CD5L is differentially expressed in the lymph node metastases of patients with 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 the scavenger receptor cysteine-rich superfamily (SRCR) molecule apoptosis inhibitor of macrophages (AIM), CD5-like, encoded by CD5L, 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 CD5L was also differentially expressed in primary breast tumors when compared to normal breast tissue. CD5L mRNA was present at increased quantities in lymph node metastases as compared to primary tumors of the breast. Importantly, expression of CD5L in primary tumors of the breast was correlated with patient recurrence-free survival, in lymph node negative patients but not in lymph node positive patients. Modulation of CD5L 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, metastatic breast cancer, CD5-like, apoptosis inhibitor of macrophages (AIM), CD5L, 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 CD5-like, CD5L, in lymph node metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE10893⁴ and GSE42568⁵ 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. GSE42568 was generated using Affymetrix Human Genome U133 Plus 2.0 array technology with $n=17$ normal breast tissue biopsies and $n=104$ primary breast tumor biopsies from patients with breast cancer; analysis was performed using platform GPL570. 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 CD5L 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 CD5L mRNA expression levels with recurrence-free survival in $n=3951$ breast cancer patients, $n=1133$ lymph node positive patients and $n=2020$ lymph node negative 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.

CD5L 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 apoptosis inhibitor of macrophages AIM/CD5-like, encoded by CD5L, 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, CD5L ranked 71 out of 18034 total transcripts (Chart 1), equating to 99.6% differential expression. Differential expression of CD5L in the lymph node metastases of patients with metastatic breast cancer was statistically significant (Chart 1; $p=4.62e-04$).

To validate transcriptome-wide differential expression of CD5L in human breast cancer, we queried a second microarray dataset⁵, here comparing normal breast tissues and primary tumors of the breast. Again, we identified CD5L as a differentially expressed 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, CD5L ranked 18975 out of 54675 total transcripts (Chart 2), equating to 65,3% differential expression. Differential expression of CD5L in the primary tumors of patients with breast cancer was statistically significant (Chart 2; $p=2.94e-02$). Thus, transcriptome-wide differential expression of CD5L, in the tumor tissues of women with breast cancer was conserved across two independent microarray datasets, both in primary and metastatic tumor tissues.

CD5L is expressed at higher levels in the lymph node metastases of patients with metastatic breast cancer.

We obtained exact mRNA expression levels for CD5L, 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 CD5L expression in lymph node metastatic tissues. We observed significantly increased expression of CD5L in the lymph node metastases of patients with breast cancer as compared to primary tumors of the breast: CD5L was expressed at -0.02 ± 0.46 arbitrary units (AU) in primary tumors of the breast, while it was expressed at 0.93 ± 1.74 AU in lymph node metastatic tissues (Figure 1). The difference in CD5L mRNA levels between primary tumors of the breast and lymph node metastases was statistically significant (Figure 1; $p=0.00054$).

CD5L expression is correlated with survival outcomes in human breast cancer.

We performed Kaplan-Meier survival analysis⁷ in $n=3951$ breast cancer patients in total, to evaluate whether CD5L tumor expression was correlated with survival outcomes in breast cancer. We observed a correlation between primary tumor expression of CD5L and recurrence-free survival (RFS) in patients with breast cancer (Figure 2). Patients whose primary tumors expressed low levels of CD5L possessed median RFS of 216.66 months, while patients whose tumors expressed high levels of CD5L possessed median RFS of 185.16 months. This difference in RFS based on CD5L tumor expression in patients with breast cancer was statistically significant (Figure 2, Chart 3; logrank p -value: 0.00074; (hazard ratio: 0.83 (0.74-0.92))).

We also performed Kaplan-Meier survival analysis⁷ in $n=1133$ lymph node positive breast cancer patients and $n=2020$ lymph node negative breast cancer patients, to evaluate whether lymph node status influenced correlation of CD5L tumor expression with survival outcomes in human breast cancer. We observed a statistically significant correlation between primary tumor expression of CD5L and recurrence-free survival (RFS) in lymph node negative breast cancer (Figure 2). Lymph node negative patients whose primary tumors expressed low levels of CD5L possessed median RFS of 216.66 months, while lymph node negative patients whose tumors expressed high levels of CD5L possessed median RFS of 191.21 months. This difference in RFS based on CD5L tumor expression in lymph node negative patients with breast cancer approached statistical significance (Figure 2, Chart 3; logrank p -value: 0.063; (hazard ratio: 1.17 (0.99-1.39))). CD5L primary tumor expression was not significantly correlated with recurrence-free survival outcomes in lymph node positive patients (Figure 2, Chart 3; logrank p -value: 0.59; hazard ratio: 0.95 (0.78-1.15)).

Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified CD5L 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 CD5L in primary tumors of the breast as compared to normal breast tissue. Further, we found a significant correlation between CD5L expression and patient survival outcomes, as

recurrence-free survival was greater in patients whose primary tumors expressed higher levels of CD5L as compared to patients whose primary tumors expressed lower levels of CD5L, and this correlation was trended towards statistical significance in lymph node negative patients but not in lymph node positive patients.

Discussion

We provided evidence here that the SRCR family glycoprotein CD5L, also known as the apoptosis inhibitor of macrophages, AIM, 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 CD5L is also differentially expressed in primary tumors of the breast in human breast cancer, that CD5L mRNA is present at significantly increased quantities in lymph node metastatic tissues as compared to primary tumors of the breast, and that primary tumor CD5L expression is correlated with patient survival outcomes in human breast cancer, in all patients and in lymph node negative patients but not in lymph node positive patients. Evaluation of the effects of genetic depletion of CD5L in mouse models of metastatic breast cancer on metastasis to the central nervous system is merited. Modulation of CD5L 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: 71
Probe ID: 7454
p-value: 4.62e-04
t: -3.6546227
B: -0.1555
Gene: CD5L
Gene name: CD5-like

Chart 1: CD5L 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: 18975
Probe ID: 206680_at
p-value: 2.94e-02
t: 2.21
B: -4.8099026
Gene: CD5L
Gene name: CD5-like

Chart 2: CD5L is differentially expressed in primary tumors of the breast in human breast cancer when compared 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: CD5L 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 CD5L 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 CD5L expression between primary tumors of the breast and lymph node metastases, a *p*-value, is $p=0.00054$.

Figure 2: CD5L primary tumor expression is correlated with recurrence-free survival in lymph node negative patients but not in lymph node positive patients.

Depicted in this Kaplan-Meier plot (**above**) is the probability of recurrence-free survival for $n=3951$ total patients stratified into two groups, based on low or high expression of CD5L in patient primary tumors, in the upper survival quartile. 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 CD5L 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. Below, Kaplan-Meier plots for probability of recurrence-free survival in $n=1133$ lymph node positive patients (**left**) and $n=2020$ lymph node negative patients (**right**).

All patients

Low CD5L expression: 216.66 months

High CD5L expression: 185.16 months

Lymph node negative patients

Low CD5L expression: 216.66 months

High CD5L expression: 191.21 months

Chart 3: Median recurrence-free survival is inferior in patients with high primary tumor expression of CD5L.

The median RFS (recurrence-free survival) of patients with low primary tumor expression of CD5L and high primary tumor expression of CD5L is listed in this chart, for $n=3951$ breast cancer patients and $n=2020$ lymph node negative patients.