CR2 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 complement component 3d receptor 2, encoded by CR2, 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. CR2 was also differentially expressed in the tumor cells of patients with triple negative breast cancer⁵. CR2 mRNA was present at increased quantities in lymph node metastatic tissues as compared to primary tumors of the breast. Importantly, expression of CR2 in primary tumors was significantly correlated with patient recurrence-free survival. Modulation of CR2 expression may be relevant to the biology by which tumor cells metastasize from the breast to the brain in humans with metastatic breast cancer.

Keywords: breast cancer, metastasis, brain metastasis, central nervous system metastasis, lymph node metastasis, the complement component 3d receptor 2, CR2, 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 patients with brain metastatic breast cancer^{4,5}. We discovered differential and increased expression of the gene encoding the complement component 3d receptor 2, CR2, in brain metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE10893⁴ and GSE38959⁵ 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. GSE38959 was generated using Agilent-014850 Whole Human Genome Microarray 4x44K G4112F with $n=13$ samples of normal mammary gland ductal cells and $n=30$ samples of primary tumor cells from patients with triple negative breast cancer; analysis was performed using platform GPL4133. 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 CR2 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 CR2 mRNA expression levels with recurrence-free 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.

CR2 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 complement component 3d receptor 2, encoded by CR2, 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, CR2 ranked 61 out of 17418 total transcripts (Chart 1), equating to 99.6% differential expression. Differential expression of CR2 in the lymph node metastases of patients with metastatic breast cancer was statistically significant (Chart 1; $p=3.56e-04$).

To attempt to validate differential transcriptome-wide differential expression of CR2 in human

breast cancer, we queried a second microarray dataset⁵, here comparing primary tumor cells from patients with triple negative breast cancer to normal mammary gland ductal cells. Again, we identified CR2 as a differentially expressed gene in the tumor tissues of patients with breast cancer (Chart 2). When sorting each of the genes expressed in triple negative breast cancer tumor cells based on significance of difference as compared to normal mammary gland ductal cells, CR2 ranked 5035 out of 45015 total transcripts (Chart 2), equating to 88.8% differential expression. Differential expression of CR2 in the tumor cells of patients with triple negative breast cancer was statistically significant (Chart 2; $p=4.58e-04$). Thus, differential expression of CR2, transcriptome-wide, in the tumor tissues of women with breast cancer was conserved across two independent microarray datasets, both in primary and metastatic tumor tissues.

CR2 is expressed at lower levels in the lymph node metastases of patients with metastatic breast cancer.

We obtained exact mRNA expression levels for CR2, 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 CR2 expression in lymph node metastatic tissues. We observed significantly lower expression of CR2 in the lymph node metastases of patients with breast cancer as compared to primary tumors of the breast: CR2 was expressed at -0.12 ± 0.23 arbitrary units (AU) in primary tumors of the breast, while it was expressed at 0.80 ± 1.24 AU in lymph node metastatic tissues. The difference in CR2 mRNA levels between primary tumors of the breast and lymph node metastatic tissues was statistically significant (Figure 1; $p=0.000415$).

CR2 expression is significantly correlated with survival outcomes in human breast cancer.

We performed Kaplan-Meier survival analysis⁸ in 4929 breast cancer patients in total, to evaluate whether CR2 tumor expression was correlated with survival outcomes in human breast cancer. We observed a statistically significant correlation between primary tumor expression of CR2 and recurrence-free survival (RFS) in patients with breast cancer (Figure 2). Patients whose primary tumors expressed low levels of CR2 possessed median RFS of 44.4 months, while patients whose tumors expressed high levels of CR2 possessed median RFS of 69 months. This difference in RFS based on CR2 tumor expression in patients with breast cancer was statistically significant (Figure 2, Chart 3; logrank p -value: $8.1e-06$; hazard ratio: 0.76 ($0.67-0.86$)).

Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified the complement component 3d receptor 2, encoded by CR2, 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 in the primary tumor cells of patients with triple negative breast cancer when compared to normal mammary gland ductal cells; we observed significantly decreased expression of CR2 in lymph node metastases as compared to primary tumors of the breast. Further, we found a significant correlation between CR2 expression and patient survival outcomes, as recurrence-free survival was significantly greater in patients whose primary tumors expressed lower levels of CR2 as compared to patients whose primary tumors expressed higher levels of CR2.

Discussion

We provided evidence here that the complement component 3d receptor 2, encoded by CR2, is among the genes whose expression is most different in the lymph node metastases of patients with metastatic breast cancer, that CR2 is also differentially expressed in the primary tumor cells of patients with triple negative breast cancer, that CR2 mRNA is present at significantly increased quantities in

1 lymph node metastatic tissues as compared to primary tumors of the breast, and that primary tumor CR2
2 expression is significantly correlated with patient survival outcomes in human breast cancer. Evaluation of
3 the effects of genetic depletion of CR2 in mouse models of metastatic breast cancer on metastasis to the
4 lymph node and to the central nervous system is merited. Modulation of CR2 expression may be relevant
5 to the processes by which breast cancer cells exit the breast, enter the vasculature and/or lymphatics, reside
6 in the lymph nodes, evade immune clearance, breach the blood-brain barrier and colonize the brain.
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Rank: 61
Probe ID: 15366
p-value: 3.56e-04
t: -3.7322283
B: 0.0661
Gene: CR2
Gene name: complement component 3d receptor 2

Chart 1: CR2 is differentially expressed in lymph node metastatic breast cancer when comparing brain 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: 5035
probe ID: 28782
p-value: 4.58e-04
t: 3.7795408
B: -0.513034
Gene: CR2
Gene name: complement component 3d receptor 2

Chart 2: CR2 is differentially expressed in the tumor cells of patients with triple negative breast cancer when compared to normal mammary gland ductal cells.

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: CR2 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 CR2 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 CR2 expression between primary tumors and lymph node metastasis, a *p*-value, is $p=0.000415$.

Figure 2: Significant correlation between CR2 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 $n=4929$ total patients stratified into two groups, based on low or high expression of CR2 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 CR2 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.

1 Low CR2 expression: 44.4 months

2 High CR2 expression: 69 months

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4 **Chart 3: Median recurrence-free survival is inferior in patients with low primary tumor expression of CR2.**

5 The median RFS (recurrence-free survival) of patients with low primary tumor expression of CR2 and
6 high primary tumor expression of CR2 is listed in this chart, for $n=4929$ breast cancer patients.