HOXC10 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 the homeobox C10, encoded by HOXC10, 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. HOXC10 mRNA was present at increased quantities in brain metastatic tissues as compared to primary tumors of the breast. Importantly, expression of HOXC10 in primary tumors was significantly correlated with patient overall survival. Modulation of HOXC10 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, the homeobox C10, HOXC10, 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 the homeobox C10, HOXC10, in brain metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE10893⁴ and GSE52604⁵ 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. GSE52604 was generated using Agilent-014850 Whole Human Genome Microarray 4x44K G4112F with $n=10$ normal breast tissues and $n=35$ 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 HOXC10 gene expression was significantly different between primary tumors of the breast and brain metastases in humans with breast cancer using an unpaired two-tailed t-test with Welch's correction; PRISM 9.0 was utilized for statistical analyses. For Kaplan-Meier survival analysis, we used the Kaplan-Meier plotter online tool⁷ for correlation of HOXC10 mRNA expression levels with overall survival in $n=1402$ breast cancer patients.

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.

HOXC10 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 the

homeobox C10, encoded by HOXC10, 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, HOXC10 ranked 143 out of 17418 total transcripts (Chart 1), equating to 99.2% differential expression. Differential expression of HOXC10 in the brain metastases of patients with metastatic breast cancer was statistically significant (Chart 1; $p=3.16E-04$).

Differential gene expression analyses that utilize primary and metastatic tumor tissues to discover genes associated with metastasis can be challenging due to the relatively heterogeneous cellular composition and constitution of the tumor as compared to normal, untransformed (benign) tissues. Thus, to attempt to validate differential transcriptome-wide differential expression of HOXC10 in brain metastases in human metastatic breast cancer, we queried a second microarray dataset⁵, here comparing normal breast tissues rather than primary tumors of the breast to brain metastases. Again, we identified HOXC10 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 normal breast tissues, HOXC10 ranked 6440 out of 41093 total transcripts (Chart 2), equating to 84.3% differential expression. Differential expression of HOXC10 in the brain metastases of patients with metastatic breast cancer was statistically significant (Chart 2; $p=6.86E-04$). Thus, differential expression of HOXC10, transcriptome-wide, in the brain metastases of women with metastatic breast cancer was conserved across two independent microarray datasets, both when compared to benign and transformed breast tissues.

HOXC10 is expressed at higher levels in the brain metastases of patients with metastatic breast cancer.

We obtained exact mRNA expression levels for HOXC10, 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 HOXC10 expression in brain metastatic tissues. HOXC10 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 1; $p=0.000949$).

HOXC10 expression is significantly correlated with survival outcomes in human breast cancer.

We performed Kaplan-Meier survival analysis⁸ in $n=1402$ breast cancer patients in total, to evaluate whether HOXC10 tumor expression was correlated with survival outcomes in breast cancer. We observed a statistically significant correlation between primary tumor expression of HOXC10 and overall survival (OS) in patients with breast cancer in the lower survival quartile (Figure 2). Patients whose primary tumors expressed low levels of HOXC10 possessed median OS of 120 months, while patients whose tumors expressed high levels of HOXC10 possessed median OS of 85.2 months. This difference in OS based on HOXC10 tumor expression in patients with breast cancer was statistically significant (Figure 2, Chart 3; logrank p -value: 0.035; hazard ratio: 1.32 (1.02-1.71)).

1 Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we
2 identified the homeobox C10, encoded by HOXC10, as among the genes whose expression was
3 most different, transcriptome-wide, in the brain metastases of patients with breast cancer, both
4 when compared to primary tumors of the breast and to normal breast tissues; we observed
5 significantly increased expression of HOXC10 in brain metastases as compared to primary
6 tumors of the breast. Further, we found a significant correlation between HOXC10 expression
and patient survival outcomes, as overall survival was significantly greater in patients whose
primary tumors expressed higher levels of HOXC10 as compared to patients whose primary
tumors expressed lower levels of HOXC10.

7 **Discussion**

8 We provided evidence here that the homeobox C10, encoded by HOXC10, is among the
9 genes whose expression is most different in the brain metastases of patients with brain metastatic
10 breast cancer, that HOXC10 mRNA is present at significantly increased quantities in brain
11 metastatic tissues as compared to primary tumors of the breast, and that primary tumor HOXC10
12 expression is significantly correlated with patient survival outcomes in human breast cancer.
13 Evaluation of the effects of genetic depletion of HOXC10 in mouse models of metastatic breast
14 cancer on metastasis to the central nervous system is merited. Modulation of HOXC10
15 expression may be relevant to the processes by which breast cancer cells exit the breast, enter the
16 vasculature and/or lymphatics, reside in the lymph nodes, evade immune clearance, breach the
17 blood-brain barrier and colonize the brain. Further, blinded identification of brain-specific
18 differential gene expression in breast cancer cells that have metastasized to the brain
19 demonstrates the power of our unbiased, systems-level discovery approach.
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Rank: 143
Probe ID: 6562
p-value: 3.16E-04
t: -4.58
B: 0.4697
Gene: HOXC10
Gene name: homeobox C10

Chart 1: HOXC10 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: 6440
probe ID: A_24_P218805
p-value: 6.86E-04
t: -3.6359314
B: -0.872878
Gene: HOXC10
Gene name: homeobox C10

Chart 2: HOXC10 is differentially expressed in brain metastatic breast cancer when comparing brain metastases 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: HOXC10 is expressed at significantly higher levels in the brain metastases of patients with metastatic breast cancer when compared to primary tumors of the breast.

The mRNA expression level of HOXC10 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 HOXC10 expression between primary tumors of the breast and brain metastases, a p -value, is $p=0.000949$.

Figure 2: Significant correlation between HOXC10 primary tumor expression and overall survival in patients with breast cancer.

Depicted in this Kaplan-Meier plot is the probability of overall survival for $n=1402$ total patients stratified into two groups, based on low or high expression of HOXC10 in patient primary tumors. The log rank p -value denoting statistical significance of difference in overall 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 HOXC10 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.

Low HOXC10 expression: 120 months
High HOXC10 expression: 85.2 months

Chart 3: Median overall survival is inferior in patients with high primary tumor expression of HOXC10.

The median OS (overall survival) of patients with low primary tumor expression of HOXC10 and high primary tumor expression of HOXC10 is listed in this chart, for $n=1402$ breast cancer patients, in the lower survival quartile.