ZNF367 is differentially expressed in metastasis to lymph nodes in human breast cancer.

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Metastasis to the brain is a clinical problem in patients with breast cancer¹⁻³. Between the brain and the breast resides 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 human breast cancer. We found that the zinc finger protein 367, encoded by ZNF367, was among the genes whose expression was most different in the lymph nodes metastases of patients with metastatic breast cancer as compared to primary tumors of the breast⁴. ZNF367 was also differentially expressed in the tumor cells of patients with triple negative breast cancer⁵. ZNF367 mRNA was present at increased quantities in lymph node metastatic tissues as compared to primary tumors of the breast. Importantly, expression of ZNF367 in primary tumors was significantly correlated with patient distant metastasis-free survival, in lymph node negative but not in lymph node positive breast cancer. Modulation of ZNF367 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 metastasis, central nervous system metastasis, lymph node metastasis, the zinc finger protein 367, ZNF367, 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 distant metastasis 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 human breast cancer^{4,5}. We discovered differential and increased expression of the gene encoding the zinc finger protein 367, ZNF367, in lymph node metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE10893⁴ and GSE38959⁵ 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. GSE38959 was generated using Agilent-014850 Whole Human Genome Microarray 4x44K G4112F with $n=13$ samples of normal mammary gland ductal cells and $n=30$ samples of primary tumor cells from patients with triple negative breast cancer; analysis was performed using platform GPL4133. 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 ZNF367 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 ZNF367 mRNA expression levels with distant metastasis-free survival in $n=4929$ 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 lymph node metastasis in human breast cancer in an unbiased fashion and for the discovery of novel therapeutic targets.

ZNF367 is differentially expressed in lymph node metastases of patients with metastatic breast cancer.

Through blind, systems-level analysis of published microarray data⁴, we identified the zinc finger protein 367, encoded by ZNF367, 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, ZNF367 ranked 120 out of 18034 total transcripts (Chart 1), equating to 99.3% differential expression. Differential expression of ZNF367 in the lymph node metastases of patients with metastatic breast cancer was statistically significant (Chart 1; $p=1.11e-03$).

To attempt to validate differential transcriptome-wide differential expression of ZNF367 in human breast cancer, we queried a second microarray dataset⁵, here comparing primary tumor cells from patients with triple negative breast cancer to normal mammary gland ductal cells. Again, we identified ZNF367 as a differentially expressed gene in the tumor tissues of patients with breast cancer (Chart 2). When sorting each of the genes expressed in triple negative breast cancer tumor cells based on significance of difference as compared to normal mammary gland ductal cells, ZNF367 ranked 207 out of 45015 total transcripts (Chart 2), equating to 99.5% differential expression. Differential expression of ZNF367 in the primary tumor cells of patients with metastatic breast cancer was statistically significant (Chart 2; $p=2.74e-11$). Thus, differential expression of ZNF367, 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.

ZNF367 is expressed at lower levels in the lymph node metastases of patients with metastatic breast cancer.

We obtained exact mRNA expression levels for ZNF367, in primary tumors of the breast and in lymph node metastasis of patients with lymph node metastatic breast cancer to determine direction and statistical significance of change in ZNF367 expression in lymph node metastatic tissues. We observed significantly lower expression of ZNF367 in the lymph node metastases of patients with breast cancer as compared to primary tumors of the breast: ZNF367 was expressed at 0.004 ± 0.22 arbitrary units (AU) in primary tumors of the breast, while it was expressed at 0.32 ± 0.28 AU in lymph node metastatic tissues. The difference in ZNF367 mRNA levels between primary tumors of the breast and lymph node metastatic tissues was statistically significant (Figure 1; $p=0.000669$).

ZNF367 expression is significantly correlated with survival outcomes in human breast cancer.

We performed Kaplan-Meier survival analysis⁸ in 240 patients with lymph node negative breast cancer and 261 patients with lymph node positive breast cancer, to evaluate whether ZNF367 tumor expression was correlated with survival outcomes in human breast cancer. We observed a statistically significant correlation between primary tumor expression of ZNF367 and distant metastasis-free survival (DMFS) in patients with lymph node negative breast cancer (Figure 2). Lymph node negative patients whose primary tumors expressed low levels of ZNF367 possessed superior median DMFS as compared to lymph node negative patients whose tumors expressed high levels of ZNF367 (Figure 2). This difference in DMFS based on ZNF367 tumor expression in patients with lymph node negative breast cancer was statistically significant (Figure 2, Chart 3; logrank p -value: 0.012; hazard ratio: 2.54 (1.2-5.38)). DMFS was not correlated with primary tumor expression of ZNF367 in patients with lymph node positive breast cancer (Figure 2, Chart 3; logrank p -value: 0.51; hazard ratio: 1.17 (0.73-1.87)).

Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified the zinc finger protein 367, encoded by ZNF367, 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 and in the primary tumor cells of patients with triple negative breast cancer when compared to normal mammary gland ductal cells; we observed significantly increased expression of ZNF367 in lymph node metastases as compared to primary tumors of the breast. Further, we found a significant correlation between ZNF367 expression and patient survival outcomes, as distant metastasis-free survival was significantly greater in patients whose primary tumors expressed low levels of ZNF367 as compared to patients whose primary tumors expressed high levels of ZNF367, in lymph node negative patients but not in lymph node positive patients.

Discussion

We provided evidence here that the zinc finger protein 367, encoded by ZNF367, is among the genes whose expression is most different in the lymph node metastases of patients with metastatic breast cancer, that ZNF367 is also differentially expressed in the primary tumor cells of patients with triple negative breast cancer, that ZNF367 mRNA is present at significantly increased quantities in lymph node metastatic tissues as compared to primary tumors of the breast, and that primary tumor ZNF367 expression is significantly correlated with patient survival outcomes in human breast cancer in lymph node negative but not lymph node positive breast cancer. Evaluation of the effects of genetic depletion of ZNF367 in mouse models of metastatic breast cancer on metastasis to the lymph node and to the central nervous system is merited. Modulation of ZNF367 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: 120
Probe ID: 16115
p-value: 1.11e-03
t: -3.3850821
B: -0.9001
Gene: ZNF367
Gene name: zinc finger protein 367

Chart 1: ZNF367 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: 207
probe ID: 29556
p-value: 2.74e-11
t: -8.7546892
B: 15.629857
Gene: ZNF367
Gene name: zinc finger protein 367

Chart 2: ZNF367 is differentially expressed in the tumor cells of patients with triple negative breast cancer when compared to normal mammary gland ductal cells.

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: ZNF367 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 ZNF367 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 ZNF367 expression between primary tumors and lymph node metastasis, a *p*-value, is *p*=0.000969.

Figure 2: Significant correlation between ZNF367 primary tumor expression and distant metastasis-free survival in patients with lymph node negative breast cancer but not in patients with lymph node positive breast cancer.

Depicted in this Kaplan-Meier plot is the probability of distant metastasis-free survival for $n=240$ patients with lymph node negative breast cancer (right) and $n=261$ patients with lymph node positive breast cancer (left) total patients stratified into two groups, based on low or high expression of ZNF367 in patient primary tumors. The log rank p -value denoting statistical significance of difference in distant metastasis-free 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 ZNF367 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.