MAGIX 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 MAGI family member, X-linked, encoded by MAGIX, 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. MAGIX mRNA was present at increased quantities in brain metastatic tissues as compared to primary tumors of the breast. Expression of MAGIX in primary tumors was significantly correlated with patient recurrence-free survival. Modulation of MAGIX 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, MAGI family member, X-linked, MAGIX, 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 MAGI family member, X-linked, MAGIX, in brain metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE10893⁴ and GSE125989⁵ 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. GSE125989 was generated using Affymetrix Human Genome U133A 2.0 array technology with $n=16$ primary breast tumors and $n=16$ brain metastases from patients with breast cancer; the samples utilized by investigators for this microarray dataset were paired, matched tumor and metastasis samples; analysis was performed using platform GPL571. 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 MAGIX 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 tool for correlation of MAGIX mRNA expression levels with recurrence-free survival in $n=3951$ 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.

MAGIX 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 MAGI family member, X-linked, encoded by MAGIX, 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, MAGIX ranked 119 out of 17418 total transcripts (Chart 1), equating to 99.3% differential expression. Differential expression of MAGIX in the brain metastases of patients with metastatic breast cancer was statistically significant (Chart 1; $p=2.36E-04$).

We queried a second microarray dataset⁵ to attempt to validate transcriptome-wide differential expression of MAGIX in human brain metastatic breast cancer. Again, we identified MAGIX 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 in patients with breast cancer, in this dataset, MAGIX ranked 2538 out of 22277 total transcripts (Chart 2), equating to 88.6% differential expression. Differential expression of MAGIX in the brain metastases of patients with metastatic breast cancer in this dataset was statistically significant (Chart 2; $p=7.84E-02$). Thus, differential expression of MAGIX, transcriptome-wide, in the brain metastases of women with metastatic breast cancer was conserved across two independent microarray datasets.

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

We obtained exact mRNA expression levels for MAGIX, 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 MAGIX expression in brain metastatic tissues. MAGIX 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; $p=0.000217$).

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

We performed Kaplan-Meier survival analysis⁷ to evaluate correlation in 3951 breast cancer patients in total, to evaluate whether MAGIX tumor expression was correlated with survival outcomes in breast cancer. We observed a statistically significant correlation between primary tumor expression of MAGIX and recurrence-free survival (RFS) (Figure 2). Patients whose primary tumors expressed low levels of MAGIX possessed median RFS of 40.44 months, while patients whose tumors expressed high levels of MAGIX possessed median RFS of 58 months. This difference in OS based on MAGIX tumor expression in breast cancer was statistically significant (Figure 2, Chart 3; logrank p -value: $9.3e-5$; hazard ratio: 0.8 (0.72-0.9)).

Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified the MAGI family member, X-linked, MAGIX, as among the genes whose expression was most different, transcriptome-wide, in the brain metastases of patients with breast cancer

1 when compared to primary tumors of the breast; we observed significantly increased expression
2 of MAGIX in brain metastases as compared to primary tumors of the breast. Further, we found a
3 significant correlation between MAGIX expression and patient survival outcomes, as
4 recurrence-free survival was significantly higher in patients whose primary tumors expressed
5 higher levels of MAGIX as compared to patients whose primary tumors expressed lower levels of
6 MAGIX.

7 **Discussion**

8 We provided evidence here that the MAGI family member, X-linked, encoded by MAGIX,
9 is among the genes whose expression is most different in the brain metastases of patients with
10 brain metastatic breast cancer, that messenger RNA for MAGIX is present at significantly
11 increased quantities in the brain metastatic tissues of patients with metastatic breast cancer as
12 compared to primary tumors of the breast and that primary tumor MAGIX expression is
13 significantly correlated with patient survival outcomes in human breast cancer. Evaluation of the
14 effects of genetic depletion of MAGIX in mouse models of metastatic breast cancer on metastasis
15 to the central nervous system is merited. Changes in MAGIX expression may be relevant to the
16 processes by which tumor cells exit the breast, enter the vasculature and/or lymphatics, reside in
17 the lymph nodes, evade immune clearance, breach the blood-brain barrier and colonize the brain.
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Rank: 119
Probe ID: 17015
p-value: 2.36E-04
t: -4.73
B: 0.738909
Gene: MAGIX
Gene name:MAGI family member, X-linked

Chart 1: MAGIX 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: 2538
Probe ID: 219965_s_at
p-value: 7.84E-02
t: 1.8091122
B: -4.4898
Gene: MAGIX
Gene name: MAGI family member, X-linked

Chart 2: MAGIX 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 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: MAGIX 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 MAGIX in primary tumors of the breast (left) and in brain metastases from women diagnosed with breast cancer (right) is graphically depicted; the result of a statistical test evaluating significance of difference in MAGIX expression between primary tumors and brain metastases is $p=0.000217$.

Figure 2: Significant correlation between MAGIX primary tumor expression and recurrence-free survival in patients with breast cancer.

Depicted in this Kaplan-Meier plot is the probability of recurrence-free survival for $n=3951$ total patients stratified into two groups, based on low or high expression of MAGIX in patient primary tumors. The log rank p -value denoting statistical significance of difference in recurrence-free 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 MAGIX 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 MAGIX expression: 228.85 months
High MAGIX expression: 216.66 months

Chart 3: Median recurrence-free survival is inferior in patients with high primary tumor expression of MAGIX.

The median RFS (recurrence-free survival) of patients with low primary tumor expression of MAGIX and high primary tumor expression of MAGIX is listed in this chart.