UPF2 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 regulator of nonsense transcripts 2, encoded by UPF2, 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. UPF2 mRNA was present at decreased quantities in brain metastatic tissues as compared to primary tumors of the breast. Expression of UPF2 in primary tumors was significantly correlated with patient post-progression survival. Modulation of UPF2 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, regulator of nonsense transcripts 2, UPF2, 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 regulator of nonsense transcripts 2, UPF2, in brain metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE10893⁴ and GSE43837⁵ 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. GSE43837 was generated using Affymetrix Human X3P Array technology with $n=19$ primary tumors of the breast and $n=19$ brain metastases from patients with breast cancer; analysis was 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 UPF2 gene expression was significantly different between primary tumors of the breast and brain metastases in humans with breast cancer using a two-tailed t-test. For Kaplan-Meier survival analysis, we used the Kaplan-Meier plotter online tool⁷ for correlation of UPF2 mRNA expression levels with post-progression survival in $n=414$ patients; auto cut-off was selected.

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 metastasis in human breast cancer in an unbiased fashion and for the discovery of novel therapeutic targets.

UPF2 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 regulator of nonsense transcripts 2, encoded by UPF2, 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, UPF2 ranked 132 out of 17418 total transcripts (Chart 1), equating to 99.2% differential expression. Differential expression of UPF2 in the brain metastases of patients with metastatic breast cancer was statistically significant (Chart 1; $p=2.84e-04$).

To attempt to validate differential transcriptome-wide differential expression of UPF2 in brain metastases in human metastatic breast cancer, we queried a second microarray dataset⁵; again, we identified UPF2 as a differentially expressed gene in the brain metastatic 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 primary tumors of the breast, UPF2 ranked 7150 out of 61359 total transcripts (Chart 2), equating to 88.3% differential expression. Differential expression of UPF2 in the brain metastases of patients with metastatic breast cancer approached statistical significance (Chart 2; $p=0.05593963$). Thus, transcriptome-wide differential expression of UPF2 in the brain metastases of women with metastatic breast cancer was conserved across two independent microarray datasets, indicating that this may likely be a *bona fide* biological phenomena associated with metastatic breast cancer in humans.

UPF2 is expressed at lower levels in the brain metastases of patients with metastatic breast cancer.

We obtained exact mRNA expression levels for UPF2, 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 UPF2 expression in brain metastatic tissues. We observed significantly lower expression of UPF2 in the brain metastases of patients with breast cancer as compared to primary tumors of the breast: UPF2 was expressed at 0.48 ± 0.48 units (AU) in primary tumors of the breast, while it was expressed at -0.69 ± 0.24 AU in brain metastatic tissues. The difference in UPF2 mRNA levels between primary tumors of the breast and brain metastatic tissues was statistically significant (Figure 1; $p=0.000498$).

UPF2 expression is significantly correlated with survival outcomes in human breast cancer.

We performed Kaplan-Meier survival analysis⁸ in 414 breast cancer patients in total, to evaluate whether UPF2 tumor expression was correlated with survival outcomes in breast cancer. We observed a statistically significant correlation between primary tumor expression of UPF2 and post-progression survival (PPS) in patients with breast cancer (Figure 2). Patients whose primary tumors expressed low levels of UPF2 possessed median PPS of 36.66 months, while patients whose tumors expressed high levels of UPF2 possessed median PPS of 26.8 months. This difference in OS based on UPF2 tumor expression in patients with breast cancer trended towards statistical significance (Figure 2, Chart 3; logrank p -value: 0.065; hazard ratio: 1.26 (0.99-1.61)).

Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified regulator of nonsense transcripts 2, encoded by UPF2, as among the genes whose expression was most different, transcriptome-wide, in the brain metastases of patients with breast cancer when compared to primary tumors of the breast; we observed significantly decreased expression of UPF2 in brain metastases as compared to primary tumors of the breast. Further, we found a significant correlation between UPF2 expression and patient survival outcomes, as post-progression survival was significantly greater in patients whose tumors expressed lower levels of UPF2 as compared to patients whose tumors expressed higher levels of UPF2.

Discussion

We provided evidence here that regulator of nonsense transcripts 2, encoded by UPF2, is among the genes whose expression is most different in the brain metastases of patients with brain metastatic breast cancer, that UPF2 mRNA is present at significantly decreased quantities in brain metastatic tissues as compared to primary tumors of the breast, and that primary tumor UPF2 expression is significantly correlated with patient survival outcomes in human breast cancer. Evaluation of the effects of genetic depletion of UPF2 in mouse models of metastatic breast cancer on metastasis to the central

nervous system is merited. Modulation of UPF2 expression may be relevant to the processes by which breast cancer cells exit the breast, enter the vasculature and/or lymphatics, reside in the lymph nodes, evade immune clearance, breach the blood-brain barrier and colonize the brain.

References

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Rank: 132
Probe ID: 20451
p-value: 2.84e-04
t: 4.64
B: 0.566274
Gene: UPF2
Gene name: regulator of nonsense transcripts homolog 2

Chart 1: UPF2 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: 7150
probe ID: g11693131_3p_a_at
p-value: 0.05593963
t: -1.9648255
B: -3.97165
Gene: UPF2
Gene name: regulator of nonsense transcripts 2

Chart 2: UPF2 is differentially expressed in brain metastatic breast cancer when comparing brain metastases to primary tumors of the breast.

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: UPF2 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 UPF2 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 test evaluating significance of difference in UPF2 expression between primary tumors of the breast and brain metastases is $p=0.000498$.

Figure 2: Significant correlation between UPF2 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 $n=414$ total patients with breast cancer stratified into two groups, based on low or high expression of UPF2 in patient primary tumors. 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 UPF2 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 UPF2 expression: 36.66 months

2 High UPF2 expression: 26.8 months

3
4 **Chart 3: Median post-progression survival is inferior in patients with high primary tumor expression of UPF2.**

5 The median PPS (post-progression survival) of patients with low primary tumor expression of UPF2 and
6 high primary tumor expression of UPF2 is listed in this chart, for $n=414$ breast cancer patients.