FCRLA is a differentially expressed gene in lymph node metastatic human 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 lymph node metastasis in humans with metastatic breast cancer. We found that Fc receptor-like A, encoded by FCRLA, 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. FCRLA mRNA was present at increased quantities in lymph node metastatic tissues as compared to primary tumors of the breast. Expression of FCRLA in primary tumors was significantly correlated with patient overall survival in lymph node negative patients. Modulation of FCRLA expression may be relevant to the biology by which tumor cells metastasize from the breast to the lymph node in humans with metastatic breast cancer.

Keywords: breast cancer, metastasis, lymph node metastases, central nervous system metastases, Fc receptor-like A, FCRLA, 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 lymph node 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 lymph node metastatic breast cancer^{4,5}. We discovered differential and increased expression of the gene encoding Fc receptor-like A, FCRLA, in lymph node metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE10893⁴ and GSE43837⁵ for this global differential gene expression analysis of lymph node 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 $n=3$ lymph node metastases from patients with breast cancer; analysis was performed using platform GPL885. GSE43837 was generated using Affymetrix Human X3P Array technology with $n=19$ primary tumors of the breast and $n=19$ lymph node metastases from patients with breast cancer; 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 FCRLA 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 FCRLA mRNA expression levels with overall survival in $n=180$ patients; auto cut-off was selected.

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.

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

Through blind, systems-level analysis of published microarray data⁴, we identified Fc receptor-like A, encoded by FCRLA, as a differentially expressed gene in the breast 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, FCRLA ranked 64 out of 17418 total transcripts (Chart 1), equating to 99.6% differential expression. Differential expression of FCRLA in the lymph node metastases of patients with metastatic breast cancer was statistically significant (Chart 1; $p=3.78e-04$).

To attempt to validate differential transcriptome-wide differential expression of FCRLA in lymph node metastases in human metastatic breast cancer, we queried a second microarray dataset⁵; again, we identified FCRLA as a differentially expressed gene in the lymph node metastatic tissues of patients with breast cancer (Chart 2). When sorting each of the genes expressed in lymph node metastases based on significance of difference as compared to primary tumors of the breast, FCRLA ranked 8067 out of 61359 total transcripts (Chart 2), equating to 86.9% differential expression. Differential expression of FCRLA in the lymph node metastases of patients with metastatic breast cancer trended towards statistical significance (Chart 2; $p=0.06480016$). Thus, transcriptome-wide differential expression of FCRLA in the lymph node metastases of women with metastatic breast cancer was conserved across two independent microarray datasets, indicating that this may likely be a *bona fide* biological phenomena associated with metastatic breast cancer in humans.

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

We obtained exact mRNA expression levels for FCRLA, 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 FCRLA expression in lymph node metastatic tissues. We observed significantly higher expression of FCRLA in the lymph node metastases of patients with breast cancer as compared to primary tumors of the breast: FCRLA was expressed at 0.37 ± 0.54 units (AU) in primary tumors of the breast, while it was expressed at 1.27 ± 1.18 AU in lymph node metastatic tissues. The difference in FCRLA mRNA levels between primary tumors of the breast and lymph node metastatic tissues was statistically significant (Figure 1; $p=0.000438$).

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

We performed Kaplan-Meier survival analysis⁸ in $n=230$ lymph node positive patients and $n=180$ lymph node negative patients, to evaluate whether FCRLA tumor expression was correlated with survival outcomes in breast cancer. We observed a statistically significant correlation between primary tumor expression of FCRLA and overall survival (OS) in lymph node negative patients with breast cancer, in the lower survival quartile (Figure 2). Lymph node negative patients whose primary tumors expressed low levels of FCRLA possessed significantly superior median OS, on average, as compared to lymph node positive patients whose tumors expressed high levels of FCRLA. This difference in OS based on FCRLA tumor expression in lymph node negative patients with breast cancer was statistically significant (Figure 2, Chart 3; logrank p -value: 0.045; hazard ratio: 3.22 (0.96-10.79)). Primary tumor expression of FCRLA was not significantly correlated with FCRLA expression in lymph node positive breast cancer patients (Figure 2, Chart 3; logrank p -value: 0.82; hazard ratio: 1.06 (0.62-1.83)).

Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified Fc receptor-like A, encoded by FCRLA, 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; we observed significantly increased expression of FCRLA in lymph node metastases as compared to primary tumors of the breast. Further, we found a significant correlation between FCRLA expression and patient survival outcomes, as overall survival was significantly greater in patients whose tumors expressed low levels of FCRLA as compared to patients whose tumors expressed high levels of FCRLA, in lymph node negative patients but not in lymph node positive patients.

Discussion

We provided evidence here that Fc receptor-like A, encoded by FCRLA, is among the genes whose

1 expression is most different in the lymph node metastases of patients with lymph node metastatic breast
2 cancer, that FCRLA mRNA is present at significantly increased quantities in lymph node metastatic
3 tissues as compared to primary tumors of the breast, and that primary tumor FCRLA expression is
4 significantly correlated with patient survival outcomes in lymph node negative human breast cancer.
5 Evaluation of the effects of genetic depletion of FCRLA in mouse models of metastatic breast cancer on
6 metastasis to the central nervous system is merited. Modulation of FCRLA expression may be relevant to
7 the processes by which breast cancer cells exit the breast, enter the vasculature and/or lymphatics, reside in
8 the lymph nodes, evade immune clearance, breach the blood-lymph node barrier and colonize the lymph
9 node.
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Rank: 64
Probe ID: 20151
p-value: 3.78e-04
t: -3.714495
B: 0.0152
Gene: FCRLA
Gene name: FCRLA Fc receptor-like A homolog

Chart 1: FCRLA 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: 8067
probe ID: Hs.266331.0.A1_3p_a_at
p-value: 0.06480016
t: 1.895383
B: -4.07965
Gene: FCRLA
Gene name: Fc receptor-like A

Chart 2: FCRLA 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, 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: FCRLA 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 FCRLA 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 FCRLA expression between primary tumors of the breast and lymph node metastases is $p=0.000438$.

Figure 2: Significant correlation between FCRLA primary tumor expression and overall survival in patients with breast cancer, in lymph node negative patients but not in lymph node positive patients.

Depicted in these Kaplan-Meier plots is the probability of overall survival in $n=230$ patients with lymph node positive breast cancer (**left**) and $n=180$ patients with lymph node negative breast cancer (**right**), stratified into two groups, based on low or high expression of FCRLA 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 each graph is the number of patients at risk (number of patients alive) per interval, after stratification based on FCRLA 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.