CCR7 is differentially expressed in the lymph nodes of patients with metastatic breast cancer.

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Metastasis to the brain is a clinical problem in patients with breast cancer¹⁻³. The lymph nodes reside between the breast and the brain. 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 the C-C chemokine receptor type 7, CCR7, 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. CCR7 mRNA was present at increased quantities in lymph node metastases as compared to primary tumors of the breast. Importantly, expression of CCR7 in primary tumors of the breast was significantly correlated with patient recurrence-free survival. Modulation of CCR7 expression may be relevant to the biology by which tumor cells metastasize from the breast to the brain in humans with metastatic breast cancer. CCR7 may function to support tissue infiltration in brain metastatic breast cancer in humans.

Keywords: breast cancer, metastasis, brain metastases, central nervous system metastases, C-C chemokine receptor type 7, CCR7, 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, comparing to primary tumors and normal breast tissues^{4,5}. We discovered transcriptome-wide differential expression of the gene encoding the C-C chemokine receptor type 7, CCR7, in lymph node metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE10893⁴ and GSE124648⁵ 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. GSE124648 was generated using Affymetrix Human Genome U133A array technology with $n=10$ normal breast tissues and $n=44$ lymph node metastases from patients with breast cancer; analysis was performed using platform GPL96. 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 CCR7 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 CCR7 mRNA expression levels with recurrence-free survival in $n=3951$ 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.

CCR7 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 the

C-C chemokine receptor type 7, encoded by CCR7, 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, CCR7 ranked 247 out of 18034 total transcripts (Chart 1), equating to 98.6% differential expression. Differential expression of CCR7 in the lymph node metastases of patients with metastatic breast cancer was statistically significant (Chart 1; $p=3.06e-03$).

We queried a second microarray dataset to attempt to validate transcriptome-wide differential expression of CCR7 in the lymph node metastases of patients with metastatic breast cancer. We again observed CCR7 to be among the genes expressed most quantitatively differently in the lymph node metastases of patients with metastatic breast cancer as compared to primary tumors of the breast. When sorting each of the genes expressed in lymph node metastases based on significance of difference as compared to normal breast tissues, CCR7 ranked 1906 out of 22283 total transcripts (Chart 2), equating to 91.4% differential expression. Differential expression of CCR7 in the lymph node metastases of patients with metastatic breast cancer approached the level of statistical significance (Chart 2; $p=4.95e-05$).

CCR7 is expressed at higher levels in the lymph node metastases of patients with metastatic breast cancer.

We obtained exact mRNA expression levels for CCR7, 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 CCR7 expression in lymph node metastatic tissues. CCR7 was expressed at higher levels in the lymph node metastases of patients with breast cancer as compared to primary tumors of the breast, and this difference was statistically significant (Figure 1; $p=0.003618$).

CCR7 expression is significantly correlated with survival outcomes in human breast cancer.

We performed Kaplan-Meier survival analysis⁸ in $n=3951$ breast cancer patients in total, to evaluate whether CCR7 tumor expression was correlated with survival outcomes in breast cancer. We observed a statistically significant correlation between primary tumor expression of CCR7 and recurrence-free survival (RFS) in patients with breast cancer (Figure 2). Patients whose primary tumors expressed low levels of CCR7 possessed median RFS of 38.7 months, while patients whose tumors expressed high levels of CCR7 possessed median RFS of 62 months. This difference in RFS based on CCR7 tumor expression in patients with breast cancer was statistically significant (Figure 2, Chart 3; logrank p -value: $9.7e-07$; hazard ratio: 0.76 (0.68-0.85)).

Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified CCR7 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 to normal breast tissues, respectively; we observed significantly increased expression of CCR7 in lymph node metastases as compared to primary tumors of the breast. Further, we found a significant correlation between CCR7 expression and patient survival outcomes, as recurrence-free survival was significantly greater in patients whose primary tumors expressed higher levels of CCR7 as compared to patients whose primary tumors expressed lower levels of CCR7.

Discussion

We provided evidence here that the chemokine receptor type 7, CCR7, 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 and to normal breast tissues, that CCR7 mRNA is present at significantly increased quantities in lymph node metastatic tissues as compared to primary tumors of the breast, and that primary tumor CCR7 expression is significantly correlated with patient survival outcomes in human breast cancer. Evaluation of the effects of genetic depletion of CCR7 in mouse models of metastatic breast cancer on metastasis to the central nervous system is merited. CCR7 has been described as a biomarker that can predict metastasis to the axillary lymph nodes in humans with breast cancer⁸. We recently described the differential expression and up-regulation of the ligand for CCR7, CCL21, in the lymph node metastases of humans with metastatic breast cancer⁹. We suggest that in human metastatic breast cancer, up-regulation of CCR7 by circulating tumor cells functions as one part of a multi-component system including CCL21¹⁰ to support recruitment to the lymph nodes. Modulation of CCR7 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: 247
Probe ID: 22410
p-value: 3.06e-03
t: -3.0561305
B: -1.7522
Gene: CCR7
Gene name: C-C chemokine receptor type 7

Chart 1: CCR7 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: 1906
Probe ID: 206337_at
p-value: 4.95e-05
t: -4.400516
B: 1.400885
Gene: CCR7
Gene name: C-C motif chemokine receptor 7

Chart 2: CCR7 is differentially expressed in lymph node metastatic breast cancer when comparing lymph node 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.

CCR7

Figure 1: CCR7 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 CCR7 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 CCR7 expression between primary tumors of the breast and lymph node metastases, a p -value, is $p=0.003618$.

Figure 2: Significant correlation between CCR7 primary tumor expression and recurrence-free survival in patients with breast cancer.

Depicted in this Kaplan-Meier plot is the probability of recurrence-free survival for $n=3951$ total patients stratified into two groups, based on low or high expression of CCR7 in patient primary tumors. 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 is the number of patients at risk (number of patients alive) per interval, after stratification based on CCR7 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.

Low CCR7 expression: 38.7 months

High CCR7 expression: 62 months

Chart 3: Median recurrence-free survival is inferior in patients with low primary tumor expression of CCR7.

The median RFS (recurrence-free survival) of patients with low primary tumor expression of CCR7 and high primary tumor expression of CCR7 is listed in this chart, for $n=3951$ breast cancer patients.