ACSL4 is a differentially expressed gene in human brain metastatic breast cancer.

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Metastasis to the brain is a clinical problem in patients with breast cancer¹⁻³. We mined published microarray data^{4,5} to compare primary and metastatic tumor transcriptomes for the discovery of genes associated with brain metastasis in humans with metastatic breast cancer. We found that acyl-CoA synthetase long-chain family member 4, encoded by ACSL4, was among the genes whose expression was most quantitatively different in the brain metastases of patients with metastatic breast cancer. ACSL4 mRNA was present at decreased quantities in brain metastatic tissues as compared to primary tumors of the breast. Importantly, expression of ACSL4 in primary tumors was significantly correlated with patient post-progression survival in patients with breast cancer. Modulation of ACSL4 expression may be relevant to the biology by which tumor cells metastasize from the breast to the brain.

Keywords: breast cancer, metastasis, brain metastases, central nervous system metastases, acyl-CoA synthetase long-chain family member 4, ACSL4, 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 decreased expression of the gene encoding acyl-CoA synthetase long-chain family member 4, ACSL4, in brain metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE10893⁴ and GS52604⁵ 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 $n=11$ primary breast tumors and $n=4$ brain metastases from patients with breast cancer; analysis was performed using platform GPL885. 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 ACSL4 gene expression was significantly different between primary tumors of the breast and brain 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 ACSL4 mRNA expression levels in primary tumors of the breast with post-progression survival in $n=414$ breast cancer patients.

Results

We performed global comparative transcriptome analysis of metastatic and primary tumor tissues of patients with metastatic breast, as well as normal tissues of the breast 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.

ACSL4 is differentially expressed in the brain metastases of patients with brain metastatic breast cancer.

Through blind, systems-level analysis of published microarray data⁴, we identified acyl-CoA synthetase long-chain family member 4, encoded by ACSL4, as a differentially expressed gene in the brain metastatic tissues of humans with breast cancer (Table 1). When sorting each of the genes expressed in brain metastases based on significance of difference as compared to primary tumors of the breast in patients with breast cancer, ACSL4 ranked 116 out of 17418 total transcripts (Chart 1), equating to 99.3% differential expression. Differential expression of ACSL4 in the brain metastases of patients with metastatic breast cancer was statistically significant (Chart 1; $p=2.32e-04$).

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 ACSL4 in brain metastases in human metastatic 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 ACSL4 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 normal breast tissues, ACSL4 ranked 14880 out of 41093 total transcripts (Chart 2), equating to 63.8% differential expression. Differential expression of ACSL4 in the brain metastases of patients with metastatic breast cancer was statistically significant (Chart 2; $p=2.75e-02$). Thus, differential expression of ACSL4, transcriptome-wide, in the brain metastases of women with metastatic breast cancer was conserved across two independent microarray datasets, both when compared to benign and transformed breast tissues. This suggests that ACSL4 differential expression in human brain metastatic breast cancer is a *bona fide* biological process.

ACSL4 is expressed at lower levels in the brain metastases of patients with metastatic breast cancer.

We obtained exact mRNA expression levels for ACSL4, in primary tumors of the breast and in brain metastasis of patients with brain metastatic breast cancer to determine direction and statistical significance of change in ACSL4 expression in brain metastatic tissues. We observed significantly decreased expression of ACSL4 in the brain metastases of patients with breast cancer as compared to primary tumors of the breast: ACSL4 was expressed at -0.83 ± 0.23 arbitrary units (AU) in primary tumors of the breast, while it was expressed at -1.64 ± 0.35 AU in brain metastatic tissues (Figure 1). The difference in ACSL4 mRNA levels between primary tumors of the breast and brain metastatic tissues was statistically significant (Figure 1; $p=0.000133$).

ACSL4 expression is correlated with survival outcomes in human breast cancer.

