Adenylyl cyclase 10 is differentially expressed in the lymph nodes of patients with metastatic 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 metastasis to the lymph nodes in humans with metastatic breast cancer. We found that adenylyl cyclase 10, encoded by ADCY10, 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. ADCY10 mRNA was present at increased quantities in lymph node metastases as compared to primary tumors of the breast. Importantly, expression of ADCY10 in primary tumors of the breast was correlated with patient recurrence-free survival. Modulation of ADCY10 expression may be relevant to the biology by which tumor cells metastasize from the breast to the lymph nodes and the brain in humans with metastatic breast cancer.

Keywords: breast cancer, metastasis, brain metastasis, central nervous system metastasis, lymph node metastasis, adenylyl cyclase 10 , ADCY10, 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 and lymph node metastatic tissues to support identification of therapeutic targets, whether they are treatment related or not. The lymph nodes reside between the breast and the brain. We performed a global comparative analysis of lymph node metastatic tumor tissues in patients with metastatic breast cancer, compared to primary tumors and normal breast tissues^{4,5}. We discovered transcriptome-wide differential expression of the gene encoding adenylyl cyclase 10, ADCY10, in lymph node metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE10893⁴ and GSE42568⁵ 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 $n=71$ primary breast tumors and $n=7$ lymph node metastases from patients with breast cancer; analysis was performed using platform GPL887. GSE42568 was generated using Affymetrix Human Genome U133 Plus 2.0 array technology with $n=17$ normal breast tissue biopsies and $n=104$ primary breast tumor biopsies from patients with breast cancer; analysis was performed using platform GPL570. 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 ADCY10 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-ADCY10t. For Kaplan-Meier survival analysis, we used the Kaplan-Meier plotter online tool⁷ for correlation of ADCY10 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 metastasis in human breast cancer in an unbiased fashion and for the discovery of novel therapeutic targets.

ADCY10 is differentially expressed in the lymph node metastases of patients with metastatic breast cancer.

Through blind, systems-level analysis of published microarray data⁴, we identified adenylyl cyclase 10, encoded by ADCY10, as a differentially expressed gene in the lymph node 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, ADCY10 ranked 22 out of 18034 total transcripts (Chart 1), equating to 99.9% differential expression. Differential expression of ADCY10 in the lymph node metastases of patients with metastatic breast cancer was statistically significant (Chart 1; $p=6.34e-05$).

To validate transcriptome-wide differential expression of ADCY10 in human breast cancer, we queried a second microarray dataset⁵, here comparing normal breast tissues and primary tumors of the breast. Here, we identified a pseudogene transcript produced at the ADCY10 locus, ADCY10P1, as a differentially expressed 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, ADCY10P1 ranked 16577 out of 54675 total transcripts (Chart 2), equating to 69.7% differential expression. Differential expression of ADCY10P1 in the primary tumors of patients with breast cancer was statistically significant (Chart 2; $p=1.43\text{e-}02$). Thus, transcriptome-wide differential expression of ADCY10, coding and non-coding, in the tumor tissues of women with breast cancer was conserved across two independent microarray datasets, both in primary and metastatic tumor tissues.

ADCY10 is expressed at higher levels in the lymph node metastases of patients with metastatic breast cancer.

We obtained exact mRNA expression levels for ADCY10, in primary tumors of the breast and in lymph node metastases of patients with metastatic breast cancer to determine direction and statistical significance of change in ADCY10 expression in lymph node metastatic tissues. We observed significantly increased expression of ADCY10 in the lymph node metastases of patients with breast cancer as compared to primary tumors of the breast: ADCY10 was expressed at 0.29 ± 0.25 arbitrary units (AU) in primary tumors of the breast, while it was expressed at 0.80 ± 0.64 AU in lymph node metastatic tissues (Figure 1). The difference in ADCY10 mRNA levels between primary tumors of the breast and lymph node metastases was statistically significant (Figure 1; $p=0.000048$).

ADCY10 expression is significantly correlated with survival outcomes in human breast cancer.

We performed Kaplan-Meier survival analysis⁷ in $n=3951$ breast cancer patients in total, to evaluate whether ADCY10 tumor expression was correlated with survival outcomes in breast cancer. We observed a statistically significant correlation between primary tumor expression of ADCY10 and recurrence-free survival (RFS) in patients with breast cancer (Figure 2). Patients whose primary tumors expressed low levels of ADCY10 possessed median RFS of 216.66 months, while patients whose tumors expressed high levels of ADCY10 possessed median RFS of 191.21 months. This difference in RFS based on ADCY10 tumor expression in patients with breast cancer was statistically significant (Figure 2, Chart 3; logrank p -value: $2\text{e-}07$; hazard ratio: $0.75 (0.67\text{-}0.84)$).

Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified ADCY10 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 ADCY10 in lymph node metastases as compared to primary tumors of the breast. Further, we found a significant correlation between ADCY10 expression and patient survival outcomes, as recurrence-free survival was significantly greater in patients whose primary tumors expressed lower levels of ADCY10 as compared to patients whose primary tumors expressed higher levels of ADCY10.

Discussion

We provided evidence here that adenylyl cyclase 10, ADCY10, is among the genes whose expression is most different in the lymph node metastases of patients with metastatic breast cancer as compared to primary tumors of the breast, that ADCY10 mRNA is present at significantly increased quantities in lymph node metastatic tissues as compared to primary tumors of the breast, and that primary

tumor ADCY10 expression is significantly correlated with patient survival outcomes in human breast cancer. Evaluation of the effects of genetic depletion of ADCY10 in mouse models of metastatic breast cancer on metastasis to the central nervous system is merited. Modulation of ADCY10 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.

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., Shabalin, 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. Clarke, C., Madden, S.F., Doolan, P., Aherne, S.T., Joyce, H., O'driscoll, L., Gallagher, W.M., Hennessy, B.T., Moriarty, M., Crown, J. and Kennedy, S., 2013. Correlating transcriptional networks to breast cancer survival: a large-scale coexpression analysis. *Carcinogenesis*, 34(10), pp.2300-2308.
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: 22
Probe ID: 8097
p-value: 6.34e-05
t: -4.2254668
B: 1.5441
Gene: ADCY10
Gene name: adenylyl cyclase 10 (soluble)

Chart 1: ADCY10 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: 16577
Probe ID: 236832_at
p-value: 1.43e-02
t: -2.49
B: -4.1782215
Gene: ADCY10P1
Gene name: adenylyl cyclase 10 (soluble), pseudogene 1

Chart 2: ADCY10 is differentially expressed in human breast cancer when comparing primary tumors of the breast to normal breast tissues.

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.

Figure 1: ADCY10 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 ADCY10 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 ADCY10t evaluating significance of difference in ADCY10 expression between primary tumors of the breast and lymph node metastases, a p -value, is $p=0.000048$.

Figure 2: Significant correlation between ADCY10 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 ADCY10 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 ADCY10 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.

1 Low ADCY10 expression: 216.66 months

2 High ADCY10 expression: 191.21 months

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4 **Chart 3: Median recurrence-free survival is inferior in patients with __ primary tumor expression of ADCY10.**

5 The median RFS (recurrence-free survival) of patients with low primary tumor expression of ADCY10
6 and high primary tumor expression of ADCY10 is listed in this chart, for $n=3951$ breast cancer patients,
7 for $n=1133$ lymph node positive patients, and $n=2020$ lymph node negative patients.
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