancer.

We obtained exact mRNA expression levels for DENND1C, 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 DENND1C expression in lymph node metastatic tissues. We observed significantly increased expression of DENND1C in the lymph node metastases of patients with breast cancer as compared to primary tumors of the breast: DENND1C was expressed at 0.64 ± 0.40 arbitrary units (AU) in primary tumors of the breast, while it was expressed at 1.63 ± 0.93 AU in lymph node metastatic tissues (Figure 1). The difference in DENND1C mRNA levels between primary tumors of the breast and lymph node metastases was statistically significant (Figure 1; $p=0.000506$).

DENND1C expression is significantly correlated with survival outcomes in human breast cancer.

We performed Kaplan-Meier survival analysis⁷ in $n=1879$ breast cancer patients in total, to evaluate whether DENND1C tumor expression was correlated with survival outcomes in breast cancer. We observed a statistically significant correlation between primary tumor expression of DENND1C and overall survival (OS) in patients with breast cancer, in the upper survival quartile (Figure 2). Patients whose primary tumors expressed low levels of DENND1C possessed median OS of 79.2 months, while patients whose tumors expressed high levels of DENND1C possessed median OS of 197.62. This difference in OS based on DENND1C tumor expression in patients with breast cancer was statistically significant (Figure 2, Chart 3; logrank p -value: 0.00019; hazard ratio: 0.63 (0.49-0.8)).

We also performed Kaplan-Meier survival analysis⁷ in $n=452$ lymph node positive breast cancer patients and $n=726$ lymph node negative breast cancer patients, to evaluate whether lymph node status influenced correlation of DENND1C tumor expression with survival outcomes in human breast cancer. We observed a correlation between primary tumor expression of DENND1C and overall survival (OS) in lymph node negative breast cancer, in the upper survival quartile (Figure 2). Lymph node negative patients whose primary tumors expressed low levels of DENND1C possessed median OS of 135.88 months, while lymph node negative patients whose tumors expressed high levels of DENND1C possessed median OS of 206.64 months. This difference in OS based on DENND1C tumor expression in lymph node negative patients with breast cancer trended towards statistical significance (Figure 2, Chart 3; logrank p -value: 0.068 (hazard ratio: 0.66 (0.43-1.03))). Thus, overall survival based on DENND1C primary tumor expression was markedly longer in lymph node negative patients than in the general breast cancer patient population. DENND1C primary tumor expression was not significantly correlated with overall survival outcomes in lymph node positive patients (Figure 2, Chart 3; logrank p -value: 0.66; hazard ratio: 0.92 (0.63-1.35)).

Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified

DENND1C 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 DENND1C in lymph node metastases as compared to primary tumors of the breast. Further, we found a significant correlation between DENND1C expression and patient survival outcomes, as overall survival was significantly greater in patients whose primary tumors expressed higher levels of DENND1C as compared to patients whose primary tumors expressed lower levels of DENND1C, and this correlation was significant in lymph node negative patients but not in lymph node positive patients.

Discussion

We provided evidence here that the DENN domain-containing protein 1C, DENND1C, 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 DENND1C mRNA is present at significantly increased quantities in lymph node metastatic tissues as compared to primary tumors of the breast, and that primary tumor DENND1C expression is significantly correlated with patient survival outcomes in human breast cancer, in all patients and in lymph node negative patients but not in lymph node positive patients. Evaluation of the effects of genetic depletion of DENND1C in mouse models of metastatic breast cancer on metastasis to the central nervous system is merited. Modulation of DENND1C 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.

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Rank: 68
Probe ID: 15796
p-value: 4.29e-04
t: -3.6770901
B: -0.0917
Gene: DENND1C
Gene name: DENN domain-containing protein 1C

Chart 1: DENND1C 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: 3784
Probe ID: 221080_s_at
p-value: 1.83e-03
t: -3.274131
B: -2.027155
Gene: DENND1C
Gene name: DENN domain-containing protein 1C

Chart 2: DENND1C 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.

Figure 1: DENND1C 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 DENND1C 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 DENND1C expression between primary tumors of the breast and lymph node metastases, a p -value, is $p=0.000506$.

Figure 2: DENND1C primary tumor expression is correlated with overall survival in all patients with breast cancer and in lymph node negative patients but not in lymph node positive patients.

Depicted in this Kaplan-Meier plot (**above**) is the probability of overall survival for $n=1879$ total patients stratified into two groups, based on low or high expression of DENND1C in patient primary tumors, in the lower survival quartile. 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 DENND1C 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. Below, Kaplan-Meier plots for probability of overall survival in $n=452$ lymph node positive patients (**left**) and $n=726$ lymph node negative patients (**right**).

All patients

Low DENND1C expression: 79.2 months

High DENND1C expression: 197.62 months

Lymph node negative patients

Low DENND1C expression: 135.88 months

High DENND1C expression: 205.64 months

Chart 3: Median overall survival is inferior in patients with low primary tumor expression of DENND1C.

The median OS (overall survival) of patients with low primary tumor expression of DENND1C and high primary tumor expression of DENND1C is listed in this chart, for $n=1879$ breast cancer patients and for $n=726$ lymph node negative patients, in the upper survival quartile.