CTC-338M12.4, an uncharacterized locus, is differentially expressed in 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 that an uncharacterized locus, CTC-338M12.4 (LOC101928649), 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. CTC-338M12.4 was also differentially expressed in primary tumors of the breast when compared to normal breast tissue. CTC-338M12.4 mRNA was present at significantly higher quantities in brain metastases as compared to primary tumors of the breast. Molecular functions and up-regulation of CTC-338M12.4 may be an important event for metastasis of primary tumor-derived cancer cells to the brain in humans with metastatic breast cancer.

Keywords: breast cancer, metastasis, brain metastases, central nervous system metastases, CTC-338M12.4, LOC101928649, systems biology of breast cancer, targeted therapeutics in breast cancer.

One report described a 34% incidence of central nervous system metastases in trastuzumab-treated patients with breast cancer². This alarmingly high frequency of CNS metastasis 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 increased expression of CTC-338M12.4 in brain metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE125989⁴ and GSE42568⁵ for this global differential gene expression analysis of brain metastatic breast cancer in conjunction with GEO2R. GSE125989 was generated using Affymetrix Human Genome U133A 2.0 Array technology with $n=16$ primary tumors from patients with breast cancer and $n=16$ brain metastases from patients with brain metastatic breast cancer. GSE42568 was generated using Affymetrix Human Genome U133 Plus 2.0 Array technology with $n=17$ normal breast tissues and $n=104$ primary tumors of the breast, analysis 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 CTC-338M12.4 gene expression was significantly between primary breast tumors 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 the primary and metastatic tumor tissues of patients with brain 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.

CTC-338M12.4 is differentially expressed in the brain metastases of patients with brain metastatic breast cancer.

We identified CTC-338M12.4, an uncharacterized locus also known as LOC101928649, 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 difference as compared to the primary tumors of patients with brain metastatic breast cancer, CTC-338M12.4 ranked 155 out of 22297 total transcripts (Table 1), equating to 99.3% differential expression. Differential expression of CTC-338M12.4 in the brain metastases of patients with brain metastatic breast cancer was statistically significant (Table 1; $p=9.11E-04$).

CTC-338M12.4 is differentially expressed in the primary tumors of patients with breast cancer.

We asked whether differential expression of CTC-338M12.4 was specific to metastatic breast cancer or whether CTC-338M12.4 expression was significantly different in primary tumors of the breast as compared to normal breast tissues. Comparison of global gene expression profiles of 17 normal breast

1 tissues to 104 primary tumors of the breast⁵ revealed that CTC-338M12.4 was also among the genes
2 whose expression was most significantly different transcriptome-wide between the normal breast tissues
3 and primary tumors of the breast in patients with breast cancer (Table 2). When sorting each of the genes
4 expressed in primary tumors of the breast based on significance of difference in expression between
5 primary tumors of the breast and normal breast tissues, CTC-338M12.4 ranked 2290 out of 54675 total
6 transcripts (Table 2), equating to 95.8% differential expression. Differential expression of
7 CTC-338M12.4 in primary tumors of the breast in human breast cancer was statistically significant (Table
8 2; $p=7.08E-03$).

9 **CTC-338M12.4 is expressed at significantly higher levels in the brain metastases of patients with** 10 **brain metastatic breast cancer.**

11 We obtained exact mRNA expression levels for CTC-338M12.4, in primary tumors of the breast
12 and in brain metastasis of patients with brain metastatic breast cancer to determine magnitude and
13 direction of change in CTC-338M12.4 expression in brain metastatic tissues. CTC-338M12.4 was
14 expressed at higher levels in the brain metastases of patients with breast cancer as compared to primary
15 tumors of the breast, and this difference was statistically significant (Figure 1; $p=0.0011$). We calculated
16 a mean fold change of 1.40 ± 0.12 in CTC-338M12.4 expression when comparing primary tumors of the
17 breast to brain metastatic tissues in patients with breast cancer (Table 1).

18 Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified
19 CTC-338M12.4 as among the genes whose expression was most different in the brain metastases of
20 patients with breast cancer when compared to primary tumors of the breast, and in primary tumors of the
21 breast when compared to normal breast tissue; CTC-338M12.4 expression was significantly higher in
22 brain metastases as compared to primary tumors of the breast.

23 **Discussion**

24 Progression of metastatic breast cancer proceeds in a linear fashion from transformation of the
25 breast, to subsequent development of a tumor in the breast - the primary tumor - and then in many
26 patients, development of metastases at distant sites including the brain. We provided evidence here that
27 CTC-338M12.4 is a transcriptional feature of human breast cancer, as its expression was among the most
28 significantly different transcriptome-wide, both in the brain metastases of patients with metastatic disease
when compared to primary tumors of the breast, and in primary tumors of the breast when compared to
normal breast tissue. There is little to no information available regarding the molecular functions of
CTC-338M12.4. Investigations surrounding the basic biology of CTC-338M12.4 may facilitate
understanding of the mechanisms by which metastasis to the brain occurs in humans with brain metastatic
breast cancer.

References

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Rank	ID	p-value	t	B	FC	Gene
155	219881_s_at	9.11E-04	3.6291241	-0.6535	1.40 ± 0.12	CTC-338M12.4

Table 1: CTC-338M12.4 is differentially expressed in brain metastases in metastatic breast cancer when compared to primary tumors of the breast.

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, fold change (FC) of CTC-338M12.4 expression between the brain metastases of patients with breast cancer and primary tumors of the breast, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
2290	219881_s_at	7.08E-03	-2.76	-2.520864	CTC-338M12.4	uncharacterized LOC101928649

Table 2: CTC-338M12.4 is differentially expressed in primary tumors of the breast 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.

CTC-338M12.4 (LOC101928649)

Figure 1: CTC-338M12.4 is expressed at significantly higher levels in the brain metastases of patients with brain metastatic breast cancer.

The mRNA expression level of CTC-338M12.4 in primary tumors of the breast (left) and in the brain metastases of brain metastatic breast cancer patients (right) is graphically represented here with mean CTC-338M12.4 levels marked and the result of a statistical test evaluating difference in mRNA expression level between brain metastases and primary tumors of the breast, a *p*-value, listed above.