COMP 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 lymph node metastasis in humans with metastatic breast cancer. We found that cartilage oligomeric matrix protein, encoded by COMP, 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 COMP was also differentially expressed in brain metastatic tissues⁵. COMP mRNA was present at increased quantities in lymph node metastatic tissues as compared to primary tumors of the breast. Expression of COMP in primary tumors was correlated with patient recurrence-free survival in lymph node negative patients but not in lymph node positive patients. Modulation of COMP 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, lymph node metastases, central nervous system metastases, cartilage oligomeric matrix protein, COMP, 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 cartilage oligomeric matrix protein, COMP, in lymph node metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE10893⁴ and GSE125989⁵ 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. GSE125989 was generated using Affymetrix Human Genome U133A 2.0 array technology with $n=16$ primary breast tumors and $n=16$ brain metastases from patients with breast cancer; the samples utilized by investigators for this microarray dataset were paired, matched tumor and metastasis samples; analysis was performed using platform GPL571. 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 COMP 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 COMP mRNA expression levels with recurrence-free survival in $n=2368$ lymph node negative patients and $n=1656$ lymph node positive 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.

COMP 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 cartilage oligomeric matrix protein, encoded by COMP, 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, COMP ranked 135 out of 18034 total transcripts (Chart 1), equating to 99.3% differential expression. Differential expression of COMP in the lymph node metastases of patients with metastatic breast cancer was statistically significant (Chart 1; $p=1.36e-03$).

To attempt to validate differential transcriptome-wide differential expression of COMP in human metastatic breast cancer, we queried a second microarray dataset⁵; here, we identified COMP as a differentially expressed gene 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 primary tumors of the breast, COMP ranked 141 out of 22277 total transcripts (Chart 2), equating to 99.4% differential expression. Differential expression of COMP in the brain metastases of patients with metastatic breast cancer was statistically significant (Chart 2; $p=4.63e-04$). Thus, transcriptome-wide differential expression of COMP in the metastatic tissues of women with metastatic breast cancer was conserved across two independent microarray datasets, in the lymph nodes and in the brain, indicating that this may likely be a *bona fide* biological phenomena associated with metastatic breast cancer in humans.

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

We obtained exact mRNA expression levels for COMP, 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 COMP expression in lymph node metastatic tissues. We observed significantly higher expression of COMP in the lymph node metastases of patients with breast cancer as compared to primary tumors of the breast: COMP was expressed at 2.90 ± 1.29 units (AU) in primary tumors of the breast, while it was expressed at 1.21 ± 1.47 AU in lymph node metastatic tissues. The difference in COMP mRNA levels between primary tumors of the breast and lymph node metastatic tissues was statistically significant (Figure 1; $p=0.001649$).

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

We performed Kaplan-Meier survival analysis⁸ in $n=1656$ lymph node positive patients and $n=2368$ lymph node negative patients, to evaluate whether COMP tumor expression was correlated with survival outcomes in breast cancer. We observed a correlation between primary tumor expression of COMP and recurrence-free survival (RFS) in lymph node negative patients with breast cancer (Figure 2), in the upper survival tertile. Lymph node negative patients whose primary tumors expressed low levels of COMP possessed median RFS of 77.23 months, as compared to 104 months for lymph node negative patients whose tumors expressed high levels of COMP. This difference in RFS based on COMP tumor expression in lymph node negative patients with breast cancer approached statistical significance (Figure 2, Chart 3; logrank p -value: 0.061; hazard ratio: 0.85 (0.71-1.01)). Primary tumor expression of COMP was not correlated with RFS in lymph node positive breast cancer patients (Figure 2, Chart 3; logrank p -value: 0.43; hazard ratio: 1.07 (0.9-1.28)).

Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified cartilage oligomeric matrix protein, encoded by COMP, 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 COMP in lymph node metastases as compared to primary tumors of the breast. Further, we observed a correlation between COMP expression and patient survival outcomes in lymph node negative patients but not in lymph node positive patients.

Discussion

We provided evidence here that cartilage oligomeric matrix protein, encoded by COMP, is among the genes whose expression is most different in the lymph node metastases of patients with lymph node metastatic breast cancer, that COMP mRNA is present at significantly increased quantities in lymph node metastatic tissues as compared to primary tumors of the breast, and that primary tumor COMP expression is correlated with patient survival outcomes in lymph node negative human breast cancer. Evaluation of the effects of genetic depletion of COMP in mouse models of metastatic breast cancer on metastasis to the lymph nodes and to the central nervous system is merited. Modulation of COMP 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: 135
Probe ID: 4626
p-value: 1.36e-03
t: 3.3207864
B: -1.0717
Gene: COMP
Gene name: cartilage oligomeric matrix protein

Chart 1: COMP 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: 141
probe ID: 205713_s_at
p-value: 4.63e-04
t: 3.8352336
B: -0.0747
Gene: COMP
Gene name: cartilage oligomeric matrix protein

Chart 2: COMP 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: COMP 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 COMP 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 COMP expression between primary tumors of the breast and lymph node metastases is $p=0.001649$.

Figure 2: COMP primary tumor expression correlates with recurrence-free survival in patients with lymph node negative breast cancer but not in lymph node positive patients.

Depicted in these Kaplan-Meier plots is the probability of recurrence-free survival in $n=1656$ patients with lymph node positive breast cancer (**left**) and $n=2368$ patients with lymph node negative breast cancer (**right**), stratified into two groups, based on low or high expression of COMP (also known as MED) in patient primary tumors, in the upper survival tertile. The log rank p -value denoting statistical significance of difference in recurrence-free 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 COMP 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 negative patients

Low COMP expression: 77.23 months

High COMP expression: 104 months

Chart 3: Median recurrence-free survival is inferior in lymph node negative breast cancer patients with low primary tumor expression of COMP.

The median RFS (recurrence-free survival) of patients with low primary tumor expression of COMP and high primary tumor expression of COMP is listed in this chart, for $n=2368$ lymph node negative breast cancer patients, in the upper survival tertile.