LRRC46 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 leucine rich repeat containing 46, encoded by LRRC46, 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. LRRC46 mRNA was present at increased quantities in brain metastatic tissues as compared to primary tumors of the breast. Expression of LRRC46 in primary tumors was significantly correlated with patient distant metastasis-free and overall survival. Modulation of LRRC46 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, leucine rich repeat containing 46, LRRC46, 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 leucine rich repeat containing 46, LRRC46, 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 LRRC46 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 LRRC46 mRNA expression levels with distant metastasis-free survival in $n=664$ breast cancer patients and overall survival in $n=626$ 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.

LRRC46 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 leucine rich repeat containing 46, encoded by LRRC46, 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, LRRC46 ranked 55 out of 17418 total transcripts (Chart 1), equating to 99.7% differential expression. Differential expression of LRRC46 in the brain metastases of patients with metastatic breast cancer was statistically significant (Chart 1; $p=1.01E-04$).

To attempt to validate differential transcriptome-wide differential expression of LRRC46 in brain metastases in human metastatic breast cancer, we queried a second microarray dataset⁵; again, we identified LRRC46 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, LRRC46 ranked 12099 out of 61359 total transcripts (Chart 2), equating to 80.3% differential expression. Differential expression of LRRC46 in the brain metastases of patients with metastatic breast cancer was not deemed statistically significant (Chart 2; $p=0.10698325$). Thus, transcriptome-wide differential expression of LRRC46 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.

LRRC46 is expressed at higher levels in the brain metastases of patients with metastatic breast cancer.

We obtained exact mRNA expression levels for LRRC46, 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 LRRC46 expression in brain metastatic tissues. LRRC46 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; $p<0.00001$).

LRRC46 expression is significantly correlated with survival outcomes in human breast cancer.

We performed Kaplan-Meier survival analysis⁸ in 626 breast cancer patients in total, to evaluate whether LRRC46 tumor expression was correlated with survival outcomes in breast cancer. We observed a statistically significant correlation between primary tumor expression of LRRC46 and overall survival (OS) in patients with breast cancer (Figure 2). Patients whose primary tumors expressed low levels of LRRC46 possessed median OS of 63.36 months, while patients whose tumors expressed high levels of LRRC46 possessed median OS of 121.2 months. This difference in OS based on LRRC46 tumor expression in patients with breast cancer was statistically significant (Figure 2, Chart 3; logrank p -value: 0.003; hazard ratio: 0.62 (0.45-0.85)). We also observed a statistically significant correlation between distant metastasis-free survival and primary tumor expression of LRRC46 in 664 breast cancer patients (Figure 3; logrank p -value: 0.0054; hazard ratio: 0.63 (0.45-0.87)).

Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified leucine rich repeat containing 46, encoded by LRRC46, 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 increased

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2 expression of LRRC46 in brain metastases as compared to primary tumors of the breast. Further,
3 we found a significant correlation between LRRC46 expression and patient survival outcomes, as
4 overall survival was significantly greater in patients whose primary tumors expressed higher
5 levels of LRRC46 as compared to patients whose primary tumors expressed lower levels of
6 LRRC46.

7 **Discussion**

8 We provided evidence here that leucine rich repeat containing 46, encoded by LRRC46, is
9 among the genes whose expression is most different in the brain metastases of patients with brain
10 metastatic breast cancer, that LRRC46 mRNA is present at significantly increased quantities in
11 brain metastatic tissues as compared to primary tumors of the breast, and that primary tumor
12 LRRC46 expression is significantly correlated with patient survival outcomes in human breast
13 cancer. Evaluation of the effects of genetic depletion of LRRC46 in mouse models of metastatic
14 breast cancer on metastasis to the central nervous system is merited. Modulation of LRRC46
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.
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Rank: 55
Probe ID: 20401
p-value: 1.01E-04
t: -5.15
B: 1.512966
Gene: LRRC46
Gene name: leucine rich repeat containing 46

Chart 1: LRRC46 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: 12099
probe ID: Hs.130767.0.S1_3p_a_at
p-value: 0.10698325
t: 1.6464683
B: -4.43995
Gene: LRRC46
Gene name: leucine rich repeat containing 46

Chart 2: LRRC46 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: LRRC46 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 LRRC46 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 LRRC46 expression between primary tumors of the breast and brain metastases is $p < 0.00001$.

Figure 2: Significant correlation between LRRC46 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=626 total patients stratified into two groups, based on low or high expression of LRRC46 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 LRRC46 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 LRRC46 expression: 63.36 months

High LRRC46 expression: 121.2 months

Chart 3: Median overall survival is inferior in patients with low primary tumor expression of LRRC46.

The median OS (overall survival) of patients with low primary tumor expression of LRRC46 and high primary tumor expression of LRRC46 is listed in this chart, for $n=626$ breast cancer patients.

Figure 2: Significant correlation between LRRC46 primary tumor expression and distant metastasis-free survival in patients with breast cancer.

Depicted in this Kaplan-Meier plot is the probability of distant metastasis-free survival for $n=664$ total patients stratified into two groups, based on low or high expression of LRRC46 in patient primary tumors. The log rank p -value denoting statistical significance of difference in distant metastasis-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 LRRC46 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.