UBXD4 is a differentially expressed gene in brain metastatic human 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 for the discovery of genes associated with brain metastasis in humans with metastatic breast cancer. We found that UBX domain containing 4, encoded by UBXD4, 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. UBXD4 mRNA was present at decreased quantities in brain metastatic tissues as compared to primary tumors of the breast. Importantly, expression of UBXD4 in primary tumors was significantly correlated with patient post-progression survival. Modulation of UBXD4 expression may be relevant to the biology by which tumor cells metastasize from the breast to the brain in humans with metastatic breast cancer.

Keywords: breast cancer, metastasis, brain metastases, central nervous system metastases, UBX domain containing 4, UBXD4, 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 trial⁶ 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 trastuzumab⁶. The alarmingly high rate of central nervous system metastasis described, as well as data, both anecdotal² and from a randomized, controlled setting⁶ 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 differential and increased expression of the gene encoding UBX domain containing 4, UBXD4, in brain metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE10893⁴ and GSE42568⁵ 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 was performed using platform GPL885. GSE42568 was generated using Affymetrix Human Genome U133 Plus 2.0 array technology with $n=17$ normal breast tissue biopsies and $n=104$ primary breast tumor biopsies from patients with breast cancer; analysis was 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 UBXD4 gene expression was significantly different between primary tumors of the breast and brain metastases in humans with breast cancer using a two-tailed test. For Kaplan-Meier survival analysis, we used the Kaplan-Meier plotter online tool⁷ for correlation of UBXD4 mRNA expression levels with post-progression survival in $n=180$ breast cancer patients.

Results

We performed global comparative transcriptome analysis of metastatic and primary tumor tissues of patients with 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.

UBXD4 is differentially expressed in the brain metastases of patients with brain metastatic breast cancer.

Through blind, systems-level analysis of published microarray data⁴, we identified UBX domain containing 4, encoded by UBXD4, 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, UBXD4 ranked 232 out of 17418 total transcripts (Chart 1), equating to 98.7% differential expression. Differential expression of UBXD4 in the brain metastases of patients with metastatic breast cancer was statistically significant (Chart 1; $p=6.81e-04$).

To validate transcriptome-wide differential expression of UBXD4 in human breast cancer, we queried a second microarray dataset⁵, here comparing normal breast tissues and primary tumors of the breast. Again, we identified UBXD4 as a differentially expressed gene in the tumor tissues of patients with breast cancer (Chart 2). When sorting each of the genes expressed in brain metastases based on significance of difference as compared to normal breast tissues, UBXD4 ranked 14733 out of 54675 total transcripts (Chart 2), equating to 73.1% differential expression. Differential expression of UBXD4 in the primary tumors of patients with breast cancer was statistically significant (Chart 2; $p=7.11\text{e-}03$). Thus, differential expression of UBXD4, transcriptome-wide, in the tumor tissues of women with breast cancer was conserved across two independent microarray datasets, both in primary and metastatic tumor tissues.

UBXD4 is expressed at lower levels in the brain metastases of patients with metastatic breast cancer.

We obtained exact mRNA expression levels for UBXD4, in primary tumors of the breast and in brain metastasis of patients with brain metastatic breast cancer to determine direction and statistical significance of change in UBXD4 expression in brain metastatic tissues. We observed significantly lower expression of UBXD4 in the brain metastases of patients with breast cancer as compared to primary tumors of the breast: UBXD4 was expressed at -0.52 ± 0.52 arbitrary units (AU) in primary tumors of the breast, while it was expressed at -1.69 ± 0.35 AU in brain metastatic tissues (Figure 1). The difference in UBXD4 mRNA levels between primary tumors of the breast and brain metastatic tissues was statistically significant (Figure 1; $p=0.001234$).

UBXD4 expression is significantly correlated with survival outcomes in human breast cancer.

We performed Kaplan-Meier survival analysis⁷ in $n=180$ breast cancer patients in total, to evaluate whether UBXD4 tumor expression was correlated with survival outcomes in breast cancer. We observed a statistically significant correlation between primary tumor expression of UBXD4 and post-progression survival (PPS) in patients with breast cancer, in the lower survival quartile (Figure 2). Patients whose primary tumors expressed low levels of UBXD4 possessed median PPS of 34.68 months, while patients whose tumors expressed high levels of UBXD4 possessed median PPS of 20.4 months. This difference in PPS based on UBXD4 tumor expression in patients with breast cancer was statistically significant (Figure 2, Chart 3; logrank p -value: 0.034; hazard ratio: 1.62 (1.03-2.56)).

Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified UBX domain containing 4, encoded by UBXD4, as among the genes whose expression was most different, transcriptome-wide, in the brain metastases of patients with breast cancer, both when compared to primary tumors of the breast and to normal breast tissues; we observed significantly decreased expression of UBXD4 in brain metastases as compared to primary tumors of the breast. Further, we found a significant correlation between UBXD4 expression and patient survival outcomes, as post-progression survival was significantly greater in patients whose primary tumors expressed higher levels of UBXD4 as compared to patients whose primary tumors expressed lower levels of UBXD4.

Discussion

We provided evidence here that UBX domain containing 4, encoded by UBXD4, is among the genes whose expression is most different in the brain metastases of patients with brain metastatic breast cancer, that UBXD4 mRNA is present at significantly decreased quantities in brain metastatic tissues as compared to primary tumors of the breast, that UBXD4 is also differentially expressed in primary tumors of patients with breast cancer, and that primary tumor expression of UBXD4 is significantly correlated

1 with post-progression survival in patients with breast cancer. Evaluation of the effects of genetic depletion
2 of UBXD4 in mouse models of metastatic breast cancer on metastasis to the central nervous system is
3 merited. Modulation of UBXD4 expression may be relevant to the processes by which breast cancer cells
4 exit the breast, enter the vasculature and/or lymphatics, reside in the lymph nodes, evade immune
5 clearance, breach the blood-brain barrier and colonize the brain.
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Rank: 232
Probe ID: 10783
p-value: 6.81e-04
t: 4.21
B: -0.238684
Gene: UBXD4
Gene name: UBX domain containing 4

Chart 1: UBXD4 is differentially expressed in brain metastatic breast cancer when comparing brain metastases to primary tumors of the breast.

The rank of global differential expression, the probe/transcript ID, the *p*-value with respect to differential expression transcriptome-wide, *t*, a moderated t-statistic, *B*, the log-odds of differential expression between the groups compared, the gene and gene name are listed in this chart.

Rank: 14733
probe ID: 228605_at
p-value: 7.11e-03
t: 2.74
B: -3.5526106
Gene: UBXN2A
Gene name: UBX domain protein 2A

Chart 2: UBXD4, also known as UBXN2A, is differentially expressed in human breast cancer when comparing primary tumors of the breast to normal breast tissues.

The rank of global differential expression, probe/transcript ID, the *p*-value with respect to differential expression transcriptome-wide, *t*, a moderated *t*-statistic, *B*, the log-odds of differential expression between the groups compared, the gene and gene name are listed in this chart.

Figure 1: UBXD4 is expressed at significantly lower levels in the brain metastases of patients with metastatic breast cancer when compared to primary tumors of the breast.

The mRNA expression level of UBXD4 in primary tumors of the breast (left) and in brain metastases of women with metastatic breast cancer (right) is graphically depicted; the result of a statistical UBXD4 evaluating significance of difference in UBXD4 expression between primary tumors of the breast and brain metastases is $p=0.001234$.

Figure 2: Significant correlation between UBXD4 primary tumor expression and post-progression survival in patients with breast cancer.

Depicted in this Kaplan-Meier plot is the probability of post-progression survival for $n=180$ total patients stratified into two groups, based on low or high expression of UBXD4 in patient primary tumors, in the lower survival quartile. The log rank p -value denoting statistical significance of difference in post-progression survival when comparing the two groups, as well as hazard ratio for this comparison is listed above. Listed below is the number of patients at risk (number of patients alive) per interval, after stratification based on UBXD4 expression; in the first interval, number at risk is number of patients alive; in each subsequent interval, number at risk is the number at risk less those who have expired or are censored.

1 Low UBXD4 expression: 34.68 months

2 High UBXD4 expression: 20.4 months

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4 **Chart 3: Median post-progression survival is inferior in patients with low primary tumor expression of UBXD4.**

5 The median PPS (post-progression survival) of patients with low primary tumor expression of UBXD4
6 and high primary tumor expression of UBXD4 is listed in this chart, for $n=180$ breast cancer patients, in
7 the lower survival quartile.