RALGPS1 is over-expressed in brain metastatic breast cancer.

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Brain metastases are a clinical problem in patients with breast cancer¹⁻³. We mined published microarray data^{4,5} to discover genes associated with brain metastasis in patients with brain metastatic breast cancer. We found that RALGPS1 was among the genes most differentially expressed in the brain metastases of patients with brain metastatic breast cancer. RALGPS1 may be of relevance to the biology underlying metastasis to the brain, and it may be of relevance as a potential therapeutic target in patients with intractable disease.

Keywords: breast cancer, brain metastases, central nervous system metastases, RALGPS1, 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 metastases events demands an enhanced understanding of the transcriptional makeup of brain metastatic tissues to support identification of therapeutic targets. We performed a global comparative analysis of transcription in the brain metastases of patients with brain metastatic breast cancer^{4,5}. We discovered significant differential and over-expression of RALGPS1 in brain metastases from patients with brain metastatic breast cancer.

Methods

We used datasets GSE125989⁴ and GSE52604⁵ 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. 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. 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 RALGPS1 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} for the discovery of novel therapeutic targets.

RALGPS1 is differentially expressed in the brain metastases of patients with brain metastatic breast cancer.

We identified the Ral GEF with PH domain and SH3 binding motif 1, RALGPS1, as among the genes whose expression was most significantly different in the brain metastases of patients with brain metastatic breast cancer⁶ (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, RALGPS1 ranked 586 out of 22297 total transcripts (Table 1), equating to 97.4% differential expression. Differential expression of RALGPS1 in the brain metastases of patients with brain metastatic breast cancer was statistically significant (Table 1; $p=1.04E-02$).

Cross-validation of differentially expressed genes across datasets in cancer can be difficult and more challenging than when cross-validating across organs and sorted cell populations due to higher levels of heterogeneity in tumors, and differing methods of tumor sampling between laboratories. Thus, we asked whether we could validate differential expression of RALGPS1 when comparing brain metastases from patients with breast cancer to normal breast tissue as opposed to primary tumors of the breast. Comparison of global gene expression profiles of 10 normal breast tissues to 35 brain metastases⁷ revealed that RALGPS1 was among the genes whose expression was most significantly different transcriptome-wide between the breast and brain metastases in patients with breast cancer (Table 2). When sorting each of the genes expressed in brain metastases based on significance of difference in

expression between brain metastases and normal breast tissues, RALGPS1 ranked 4878 out of 41093 total transcripts (Table 2), equating to 88.1% differential expression. Differential expression of RALGPS1 in brain metastases from patients with brain metastatic breast cancer in this dataset was statistically significant (Table 2; $p=2.01E-04$).

RALGPS1 is expressed at significantly higher levels in the brain metastases of patients with brain metastatic breast cancer.

We obtained exact mRNA expression levels for RALGPS1, in primary tumors of the breast and in brain metastasis of patients with brain metastatic breast cancer to determine magnitude and direction of change in RALGPS1 expression in brain metastatic tissues. RALGPS1 was expressed at higher levels in the brain metastases of patients with breast cancer as compared to primary tumors of the breast, and this difference was statistically significant (Figure 1; $p=0.0020$). We calculated a mean fold change of 1.32 ± 0.29 in RALGPS1 expression when comparing primary tumors of the breast to brain metastatic tissues in patients with breast cancer (Table 1).

Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified RALGPS1 as among the genes whose expression was most different in the brain metastases of patients with breast cancer both when compared to primary tumors of the breast and to the normal breast; RALGPS1 was expressed at significantly higher levels in brain metastases as compared to primary tumors of the breast.

Discussion

RALGPS1 is a GEF, or guanine nucleotide exchange factor for the small G protein Ral⁶. When expressed in HEK 293T cells, RALGPS1 catalyzes the guanine nucleotide exchange of Ral, loading GTP onto to the RalGTPase⁶. Structurally, the protein contains a Src homology proline-rich motif in its middle region resembling that found in Grb2 and Nck, as well as a pleckstrin homology (PH) domain at its carboxy-terminus⁷. A comparative genomic hybridization analysis of breast cancer cell lines revealed an area of genomic gain at 9q33, where RALGPS1 is located; this was associated with increased expression of RALGPS1 at the mRNA level⁷. Information describing functions and roles for RALGPS1 is otherwise limited.

We report here that RALGPS1 is among the genes whose expression is most different in the brain metastases of patients with brain metastatic brain cancer. RALGPS1 may be of relevance to the biology underlying metastasis to the brain in patients with brain metastatic breast cancer, a disease with limited treatment options.

References

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Rank	ID	p-value	t	B	FC	Gene	Gene name
586	204199_at	1.04E-02	2.7109769	-2.7637	1.32 ± 0.29	RALGPS1	Ral GEF with PH domain and SH3 binding motif 1

Table 1: RALGPS1 is differentially expressed in brain metastases in brain metastatic breast cancer.

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 RALGPS1 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
4878	A_24_P53824	2.01E-04	4.0324216	0.287104	RALGPS1	Ral GEF with PH domain and SH3 binding motif 1

Table 2: RALGPS1 is differentially expressed in brain metastases in brain metastatic breast cancer.

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: RALGPS1 is expressed at significantly higher levels in the brain metastases of patients with brain metastatic breast cancer.

The mRNA expression level of RALGPS1 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 RALGPS1 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.