Glioma tumor suppressor candidate region gene 2 is differentially expressed in the lymph node and brain metastases of patients with 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 to discover genes associated with brain metastasis in patients with metastatic breast cancer. We found that glioma tumor suppressor candidate region gene 2, encoded by GLTSCR2, was among the genes whose expression was most different in the brain and lymph node metastases of patients with metastatic breast cancer as compared to normal breast tissues. GLTSCR2 mRNA was present at significantly reduced quantities in brain metastatic tissues as compared to normal breast tissues. Down-regulation of glioma tumor suppressor candidate region gene 2 may be an important event for metastasis of primary tumor-derived cancer cells to the brain in humans with metastatic breast cancer. These data combined suggest some level of common origin for metastases that reside in the lymph nodes and colonize the brain.

Keywords: breast cancer, metastasis, brain metastases, central nervous system metastases, lymph node metastasis, GLTSCR2, glioma tumor suppressor candidate region gene 2, 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 trial 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 trastuzumab. 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 significant differential and decreased expression of glioma tumor suppressor candidate region gene 2, encoded by GLTSCR2, in brain metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE52604⁴ and GSE124648⁵ and for this global differential gene expression analysis of brain metastatic breast cancer in conjunction with GEO2R. GSE52604 was generated using Whole Human Genome Microarray 4x44K G4112F technology with $n=10$ normal breast tissues and $n=35$ brain metastases from patients with breast cancer, analysis performed using platform GPL6480. GSE124648, a microarray dataset, 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; platform GPL96 was used for this analysis. 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 GLTSCR2 gene expression was significantly between normal breast tissues and brain metastases in humans with breast cancer using a two-tailed, unpaired t-test with Welch's correction. We used PRISM for all statistical analyses of differential gene expression in human breast cancer (Version 8.4.0)(455).

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 and lymph node metastasis in human breast cancer in an unbiased fashion and for the discovery of novel therapeutic targets.

GLTSCR2 is differentially expressed in the brain metastases of patients with brain metastatic breast cancer.

Identification of differentially expressed genes across datasets in cancer can be difficult and more challenging than when doing so in benign organ tissue and sorted cell populations due to higher levels of heterogeneity in tumors, and differing methods of tumor sampling between laboratories. Thus, we asked how gene expression was most significantly different between brain metastases from patients with breast cancer and normal breast tissue as opposed to primary tumors of the breast, by comparing global gene expression profiles of 10 normal breast tissues to 35 brain metastases⁴. Using this strategy, we identified the glioma tumor suppressor candidate region gene 2, GLTSCR2, as among the genes whose expression was most significantly different in the brain metastases of patients with brain metastatic breast cancer⁴ (Table 1). When sorting each of the genes expressed in brain metastases based on significance of

1 difference in expression between brain metastases and normal breast tissues, GLTSCR2 ranked 26 out of
2 41093 total transcripts (Table 1), equating to 99.94% differential expression. Differential expression of
3 GLTSCR2 in brain metastases from patients with brain metastatic breast cancer in this dataset was
4 statistically significant ($p=8.02E-17$).

5 **GLTSCR2 is differentially expressed in the lymph node metastases of patients with metastatic** 6 **breast cancer.**

7 Next, we queried a second microarray dataset⁵ generated using lymph node metastases and
8 normal breast tissues from patients with breast cancer to determine whether we could validate differential
9 expression of GLTSCR2 in metastatic tissues of humans with breast cancer, and whether differential
10 expression of GLTSCR2 was specific to brain metastases or could be observed in metastases to other sites
11 in patients with metastatic breast cancer. We observed significant differential expression of a transcript
12 detected by a probe mapping to both small nucleolar RNA, C/D box 23 SNORD23 and GLTSCR2 in
13 lymph node metastases of patients with metastatic breast cancer. When sorting each of the genes
14 expressed in lymph node metastases based on significance of difference as compared to normal breast
15 tissues in patients with breast cancer, GLTSCR2 / SNORD23 ranked 1974 out of 22283 total transcripts
16 (Table 2), equating to 91.1% differential expression. Differential expression of GLTSCR2 / SNORD23 in
17 the lymph node metastases of patients with metastatic breast cancer was statistically significant (Table 2;
18 $p=6.04E-05$). Thus, differential expression of GLTSCR2 was conserved in the brain and lymph node
19 metastases of patients with metastatic breast cancer in two independent microarray datasets^{4,5}.

20 **GLTSCR2 is expressed at significantly lower levels in the brain metastases of patients with brain** 21 **metastatic breast cancer.**

22 We obtained exact mRNA expression levels for GLTSCR2, in normal breast tissues and in brain
23 metastasis of patients with brain metastatic breast cancer to determine direction and statistical
24 significance of change in GLTSCR2 expression in brain metastatic tissues. GLTSCR2 was expressed at
25 lower levels in the brain metastases of patients with breast cancer as compared to normal breast tissues,
26 and this difference was statistically significant (Figure 1; $p<0.0001$).

27 Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified
28 the interleukin GLTSCR2 as among the genes whose expression was most different, transcriptome-wide,
in the brain and lymph node metastases of patients with breast cancer when compared to the normal
breast; GLTSCR2 expression, as compared to the benign breast, was significantly lower in metastasis to
the brain.

29 **Discussion**

30 We provided evidence here that glioma tumor suppressor candidate region gene 2⁷⁻⁹, encoded by
31 GLTSCR2, is among the genes whose expression is most different in the brain and lymph node metastases
32 of patients with brain metastatic brain cancer. Evaluation of the effects of ectopic expression of
33 GLTSCR2 in mouse models of metastatic breast cancer on metastasis to the central nervous system are
34 merited. Differential expression of glioma tumor suppressor candidate region gene 2 in metastasis to the
35 lymph nodes and to the brain suggests some level of common origin for metastases that reside in the
36 lymph nodes, successfully evade immune clearance, and subsequently colonize the brain.

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Rank	ID	p-value	t	B	Gene	Gene name
26	A_23_P39125	8.02E-17	-12.7223735	27.910184	GLTSCR2	glioma tumor suppressor candidate region gene 2

Table 1: GLTSCR2 is differentially expressed in brain metastases in patients with metastatic breast cancer when compared to normal breast tissues.

Rank of differential expression, probe ID, *p*-value with respect to differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the two groups compared, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
1974	217807_s_at	6.04E-05	4.342246	1.210243	SNORD23/// GLTSCR2	small nucleolar RNA, C/D box 23///glioma tumor suppressor candidate region gene 2

Table 2: GLTSCR2 / SNORD23 is differentially expressed in lymph node metastases of patients with metastatic breast cancer when compared to normal breast tissue.

Rank of differential expression, probe ID, *p*-value with respect to differential expression, *t*, a moderated *t*-statistic, *B*, the log-odds of differential expression between the two groups compared, gene and gene name are listed in this chart.

Figure 1: GLTSCR2 is expressed at significantly lower levels in the brain metastases of patients with brain metastatic breast cancer as compared to normal breast tissues.

The mRNA expression level of GLTSCR2 in normal breast tissues (left) and in the brain metastases of brain metastatic breast cancer patients (right) is graphically represented here with mean GLTSCR2 levels marked and the result of a statistical test evaluating difference in mRNA expression level between brain metastases and normal tissues of the breast, a *p*-value, listed above.