Bernardinelli-Seip congenital lipodystrophy 2 (seipin) is a differentially expressed gene in brain metastatic human 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 Bernardinelli-Seip congenital lipodystrophy 2, also known as seipin, encoded by BSCL2, was among the genes whose expression was most different in the brain metastases of patients with metastatic breast cancer as compared to primary tumors of the breast and to normal breast tissues. BSCL2 mRNA was present at increased quantities in brain metastatic tissues as compared to primary tumors of the breast. Expression of BSCL2 in primary tumors was significantly correlated with patient distant metastasis-free survival. Modulation of BSCL2 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 metastases, central nervous system metastases, Bernardinelli-Seip congenital lipodystrophy 2, seipin, BSCL2, 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 Bernardinelli-Seip congenital lipodystrophy 2, also known as seipin, BSCL2, in brain metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE10893⁴ and GSE52604⁵ 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 $n=3$ 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 BSCL2 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 tool for correlation of BSCL2 mRNA expression levels with distant metastasis-free survival in $n=1803$ 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.

BSCL2 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 Bernardinelli-Seip congenital lipodystrophy 2, also known as seipin, encoded by BSCL2, as a

1 differentially expressed gene in the breast metastatic tissues of humans with breast cancer (Table
2 1). When sorting each of the genes expressed in brain metastases based on significance of
3 difference as compared to primary tumors of the breast in patients with breast cancer, BSCL2
4 ranked 8 out of 17418 total transcripts (Chart 1), equating to 99.95% differential expression.
5 Differential expression of BSCL2 in the brain metastases of patients with metastatic breast cancer
6 was statistically significant (Chart 1; $p=2.59e-06$).

7 Differential gene expression analyses that utilize primary and metastatic tumor tissues to
8 discover genes associated with metastasis can be challenging due to the relatively heterogenous
9 cellular composition and constitution of the tumor as compared to normal, untransformed tissues.
10 Thus, to attempt to validate differential transcriptome-wide differential expression of BSCL2 in
11 brain metastases in human metastatic breast cancer, we queried a second microarray dataset⁵ this
12 comparing normal breast tissues rather than primary tumors of the breast to brain metastases.
13 Again, we identified BSCL2 as a differentially expressed gene in the brain metastatic tissues of
14 patients with breast cancer (Chart 2). When sorting each of the genes expressed in brain
15 metastases based on significance of difference as compared to normal breast tissues, BSCL2
16 ranked 2078 out of 41093 total transcripts (Chart 2), equating to 94.9% differential expression.
17 Differential expression of BSCL2 in the brain metastases of patients with metastatic breast cancer
18 was statistically significant (Chart 2; $p=4.62e-06$). Thus, differential expression of BSCL2,
19 transcriptome-wide, in the brain metastases of women with metastatic breast cancer was
20 conserved across two independent microarray datasets, both when compared to benign and
21 transformed breast tissues.

22 **BSCL2 expression is significantly correlated with survival outcomes in human breast cancer.**

23 We performed Kaplan-Meier survival analysis⁷ in 1803 breast cancer patients in total, to
24 evaluate whether BSCL2 tumor expression was correlated with survival outcomes in breast
25 cancer. We observed a statistically significant correlation between primary tumor expression of
26 BSCL2 and distant metastasis-free survival (DMFS) (Figure 2). Patients whose primary tumors
27 expressed low levels of BSCL2 possessed median DMFS of 81.6 months, while patients whose
28 tumors expressed high levels of BSCL2 possessed median DMFS of 122 months. This difference
in DMFS based on BSCL2 tumor expression in breast cancer was statistically significant (Figure
2, Chart 3; logrank p -value: 0.049; hazard ratio: 0.83 (0.68-1)).

29 **BSCL2 is expressed at higher levels in the brain metastases of patients with metastatic breast cancer.**

30 We obtained exact mRNA expression levels for BSCL2, in primary tumors of the breast
31 and in brain metastasis of patients with brain metastatic breast cancer to determine direction and
32 statistical significance of change in BSCL2 expression in brain metastatic tissues. BSCL2 was
33 expressed at higher levels in the brain metastases of patients with breast cancer as compared to
34 primary tumors of the breast, and this difference was statistically significant (Figure 1;
35 $p<0.00001$).

Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified seipin, encoded by BSCL2, as among the genes whose expression was most different, transcriptome-wide, in the brain metastases of patients with breast cancer, both when compared to primary tumors of the breast and to normal breast tissues; we observed significantly increased expression of BSCL2 in brain metastases as compared to primary tumors of the breast. Further, we found a significant correlation between BSCL2 expression and patient survival outcomes, as distant metastasis-free survival was significantly higher in patients whose primary tumors expressed higher levels of BSCL2 as compared to patients whose primary tumors expressed lower levels of BSCL2.

Discussion

We provided evidence here that Bernardinelli-Seip congenital lipodystrophy 2, also known as seipin, encoded by BSCL2, is among the genes whose expression is most different in the brain metastases of patients with brain metastatic breast cancer, that BSCL2 mRNA is present at significantly increased quantities in brain metastatic tissues as compared to primary tumors of the breast, and that primary tumor BSCL2 expression is significantly correlated with patient survival outcomes in human breast cancer. Evaluation of the effects of genetic depletion of BSCL2 in mouse models of metastatic breast cancer on metastasis to the central nervous system is merited. Functions of BSCL2 at the endoplasmic reticulum 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, successfully evade immune clearance, breach the blood-brain barrier and colonize the brain.

References

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Rank: 8
Probe ID: 16877
p-value: 2.59e-06
t: -7.13
B: 4.794862
Gene: BSCL2
Gene name: Bernardinelli-Seip congenital lipodystrophy 2 (seipin)

Chart 1: BSCL2 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: 2078
probe ID: A_24_P244442
p-value: 4.62e-06
t: -5.177635
B: 3.911121
Gene: BSCL2
Gene name: Bernardinelli-Seip congenital lipodystrophy 2 (seipin)

Chart 2: BSCL2 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: BSCL2 is expressed at significantly higher levels in the brain metastases of patients with metastatic breast cancer when compared to primary tumors of the breast.

The mRNA expression level of BSCL2 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 BSCL2 expression between primary tumors and brain metastases is $p < 0.00001$.

Figure 2: Significant correlation between BSCL2 primary tumor expression and distant metastasis-free survival in patients with breast cancer.

Depicted in this Kaplan-Meier plot is the probability of distant metastasis-free survival for $n=3951$ total patients stratified into two groups, based on low or high expression of BSCL2 in patient primary tumors. The log rank p -value denoting statistical significance of difference in distant metastasis-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 BSCL2 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 BSCL2 expression: 81.6 months

High BSCL2 expression: 122 months

Chart 3: Median distant metastasis-free survival is inferior in patients with low primary tumor expression of BSCL2.

The median DMFS (distant metastasis-free survival) of patients with low primary tumor expression of BSCL2 and high primary tumor expression of BSCL2 is listed in this chart.