The UBR2 E3 ubiquitin ligase is over-expressed in brain metastatic breast cancer.

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Post-translational modifications of proteins include the covalent conjugation of ubiquitin moieties to proteins by the catalytic function of E3 ligases¹. We found that the brain metastases of patients with brain metastatic breast cancer express significantly higher levels of the UBR2 E3 ligase as compared to primary tumors of the breast, through mining of published microarray data^{2,3}. The substrates of UBR2 ubiquitination may be important for the underlying biology of brain metastasis, and UBR2 could potentially represent a novel therapeutic target in a disease with limited treatment options.

Keyword: breast cancer, brain metastasis, central nervous system metastasis, ubiquitin, E3 ligase, UBR2, N-end rule pathway, systems biology of breast cancer, targeted therapeutics in breast cancer.

1 Patients treated with trastuzumab (Herceptin) face an incidence of central nervous system
2 metastasis of 34%⁴. This alarmingly high frequency of CNS metastases events demands an enhanced
3 understanding of the transcriptional makeup of brain metastatic tissues to support identification of
4 therapeutic targets. We performed a global comparative analysis of transcription in the brain metastases
5 of patients with brain metastatic breast cancer using published microarray data^{2,3}. We discovered
6 transcriptome-wide differential expression and over-expression of the UBR2 ubiquitin ligase in the brain
7 metastatic tissues of patients with breast cancer.

8 **Methods**

9 We utilized microarray datasets GSE43837² and GSE100534³ for this differential gene expression
10 analysis of brain metastatic breast cancer in conjunction with GEO2R. GSE43837 was generated using
11 Affymetrix Human X3P Array technology with $n=19$ primary breast tumors and $n=19$ brain metastases
12 from patients with breast cancer, analysis performed using platform GPL1352. GSE100534 was
13 generated using Affymetrix Human Gene 1.0 ST Array technology with $n=16$ primary tumors from
14 patients with breast cancer and $n=3$ brain metastases from patients with brain metastatic breast cancer.
15 The Benjamini and Hochberg method of p -value adjustment was used for ranking of differential
16 expression but raw p -values were used to assess statistical significance of global differential expression.
17 Log-transformation of data was auto-detected, and the NCBI generated category of platform annotation
18 was used. A statistical test was performed to evaluate whether UBR2 gene expression was significantly
19 between primary breast tumors and brain metastases in humans with breast cancer using a two-tailed,
20 unpaired t-test with Welch's correction. We used PRISM for all statistical analyses of differential gene
21 expression in human breast cancer (Version 8.4.0)(455).

22 **Results**

23 We mined published microarray data^{2,3} to identify genes most transcriptionally perturbed in the
24 brain metastases of patients with brain metastatic breast cancer.

25 **UBR2 is differentially expressed in the brain metastases of patients with brain metastatic breast cancer.**

26 We identified UBR2 as among the genes whose expression was most significantly different in the
27 brain metastases of patients with brain metastatic breast cancer when compared to primary tumors of the
28 breast. When sorting each of the genes expressed in brain metastases based on significance of change as
compared to primary tumors of the breast, UBR2 ranked 20 out of 61359 total transcripts (Table 1).
Differential expression of UBR2 in brain metastatic breast cancer was statistically significant (Table 1;
 $p=0.0000678$).

We queried a second microarray dataset to validate transcriptome-wide differential of UBR2 in
brain metastatic breast cancer. When sorting each of the genes expressed in brain metastases based on
significance of change as compared to primary tumors of the breast, UBR2 ranked 7256 out of 33297
total transcripts (Table 2). Differential expression of UBR2 in brain metastatic breast cancer in this
second dataset was statistically significant (Table 2; $p=4.05E-02$).

29 **UBR2 is expressed at significantly higher levels in the brain metastases of patients with brain metastatic breast cancer.**

1 We obtained exact mRNA expression levels of UBR2 to evaluate magnitude and direction of
2 change in UBR2 gene expression between brain metastases and primary tumors of the breast. UBR2 was
3 expressed at higher levels in brain metastases as compared to primary tumors of the breast, and this
4 difference was statistically significant (Figure 1; $p=0.0024$). We calculated a mean fold change of $2.82 \pm$
5 2.14 in UBR2 expression in brain metastases relative to primary breast tumors in patients with breast
6 cancer (Table 1).

7 Thus, we identified the E3 ubiquitin ligase UBR2 as among the genes whose expression was most
8 significantly different transcriptome-wide when comparing brain metastases and primary tumors of the
9 breast from patients with breast cancer. UBR2 was expressed at significantly higher levels in brain
10 metastases as compared to primary tumors of the breast.

11 Discussion

12 We recently reported that the deubiquitinases CYLD and USP2 were among the genes most
13 differentially expressed in the brain metastases of patients with brain metastatic breast cancer. We report
14 here that UBR2 was among the genes most differentially expressed in the brain metastases of patients
15 with brain metastatic breast cancer. Together these data point to dysregulation of the ubiquitin system in
16 central nervous metastases in patients with breast cancer.

17 UBR2⁵⁻⁸ functions as an E3 ligase for substrates in the N-end rule pathway, wherein substrates
18 with relatively short half-lives are targeted for proteasomal degradation through covalently modified by
19 N-recognins: UBR E3 ligases like UBR2, via a degradation signal termed an N-degron at the amino-
20 terminus of the substrate. As UBR2 was over-expressed in brain metastasis tissues relative to primary
21 breast tumors, UBR2 small molecule inhibition can be explored as a means to pursuing novel therapeutic
22 targets in patients with brain metastatic breast cancer. The substrates of UBR2 may be of relevance to the
23 biology by which metastatic breast cancer cells home to and colonize the brain.

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Rank	ID	p-value	t	B	FC	Gene	Gene name
20	Hs.15303.0.S1_3p_a_at	0.0000678	4.4126419	1.31645	2.82 ± 2.14	UBR2	ubiquitin protein ligase E3 component n-recognin 2

Table 1: UBR2 is differentially expressed in brain metastases in patients with 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 UBR2 expression between the brain metastases of patients with breast cancer and primary tumors of the breast, and gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
7256	8119529	4.05E-02	-2.1905555	-4.274424	UBR2	ubiquitin protein ligase E3 component n-recognin 2

Table 2: UBR2 is differentially expressed in brain metastases in patients with 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: UBR2 is expressed at significantly higher levels in the brain metastases of patients with breast cancer as compared to primary tumors of the breast.

The mRNA expression level of UBR2 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 UBR2 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.