LRRC8C is differentially expressed in lymph node metastasis in human breast cancer.

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Metastasis to the brain is a clinical problem in patients with breast cancer¹⁻³. Between the breast and the brain reside the secondary lymphoid organ, the lymph nodes. We mined published microarray data^{4,5} to compare primary and metastatic tumor transcriptomes for the discovery of genes associated with lymph node metastasis in humans with metastatic breast cancer. We found that leucine rich repeat containing 8 family member C, encoded by LRRC8C, was among the genes whose expression was most different in the lymph node metastases of patients with metastatic breast cancer as compared to primary tumors of the breast. Analysis of a separate microarray dataset revealed that LRRC8C was also differentially expressed in brain metastatic tissues⁵. LRRC8C mRNA was present at increased quantities in lymph node metastatic tissues as compared to primary tumors of the breast. Expression of LRRC8C in primary tumors was correlated with patient overall survival in lymph node positive patients but not in lymph node negative patients. Modulation of LRRC8C expression may be relevant to the biology by which tumor cells metastasize from the breast to the lymph node in humans with metastatic breast cancer.

Keywords: breast cancer, metastasis, lymph node metastases, central nervous system metastases, leucine rich repeat containing 8 family member C, LRRC8C, 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 lymph node 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 lymph node metastatic breast cancer^{4,5}. We discovered differential and increased expression of the gene encoding leucine rich repeat containing 8 family member C, LRRC8C, in lymph node metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE10893⁴ and GSE43837⁵ for this global differential gene expression analysis of lymph node 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 $n=3$ lymph node 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 $n=19$ lymph node 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 LRRC8C gene expression was significantly different between primary tumors of the breast and lymph node 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 LRRC8C mRNA expression levels with overall survival in $n=180$ lymph node negative patients and $n=230$ lymph node positive 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 lymph node metastasis in human breast cancer in an unbiased fashion and for the discovery of novel therapeutic targets.

LRRC8C is differentially expressed in the lymph node metastases of patients with lymph node metastatic breast cancer.

Through blind, systems-level analysis of published microarray data⁴, we identified leucine rich repeat containing 8 family member C, encoded by LRRC8C, 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 lymph node metastases based on significance of difference as compared to primary tumors of the breast in patients with breast cancer, LRRC8C ranked 123 out of 18034 total transcripts (Chart 1), equating to 99.3% differential expression. Differential expression of LRRC8C in the lymph node metastases of patients with metastatic breast cancer was statistically significant (Chart 1; $p=1.16e-03$).

To attempt to validate differential transcriptome-wide differential expression of LRRC8C in human metastatic breast cancer, we queried a second microarray dataset⁵; here, we identified LRRC8C 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, LRRC8C ranked 8067 out of 61359 total transcripts (Chart 2), equating to 86.9% differential expression. Differential expression of LRRC8C in the brain metastases of patients with metastatic breast cancer trended towards statistical significance (Chart 2; $p=0.06480016$). Thus, transcriptome-wide differential expression of LRRC8C in the metastatic tissues of women with metastatic breast cancer was conserved across two independent microarray datasets, in the lymph nodes and in the brain, indicating that this may likely be a *bona fide* biological phenomena associated with metastatic breast cancer in humans.

LRRC8C is expressed at higher levels in the lymph node metastases of patients with metastatic breast cancer.

We obtained exact mRNA expression levels for LRRC8C, in primary tumors of the breast and in lymph node metastasis of patients with lymph node metastatic breast cancer to determine direction and statistical significance of change in LRRC8C expression in lymph node metastatic tissues. We observed significantly higher expression of LRRC8C in the lymph node metastases of patients with breast cancer as compared to primary tumors of the breast: LRRC8C was expressed at -0.05 ± 0.56 units (AU) in primary tumors of the breast, while it was expressed at 0.70 ± 0.51 AU in lymph node metastatic tissues. The difference in LRRC8C mRNA levels between primary tumors of the breast and lymph node metastatic tissues was statistically significant (Figure 1; $p=0.001282$).

LRRC8C expression is significantly correlated with survival outcomes in human breast cancer.

We performed Kaplan-Meier survival analysis⁸ in $n=230$ lymph node positive patients and $n=180$ lymph node negative patients, to evaluate whether LRRC8C tumor expression was correlated with survival outcomes in breast cancer. We observed a correlation between primary tumor expression of LRRC8C and overall survival (OS) in lymph node positive patients with breast cancer, in the upper survival quartile (Figure 2). Lymph node positive patients whose primary tumors expressed low levels of LRRC8C possessed median OS of 58.68 months, as compared to 36 months for lymph node positive patients whose tumors expressed high levels of LRRC8C. This difference in OS based on LRRC8C tumor expression in lymph node positive patients with breast cancer trended towards statistical significance (Figure 2, Chart 3; logrank p -value: 0.098; hazard ratio: 1.57 (0.92-2.68)). Primary tumor expression of LRRC8C was not correlated with LRRC8C expression in lymph node negative breast cancer patients (Figure 2, Chart 3; logrank p -value: 0.6; hazard ratio: 0.75 (0.26-2.21)).

Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified leucine rich repeat containing 8 family member C, encoded by LRRC8C, as among the genes whose expression was most different, transcriptome-wide, in the lymph node metastases of patients with breast cancer when compared to primary tumors of the breast; we observed significantly increased expression of LRRC8C in lymph node metastases as compared to primary tumors of the breast. Further, we observed a correlation between LRRC8C expression and patient survival outcomes, as overall survival was greater in patients whose tumors expressed low levels of LRRC8C as compared to patients whose tumors expressed high levels of LRRC8C, in lymph node positive patients but not in lymph node negative patients.