We performed Kaplan-Meier survival analysis⁷ in $n=414$ breast cancer patients in total, to evaluate whether ACSL4 tumor expression was correlated with survival outcomes in breast cancer. We observed a correlation between primary tumor expression of ACSL4 and post-progression survival (PPS) in patients with breast cancer (Figure 2). Patients whose primary tumors expressed low levels of ACSL4 possessed median PPS of 36 months, while patients whose tumors expressed high levels of ACSL4 possessed median PPS of 26.04 months. This difference in PPS based on ACSL4 tumor expression in patients with breast cancer was statistically significant (Figure 2, Chart 3; logrank p -value 0.033; hazard ratio: 1.3 (1.02-1.67)).

Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified acyl-CoA synthetase long-chain family member 4, encoded by ACSL4, as among the genes whose expression was most different, transcriptome-wide, in the brain metastases of patients with breast cancer, when compared to primary tumors of the breast and to normal breast tissues, respectively; we observed significantly decreased expression of ACSL4 in brain metastases as compared to primary tumors of the breast. Further, we found a correlation between ACSL4 expression and patient survival outcomes, as post-progression survival was greater in patients whose primary tumors expressed lower levels of ACSL4 as compared to patients whose primary tumors expressed higher levels of ACSL4.

Discussion

We provided evidence here that ACSL4 is among the genes whose expression is most different in the brain metastases of patients with metastatic breast cancer, that ACSL4 mRNA is present at significantly decreased quantity in brain metastatic tissues as compared to primary tumors of the breast, and that primary tumor ACSL4 expression is correlated with patient survival outcomes in human breast cancer. ACSL4 appears to be involved in a form of cell death known as ferroptosis, as mice deficient in both GPX4, a central effector of ferroptosis, and ACSL4 display marked resistance to ferroptotic cell death⁸. ACSL4 was shown to function in the enrichment of cell membranes with long polyunsaturated ω 6 fatty acids and found to be preferentially expressed in a panel of basal-like breast cancer cell lines⁸. Thus, down-regulation of ACSL4 in brain metastatic tissues in human breast cancer may function to limit ferroptotic cell death during metastatic progression, but this remains to be demonstrated. Evaluation of the effects of genetic depletion of ACSL4 in mouse models of metastatic breast cancer on metastasis to the central nervous system is merited. Modulation of ACSL4 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: 116
Probe ID: 1157
p-value: 2.32e-04
t: 4.74
B: 0.753269
Gene: ACSL4
Gene name: acyl-CoA synthetase long-chain family member 4

Chart 1: ACSL4 is differentially expressed in brain 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: 14880
probe ID: A_23_P217564
p-value: 2.75e-02
t: 2.2759461
B: -4.253834
Gene: ACSL4
Gene name: acyl-CoA synthetase long-chain family member 4

Chart 2: ACSL4 is differentially expressed in brain metastatic breast cancer when comparing brain metastases to normal breast tissues.

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: ACSL4 is expressed at significantly lower levels in the brain metastases of patients with metastatic breast cancer when compared to primary tumors of the breast.

The mRNA expression level of ACSL4 in primary tumors of the breast (left) and in brain metastases of women with metastatic breast cancer (right) is graphically depicted; the result of a statistical test evaluating significance of difference in ACSL4 expression between primary tumors and brain metastases is $p=0.000133$.

Figure 2: ACSL4 primary tumor expression is correlated with post-progression survival in patients with breast cancer.

Depicted in this Kaplan-Meier plot is the probability of post-progression survival for $n=414$ total breast cancer patients stratified into two groups, based on low or high expression of ACSL4 in patient primary tumor. The log rank p -value denoting statistical significance of difference in post-progression 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 ACSL4 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 ACSL4 expression: 36 months
High ACSL4 expression: 26.04 months

Chart 3: Median post-progression survival is inferior in patients with high primary tumor expression of ACSL4.

The median PPS (post-progression survival) of patients with low primary tumor expression of ACSL4 and high primary tumor expression of ACSL4 is listed in this chart, for $n=414$ breast cancer patients.