Rac3 is differentially expressed in the 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 Rac3 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. Rac3 mRNA was present at higher quantities in brain metastatic tissues as compared to primary tumors of the breast. Rac3 appears to be involved in the invasive properties of breast cancer cells⁶⁻⁹. Rac3 may be a molecule of interest when understanding the molecular mechanisms by which primary tumor-derived cells metastasize to the brain.

Keywords: breast cancer, metastasis, brain metastases, central nervous system metastases, lymph node metastasis, Rac3, 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 trial¹⁰ 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 setting¹⁰ 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 increased expression of Rac3, in brain metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE10893⁴ and GSE100534⁵ and 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 performed using platform GPL885. GSE100534⁵ was generated using Affymetrix Human Gene 1.0 ST Array technology with $n=16$ primary tumors from patients with breast cancer and $n=3$ brain metastases from patients with brain metastatic breast cancer. 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 Rac3 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.

Rac3 is differentially expressed in the brain metastases of patients with brain metastatic breast cancer.

Through analysis of published microarray data⁴, we identified Rac3 as a differentially expressed gene in the breast 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, Rac3 ranked 111 out of 22575 total transcripts (Table 1), equating to 99.5% differential expression. Differential expression of Rac3 in the brain metastases of patients with metastatic breast cancer was statistically significant (Table 1; $p=2.59E-04$).

Next, we queried a second microarray dataset⁵ to determine whether we could validate differential expression of Rac3 in brain metastatic tissues of humans with breast cancer. We again observed significant differential expression of Rac3 in brain metastases of patients with metastatic breast

1 cancer. When sorting each of the genes expressed in brain metastases based on significance of difference
2 as compared to primary tumors of the breast, Rac3 ranked 1286 out of 33297 total transcripts (Table 2),
3 equating to 96.1% differential expression. Differential expression of Rac3 in the brain metastases of
4 patients with metastatic breast cancer was statistically significant (Table 2; $p=4.62E-04$). Thus,
5 differential expression of Rac3 was conserved in the brain metastases of patients with metastatic breast
6 cancer in two independent microarray datasets^{4,5}.

7 **Rac3 is expressed at higher levels in the brain metastases of patients with brain metastatic breast** 8 **cancer.**

9 We obtained exact mRNA expression levels for Rac3, in brain metastasis and in primary tumors
10 of patients with brain metastatic breast cancer to determine direction and statistical significance of change
11 in Rac3 expression in brain metastatic tissues. Rac3 was expressed at higher levels in the brain
12 metastases of patients with breast cancer as compared to primary tumors of the breast, and this difference
13 approached the level of statistical significance (Figure 1; $p=0.0622$).

14 Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified
15 the interleukin Rac3 as among the genes whose expression was most different, transcriptome-wide, in the
16 brain metastases of patients with breast cancer when compared to primary tumors of the breast; Rac3
17 expression, as compared to the transformed breast, was higher in metastasis to the brain.

18 **Discussion**

19 We provided evidence here that Rac3 is among the genes whose expression is most different in
20 the primary tumors and brain metastases of patients with brain metastatic breast cancer. Rac3 is a small
21 GTP-binding protein with functions in regulation of cytoskeletal dynamics; Rac3 has been repeatedly
22 implicated in the invasiveness and aggressiveness of breast cancer cells⁶⁻⁹. Evaluation of the effects of
23 genetic depletion of Rac3 in mouse models of metastatic breast cancer on metastasis to the central
24 nervous system is merited.
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Rank	ID	p-value	t	B	Gene	Gene name
111	22172	2.59E-04	4.78	0.67799	RAC3	ras-related C3 botulinum toxin substrate 3 (rho family, small GTP binding protein Rac3)

Table 1: Rac3 is differentially expressed in brain metastases in patients with 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, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
1286	8010737	4.62E-04	4.1767647	-0.095527	RAC3	ras-related C3 botulinum toxin substrate 3 (rho family, small GTP binding protein Rac3)

Table 2: Rac3 is differentially expressed in brain metastases of patients with 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, gene and gene name are listed in this chart.

Figure 1: Rac3 is expressed at higher levels in the brain metastases of patients with brain metastatic breast cancer as compared to primary tumors of the breast.

The mRNA expression level of Rac3 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 Rac3 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.