Discussion

We provided evidence here that leucine rich repeat containing 8 family member C, encoded by LRRC8C, is among the genes whose expression is most different in the lymph node metastases of patients with lymph node metastatic breast cancer, that LRRC8C mRNA is present at significantly increased quantities in lymph node metastatic tissues as compared to primary tumors of the breast, and that primary tumor LRRC8C expression is correlated with patient survival outcomes in lymph node positive human breast cancer. Evaluation of the effects of genetic depletion of LRRC8C in mouse models of metastatic breast cancer on metastasis to the lymph nodes and to the central nervous system is merited. Modulation of LRRC8C 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-lymph node barrier and colonize the lymph node.

References

1. Lin, N.U., Amiri-Kordestani, L., Palmieri, D., Liewehr, D.J. and Steeg, P.S., 2013. CNS metastases in breast cancer: old challenge, new frontiers.
2. Bendell, J.C., Domchek, S.M., Burstein, H.J., Harris, L., Younger, J., Kuter, I., Bunnell, C., Rue, M., Gelman, R. and Winer, E., 2003. Central nervous system metastases in women who receive trastuzumab-based therapy for metastatic breast carcinoma. *Cancer*, 97(12), pp.2972-2977.
3. Tsukada, Y., Fouad, A., Pickren, J.W. and Lane, W.W., 1983. Central nervous system metastasis from breast carcinoma autopsy study. *Cancer*, 52(12), pp.2349-2354.
4. Weigman, V.J., Chao, H.H., Shabalín, A.A., He, X., Parker, J.S., Nordgard, S.H., Grushko, T., Huo, D., Nwachukwu, C., Nobel, A. and Kristensen, V.N., 2012. Basal-like Breast cancer DNA copy number losses identify genes involved in genomic instability, response to therapy, and patient survival. *Breast cancer research and treatment*, 133(3), pp.865-880.
5. McMullin, R.P., Wittner, B.S., Yang, C., Denton-Schneider, B.R., Hicks, D., Singavarapu, R., Moulis, S., Lee, J., Akbari, M.R., Narod, S.A. and Aldape, K.D., 2014. A BRCA1 deficient-like signature is enriched in breast cancer lymph node metastases and predicts DNA damage-induced poly (ADP-ribose) polymerase inhibitor sensitivity. *Breast Cancer Research*, 16(2), pp.1-10.
6. Awada, A., Colomer, R., Inoue, K., Bondarenko, I., Badwe, R.A., Demetriou, G., Lee, S.C., Mehta, A.O., Kim, S.B., Bachelot, T. and Goswami, C., 2016. Neratinib plus paclitaxel vs trastuzumab plus paclitaxel in previously untreated metastatic ERBB2-positive breast cancer: the NEfERT-T randomized clinical trial. *JAMA oncology*, 2(12), pp.1557-1564.
7. Györfy, B., Lanczky, A., Eklund, A.C., Denkert, C., Budczies, J., Li, Q. and Szallasi, Z., 2010. An online survival analysis tool to rapidly assess the effect of 22,277 genes on breast cancer prognosis using microarray data of 1,809 patients. *Breast cancer research and treatment*, 123(3), pp.725-731.

Rank: 123

Probe ID: 1747

p-value: 1.16e-03

t: -3.3725755

B: -0.9337

Gene: LRRC8C

Gene name: LRRC8C leucine rich repeat containing 8 family member C homolog

Chart 1: LRRC8C is differentially expressed in lymph node metastatic breast cancer when comparing lymph node 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: 8067

probe ID: Hs.266331.0.A1_3p_a_at

p-value: 0.06480016

t: 1.895383

B: -4.07965

Gene: LRRC8C

Gene name: leucine rich repeat containing 8 family member C

Chart 2: LRRC8C is differentially expressed in lymph node metastatic breast cancer when comparing lymph node 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: LRRC8C is expressed at significantly higher levels in the lymph node metastases of patients with metastatic breast cancer when compared to primary tumors of the breast.

The mRNA expression level of LRRC8C in primary tumors of the breast (left) and in lymph node metastases of women with metastatic breast cancer (right) is graphically depicted; the result of a statistical test evaluating significance of difference in LRRC8C expression between primary tumors of the breast and lymph node metastases is $p=0.001282$.

Figure 2: LRRC8C primary tumor expression correlates with overall survival in patients with lymph node positive breast cancer but not in lymph node negative patients.

Depicted in these Kaplan-Meier plots is the probability of overall survival in $n=230$ patients with lymph node positive breast cancer (**left**) and $n=180$ patients with lymph node negative breast cancer (**right**), stratified into two groups, based on low or high expression of LRRC8C in patient primary tumors, in the upper survival quartile. 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 each graph is the number of patients at risk (number of patients alive) per interval, after stratification based on LRRC8C 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.

Lymph node positive patients

Low LRRC8C expression: 58.68 months

High LRRC8C expression: 36 months

Chart 3: Median overall survival is inferior in lymph node positive breast cancer patients with high primary tumor expression of LRRC8C.

The median OS (overall survival) of patients with low primary tumor expression of LRRC8C and high primary tumor expression of LRRC8C is listed in this chart, for $n=230$ lymph node positive breast cancer patients.