SIX1 is a differentially expressed gene in human metastatic breast cancer, in the brain and in the lymph nodes.

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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 six homeobox 1, encoded by SIX1, was among the genes whose expression was most different in the brain and lymph node metastases of patients with metastatic breast cancer. SIX1 mRNA was present at increased quantities in brain metastatic tissues as compared to primary tumors of the breast. Importantly, expression of SIX1 in primary tumors was significantly correlated with patient overall survival in patients with breast cancer. Modulation of SIX1 expression may be relevant to the biology by which tumor cells metastasize from the breast to the brain while evading immune clearance in the lymph nodes in humans with metastatic breast cancer. These data are one piece of evidence suggesting a common ancestor or tumor clone for brain and lymph node metastases that originate from the primary tumor, alluding to patterns in developmental origin and migratory pathways through the lymph node in human brain metastatic breast cancer.

Keywords: breast cancer, metastasis, brain metastases, central nervous system metastases, lymph node metastases, six homeobox 1, SIX1, 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 six homeobox 1, SIX1, in brain metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE10893⁴ and GSE124648⁵ 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. GSE124648 was generated using Affymetrix Human Genome U133A array technology with $n=10$ normal breast tissues and $n=44$ lymph node metastases from patients with breast cancer; analysis was performed using platform GPL96. 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 SIX1 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 SIX1 mRNA expression levels in primary tumors of the breast with overall survival in $n=1402$ breast cancer patients, with auto cut-off selected.

Results

We performed global comparative transcriptome analysis of metastatic and primary tumor tissues of patients with metastatic breast, as well as normal tissues of the breast 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.

SIX1 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 six homeobox 1, encoded by SIX1, as a differentially expressed gene in the brain 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, SIX1 ranked 201 out of 17418 total transcripts (Chart 1), equating to 98.8% differential expression. Differential expression of SIX1 in the brain metastases of patients with metastatic breast cancer was statistically significant (Chart 1; $p=8.89e-04$).

SIX1 is differentially expressed in the lymph node metastases of patients with breast cancer.

To determine whether differential expression of SIX1 in human breast cancer could be validated in a second microarray dataset, and whether this was specific to brain metastases or could be observed in other anatomical locations, we queried a second microarray dataset⁵, here comparing normal breast tissues to lymph node metastases. Again, we identified SIX1 as a differentially expressed gene in the lymph node metastatic tissues of patients with breast cancer (Chart 2). When sorting each of the genes expressed in lymph node metastases based on significance of difference as compared to normal breast tissues, SIX1 ranked 6390 out of 22283 total transcripts (Chart 2), equating to 71.3% differential expression. Differential expression of SIX1 in the lymph node metastases of patients with metastatic breast cancer approached the level of statistical significance (Chart 2; $p=1.84\text{e-}02$). Thus, differential expression of SIX1, transcriptome-wide, in metastatic tissues of women with metastatic breast cancer was conserved across two independent microarray datasets, in the brain and in the lymph nodes.

SIX1 is expressed at higher levels in the brain metastases of patients with metastatic breast cancer.

We obtained exact mRNA expression levels for SIX1, 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 SIX1 expression in brain metastatic tissues. We observed significantly higher expression of SIX1 in the brain metastases of patients with breast cancer as compared to primary tumors of the breast: SIX1 was expressed at -0.12 ± 1.21 arbitrary units (AU) in primary tumors of the breast, while it was expressed at 2.65 ± 0.49 AU in brain metastatic tissues (Figure 1). The difference in SIX1 mRNA levels between primary tumors of the breast and brain metastatic tissues was statistically significant (Figure 1; $p=0.002547$).

SIX1 expression is correlated with survival outcomes in human breast cancer.

We performed Kaplan-Meier survival analysis⁷ in 1402 breast cancer patients in total, to evaluate whether SIX1 tumor expression was correlated with survival outcomes in breast cancer. We observed a correlation between primary tumor expression of SIX1 and overall survival (OS) in patients with breast cancer (Figure 2). Patients whose primary tumors expressed low levels of SIX1 possessed median OS of 130 months, while patients whose tumors expressed high levels of SIX1 possessed median OS of 80.65 months. This difference in OS based on SIX1 tumor expression in patients with breast cancer was statistically significant (Figure 2, Chart 3; logrank p -value 0.0034; hazard ratio: 1.38 (1.11-1.71)).

Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified six homeobox 1, encoded by SIX1, as among the genes whose expression was most different, transcriptome-wide, in the brain and lymph node metastases of patients with breast cancer, when compared to primary tumors of the breast and to normal breast tissues, respectively; we observed significantly increased expression of SIX1 in brain metastases as compared to primary tumors of the breast. Further, we found a correlation between SIX1 expression and patient survival outcomes, as overall survival was greater in patients whose primary tumors expressed lower levels of SIX1 as compared to patients whose primary tumors expressed higher levels of SIX1.

Discussion

We provided evidence here that SIX1 is among the genes whose expression is most different in the brain and lymph node metastases of patients with metastatic breast cancer, that SIX1 mRNA is present at significantly increased quantity in brain metastatic tissues as compared to primary tumors of the breast, and that primary tumor SIX1 expression is significantly correlated with patient survival outcomes in human breast cancer. Evaluation of the effects of genetic depletion of SIX1 in mouse models of metastatic breast cancer on metastasis to the central nervous system is merited. Modulation of SIX1 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. These data are one piece of evidence that allude to a common cell or cell(s) of origin for brain and lymph node metastases in human metastatic breast cancer.

References

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Rank: 201
Probe ID: 3311
p-value: 8.89e-04
t: -4.15
B: -0.4574
Gene: SIX1
Gene name: six homeobox 1

Chart 1: SIX1 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: 6390
probe ID: 205817_at
p-value: 1.84e-02
t: -2.429163
B: -4.140376
Gene: SIX1
Gene name: six homeobox 1

Chart 2: SIX1 is differentially expressed in the lymph node metastases of patients with breast cancer when comparing lymph node 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: SIX1 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 SIX1 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 SIX1 expression between primary tumors and brain metastases is $p=0.002547$.

Figure 2: Significant correlation between SIX1 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 breast cancer patients stratified into two groups, based on low or high expression of SIX1 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 SIX1 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 SIX1 expression: 130 months
High SIX1 expression: 80.65 months

Chart 3: Median overall survival is inferior in patients with high primary tumor expression of SIX1.

The median OS (overall survival) of patients with low primary tumor expression of SIX1 and high primary tumor expression of SIX1 is listed in this chart, for $n=1402$ breast cancer patients.