Deubiquitinases USP2 and CYLD are differentially expressed in brain metastatic breast cancer.

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The ubiquitin pathway is an elaborate system by which a single molecule can adopt multiple configurations based on the manner in which it is adjoined to create varied “chain” types thus conferring specificity to its generation and destruction based on chain type¹. The best known type of ubiquitin is the lysine 48 (K48)-linked variant, which is the canonical cellular signal for proteasomal degradation²⁻¹⁰. We found here using published microarray data^{11,12} that the deubiquitinases CYLD and USP2 are among the genes most differentially expressed in the brain metastases of patients with breast cancer. We observed significant over-expression of CYLD and repression of USP2 in brain metastases; thus, modulation of activity or expression levels of these deubiquitinases may represent viable therapeutic avenues in brain metastatic breast cancer.

Keywords: breast cancer, ubiquitin, deubiquitinases, brain metastases, central nervous system metastases, USP2, CYLD, 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 cancer¹³. 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^{11,12}. We discovered significant differential expression of USP2 and CYLD, two deubiquitinase enzymes important for the cleavage of ubiquitin chains, in brain metastases from patients with brain metastatic breast cancer.

Methods

We used datasets GSE125989¹¹ and GSE100534¹² 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. GSE100534 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 USP2 or CYLD 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 for the discovery of novel therapeutic targets.

USP2 is differentially expressed in the brain metastases of patients with brain metastatic breast cancer.

We identified the deubiquitinase USP2¹⁴⁻¹⁵ as among the genes whose expression was most significantly different in the brain metastases of patients with brain metastatic breast cancer (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, USP2 ranked 92 out of 22297 total transcripts (Table 1). Differential expression of USP2 in the brain metastases of patients with brain metastatic breast cancer was statistically significant (Table 1; $p=8.77E-03$).

We mined a second microarray dataset to attempt to validate global differential expression of USP2 in brain metastatic breast cancer. Again, we found that USP2 was among the genes whose expression changed most significantly in the brain metastases of patients with brain metastatic breast cancer. 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, USP2 ranked 3923 out of 33297 total transcripts (Table 2). Differential expression of USP2 in the brain metastases of patients with brain metastatic breast cancer was again statistically significant (Table 2; $p=3.12E-04$).

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

We also identified the deubiquitinase and cylindromatosis tumor suppressor CYLD¹⁶⁻¹⁹ as among the genes whose expression was most significantly different in the brain metastases of patients with brain metastatic breast cancer (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, USP2 ranked 471 out of 22297 total transcripts (Table 3). Differential expression of CYLD in the brain metastases of patients with brain metastatic breast cancer was statistically significant (Table 3; $p=7.23\text{E-}03$).

We mined a second microarray dataset to attempt to validate global differential expression of CYLD in brain metastatic breast cancer. Again, we found that CYLD was among the genes whose expression changed most significantly in the brain metastases of patients with brain metastatic breast cancer. 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, CYLD ranked 456 out of 33297 total transcripts (Table 4). Differential expression of CYLD in the brain metastases of patients with brain metastatic breast cancer was again statistically significant (Table 4; $p=2.88\text{E-}05$).

USP2 is expressed at significantly lower levels in the brain metastases of patients with brain metastatic breast cancer.

We obtained exact mRNA expression levels for USP2, 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 USP2 expression in brain metastatic tissues. USP2 was expressed at lower 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; $p=0.0004$). We calculated a mean fold change of 0.655 ± 0.274 in USP2 expression when comparing primary tumors of the breast to brain metastatic tissues in patients with breast cancer (Table 1).

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

We obtained exact mRNA expression levels for CYLD, 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 CYLD expression in brain metastatic tissues. CYLD 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 2; $p=0.00023$). We calculated a mean fold change of 1.328 ± 0.277 in CYLD expression when comparing primary tumors of the breast to brain metastatic tissues in patients with breast cancer (Table 2).

Thus, by mining published microarray data in an unbiased and systematic fashion, we identified the deubiquitinases CYLD and USP2 as among the genes whose expression was most significantly different in the brain metastases of patients with breast cancer; while CYLD was expressed at significantly higher levels, USP2 was expressed at significantly lower levels in brain metastases as compared to primary tumors of the breast.

Discussion

CYLD has been described as a tumor suppressor¹⁷ but molecules can have varying functions based on cellular context. We found that CYLD was expressed at significantly higher levels in the brain metastases of patients with brain metastatic breast cancer; it is possible this is a reflection of an unsuccessful attempt to control tumor cell proliferation; it is also possible this implies that CYLD may not always function in a tumor suppressive manner.

We report here that the deubiquitinating enzymes USP2 and CYLD are both among the genes whose expression is most different in the brain metastases of patients with brain metastatic brain cancer.

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Rank	ID	p-value	t	B	FC	Gene	Gene name
92	207211_at	3.12E-04	-4.0058919	0.2878	0.655 ± 0.274	USP2	ubiquitin specific peptidase 2

Table 1: USP2 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 USP2 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
3923	7952249	8.77E-03	2.902947	-2.883578	USP2	ubiquitin specific peptidase 2

Table 2: USP2 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.

Rank	ID	p-value	t	B	FC	Gene	Gene name
471	60084_at	7.23E-03	2.8552126	-2.4544	1.328 ± 0.277	CYLD	CYLD lysine 63 deubiquitinase

Table 3: CYLD 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 CYLD 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
456	7995552	2.88E-05	-5.3755224	2.574614	CYLD	CYLD lysine 63 deubiquitinase

Table 4: CYLD 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: USP2 is expressed at significantly lower levels in the brain metastases of patients with brain metastatic breast cancer.

The mRNA expression level of USP2 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 USP2 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.

Figure 2: CYLD is expressed at significantly higher levels in the brain metastases of patients with brain metastatic breast cancer.

The mRNA expression level of CYLD 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 CYLD 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.