CBLL1 is differentially expressed in lymph node metastasis in 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 metastasis to the lymph nodes in humans with metastatic breast cancer. We found that cbl proto-oncogene like 1, CBLL1, 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. Analysis of a separate microarray dataset revealed that CBLL1 was also differentially expressed in brain metastatic tissues. CBLL1 mRNA was present at increased quantities in lymph node metastases as compared to primary tumors of the breast. Importantly, expression of CBLL1 in primary tumors of the breast was correlated with patient overall survival, in lymph node positive patients but not in lymph node negative patients. Modulation of CBLL1 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, cbl proto-oncogene like 1, CBLL1, 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 cbl proto-oncogene like 1, CBLL1, in lymph node metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE10893⁴ and GSE52604⁵ 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. GSE52604 was generated using Agilent-014850 Whole Human Genome Microarray 4x44K G4112F with $n=10$ normal breast tissues and $n=35$ brain 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 CBLL1 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 CBLL1 mRNA expression levels with overall survival in $n=726$ lymph node positive patients and $n=452$ lymph node negative 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.

CBLL1 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 cbl proto-oncogene like 1, encoded by CBLL1, 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, CBLL1 ranked 181 out of 18034 total transcripts (Chart 1), equating to 99.0% differential expression. Differential expression of CBLL1 in the lymph node metastases of patients with metastatic breast cancer was statistically significant (Chart 1; $p=2.08e-03$).

Differential gene expression analyses that utilize primary and metastatic tumor tissues to discover genes associated with metastasis can be challenging due to the relatively heterogeneous cellular composition and constitution of the tumor as compared to normal, untransformed (benign) tissues. Thus, to attempt to validate differential transcriptome-wide differential expression of CBLL1 in metastatic tissues in human breast cancer, we queried a second microarray dataset⁵, here comparing normal breast tissues rather than primary tumors of the breast, to brain metastases. Again, we identified CBLL1 as a differentially expressed gene in the metastatic tissues of humans with breast cancer, here, in the brain metastatic tissues of patients with breast cancer (Chart 2). When sorting each of the genes expressed in brain metastases based on significance of difference as compared to normal breast tissues, CBLL1 ranked 17321 out of 41093 total transcripts (Chart 2), equating to 57.8% differential expression. Differential expression of CBLL1 in the brain metastases of patients with metastatic breast cancer was statistically significant (Chart 2; $p=4.98e-02$). Thus, differential expression of CBLL1, transcriptome-wide, in the metastatic tissues of women with metastatic breast cancer was conserved across two independent microarray datasets, both in lymph node and brain metastases. This suggests that CBLL1 differential expression in human metastatic breast cancer is a *bona fide* biological process and that differential expression of CBLL1 in metastatic breast cancer is shared between the brain and the lymph nodes.

CBLL1 is expressed at higher levels in the lymph node metastases of patients with metastatic breast cancer.

We obtained exact mRNA expression levels for CBLL1, 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 CBLL1 expression in lymph node metastatic tissues. We observed significantly increased expression of CBLL1 in the lymph node metastases of patients with breast cancer as compared to primary tumors of the breast: CBLL1 was expressed at -0.39 ± 0.36 arbitrary units (AU) in primary tumors of the breast, while it was expressed at 0.06 ± 0.35 AU in lymph node metastatic tissues (Figure 1). The difference in CBLL1 mRNA levels between primary tumors of the breast and lymph node metastases was statistically significant (Figure 1; $p=0.001948$).

CBLL1 expression is significantly correlated with survival outcomes in human breast cancer.

We performed Kaplan-Meier survival analysis⁷ in $n=726$ lymph node positive breast cancer patients and $n=452$ lymph node negative breast cancer patients, to evaluate whether CBLL1 tumor expression was correlated with survival outcomes in breast cancer and to evaluate whether lymph node status influenced correlation of CBLL1 tumor expression with survival outcomes in human breast cancer. We observed a correlation between primary tumor expression of CBLL1 and overall survival (OS) in lymph node positive breast cancer (Figure 2). Lymph node positive patients whose primary tumors expressed low levels of CBLL1 possessed median OS of 40.77 months, while lymph node positive patients whose tumors expressed high levels of CBLL1 possessed median OS of 56.16 months. This difference in OS based on CBLL1 tumor expression in lymph node positive patients with breast cancer was statistically significant (Figure 2, Chart 3; logrank p -value: 0.041; hazard ratio: 0.71 (0.5-0.99)). CBLL1 primary tumor expression was not correlated with overall survival outcomes in lymph node negative patients (Figure 2, Chart 3; logrank p -value: 0.14; hazard ratio: 1.29 (0.92-1.82)).

Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified CBLL1 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 CBLL1 in lymph node metastases as compared to primary tumors of the breast. Further, we found correlation between CBLL1 expression and overall survival in lymph node positive patients but not in lymph node negative patients.

Discussion

We provided evidence here that cbl proto-oncogene like 1, CBLL1, 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 CBLL1 is also differentially expressed in brain metastatic tissues in human breast cancer, that CBLL1 mRNA is present at significantly increased quantities in lymph node metastatic tissues as compared to primary tumors of the breast, and that primary tumor CBLL1 expression is correlated with patient survival outcomes in human breast cancer in lymph node positive patients but not in lymph node negative patients. Evaluation of the effects of genetic depletion of CBLL1 in mouse models of metastatic breast cancer on metastasis to the lymph nodes and to the central nervous system is merited. Modulation of CBLL1 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: 181
Probe ID: 2466
p-value: 2.08e-03
t: -3.1845768
B: -1.4272
Gene: CBLL1
Gene name: cbl proto-oncogene like 1

Chart 1: CBLL1 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: 17321
Probe ID: A_23_P146004
p-value: 4.98e-02
t: -2.0132632
B: -4.76958
Gene: CBLL1
Gene name: cbl proto-oncogene like 1

Chart 2: CBLL1 is differentially expressed in brain metastatic breast cancer when comparing brain 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: CBLL1 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 CBLL1 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 CBLL1 expression between primary tumors of the breast and lymph node metastases, a p -value, is $p=0.001948$.

Figure 2: CBL1 primary tumor expression is correlated with overall survival in patients with breast cancer, in lymph node positive patients but not in lymph node negative patients.

Depicted in these Kaplan-Meier plots is the probability of overall survival in $n=726$ lymph node positive patients (**left**) and $n=452$ lymph node negative patients (**right**), based on low or high expression of CBL1 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 CBL1 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.

Lymph node positive patients

Low CBLL1 expression: 40.77 months

High CBLL1 expression: 56.16 months

Chart 3: In lymph node positive patients, median overall survival is inferior in patients with low primary tumor expression of CBLL1.

The median OS (overall survival) of patients with low primary tumor expression of CBLL1 and high primary tumor expression of CBLL1 is listed in this chart, for $n=452$ lymph node positive breast cancer patients.