DEFB123 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 defensin beta 123, encoded by DEFB123, 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. DEFB123 was also differentially expressed in the primary tumors of the breast when compared to normal mammary epithelium⁵. DEFB123 mRNA was present at increased quantities in lymph node metastatic tissues as compared to primary tumors of the breast. Importantly, expression of DEFB123 in primary tumors was significantly correlated with patient overall survival in patients with lymph node negative breast cancer. Modulation of DEFB123 expression may be relevant to the biology by which tumor cells metastasize from the breast to the lymph nodes in humans with metastatic breast cancer.

Keywords: breast cancer, metastasis, brain metastasis, central nervous system metastasis, lymph node metastasis, the defensin beta 123, DEFB123, 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 human breast cancer^{4,5}. We discovered differential and increased expression of the gene encoding the defensin beta 123, DEFB123, in lymph node metastatic tissues of patients with breast cancer.

Methods

We used datasets GSE10893⁴ and GSE72653⁵ 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. GSE72653 was generated using Agilent-014850 Whole Human Genome Microarray 4x44K G4112F with $n=9$ samples of normal mammary epithelium and $n=9$ primary tumors of the breast; analysis was performed using platform GPL6480. 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 DEFB123 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 DEFB123 mRNA expression levels with overall 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 brain metastasis in human breast cancer in an unbiased fashion and for the discovery of novel therapeutic targets.

DEFB123 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 defensin beta 123, encoded by DEFB123, 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, DEFB123 ranked 115 out of 18034 total transcripts (Chart 1), equating to 99.4% differential expression. Differential expression of DEFB123 in the lymph node metastases of patients with metastatic breast cancer was statistically significant (Chart 1; $p=1.00e-03$).

To attempt to validate differential transcriptome-wide differential expression of DEFB123 in human breast cancer, we queried a second microarray dataset⁵, here comparing primary tumors of the breast to normal mammary epithelium. Again, we identified DEFB123 as a differentially expressed gene in the tumor tissues of patients with breast cancer (Chart 2). When sorting each of the genes expressed in primary tumors of the breast based on significance of difference as compared to normal mammary epithelium, DEFB123 ranked 15014 out of 41093 total transcripts (Chart 2), equating to 63.5% differential expression. Differential expression of DEFB123 in the primary tumor tissue of patients with breast cancer trended towards statistical significance (Chart 2; $p=9.70\text{e-}02$). Thus, differential expression of DEFB123, transcriptome-wide, in the tumor tissues of women with breast cancer was conserved across two independent microarray datasets, both in primary tumors of the breast and in metastasis to the lymph nodes.

DEFB123 is expressed at lower levels in the lymph node metastases of patients with metastatic breast cancer.

We obtained exact mRNA expression levels for DEFB123, in primary tumors of the breast and in brain metastasis of patients with lymph node metastatic breast cancer to determine direction and statistical significance of change in DEFB123 expression in lymph node metastatic tissues. We observed significantly lower expression of DEFB123 in the lymph node metastases of patients with breast cancer as compared to primary tumors of the breast: DEFB123 was expressed at 0.09 ± 0.20 arbitrary units (AU) in primary tumors of the breast, while it was expressed at 0.41 ± 0.40 AU in lymph node metastatic tissues. The difference in DEFB123 mRNA levels between primary tumors of the breast and lymph node metastatic tissues was statistically significant (Figure 1; $p=0.000583$).

DEFB123 expression is significantly correlated with survival outcomes in human breast cancer.

We performed Kaplan-Meier survival analysis⁸ in $n=180$ lymph node negative breast cancer patients and $n=230$ lymph node positive breast cancer patients, to evaluate whether DEFB123 tumor expression was correlated with survival outcomes in human breast cancer and to evaluate whether lymph node status of the patient influenced this outcome. We observed a statistically significant correlation between primary tumor expression of DEFB123 and overall survival (OS) in patients with lymph node negative breast cancer (Figure 2). Lymph node negative patients whose primary tumors expressed low levels of DEFB123 possessed inferior median OS of as compared to lymph node negative patients whose tumors expressed high levels of DEFB123 (Figure 2). This difference in OS based on DEFB123 tumor expression in lymph node negative patients with breast cancer was statistically significant (Figure 2, Chart 3; logrank p -value: 0.018; hazard ratio: 0.35 (0.14-0.87)). Primary tumor expression of DEFB123 was not correlated with overall survival in patients with lymph node positive breast cancer (Figure 2, Chart 3; logrank p -value: 0.39; hazard ratio: 1.23 (0.76-1.99)).

Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified the defensin beta 123, encoded by DEFB123, as among the genes whose expression was most different, transcriptome-wide, in the brain metastases of patients with breast cancer, when compared to primary tumors of the breast and in the primary tumors of patients with breast cancer when compared to normal mammary epithelium; we observed significantly increased expression of DEFB123 in lymph node metastases as compared to primary tumors of the breast. Further, we found a significant correlation between DEFB123 expression and patient survival outcomes, as overall survival was significantly greater in patients whose primary tumors expressed high levels of DEFB123 as compared to patients whose primary tumors expressed low levels of DEFB123, in lymph node negative patients but not in lymph node positive patients.

Discussion

We provided evidence here that the defensin beta 123, encoded by DEFB123, is among the genes whose expression is most different in the lymph node metastases of patients with metastatic breast cancer, that DEFB123 is also differentially expressed in the primary tumors of patients with breast cancer when compared to matched normal mammary epithelium, that DEFB123 mRNA is present at significantly increased quantities in lymph node metastatic tissues as compared to primary tumors of the breast, and that primary tumor DEFB123 expression is significantly correlated with overall survival in patients with lymph node negative breast cancer. Evaluation of the effects of genetic depletion of DEFB123 in mouse models of metastatic breast cancer on metastasis to the lymph node and to the central nervous system is merited. Modulation of DEFB123 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: 115
Probe ID: 2936
p-value: 1.00e-03
t: -3.4178601
B: -0.8117
Gene: DEFB123
Gene name: defensin beta 123

Chart 1: DEFB123 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: 15014
probe ID: A_23_P356694
p-value: 9.70e-02
t: 1.7458075
B: -4.68994
Gene: DEFB123
Gene name: defensin beta 123

Chart 2: DEFB123 is differentially expressed in the primary tumors of patients with breast cancer when compared to matched, normal mammary epithelium.

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: DEFB123 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 DEFB123 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 DEFB123 expression between primary tumors and lymph node metastasis, a *p*-value, is *p*=0.000583.

Figure 2: Significant correlation between DEFB123 primary tumor expression and overall survival in patients with lymph node negative breast cancer.

Depicted in these Kaplan-Meier plots is the probability of overall survival for $n=180$ patients with lymph node negative breast cancer (**right**) or $n=230$ patients with lymph node positive breast cancer (**left**) stratified into two groups, based on low or high expression of DEFB123 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 DEFB123 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.