The histone lysine acetyltransferase KAT2B is differentially expressed in the lymph node metastases of patients with 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 histone lysine acetyltransferase 2B, KAT2B, 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 KAT2B was also differentially expressed in brain metastatic tissues. KAT2B mRNA was present at increased quantities in lymph node metastases as compared to primary tumors of the breast. Importantly, expression of KAT2B in primary tumors of the breast was correlated with patient recurrence-free survival, in lymph node positive patients but not in lymph node negative patients. Modulation of KAT2B 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, metastatic breast cancer, histone lysine acetyltransferase 2B, KAT2B, 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 as compared to primary tumors of the breast^{4,5}. We discovered transcriptome-wide differential expression of the gene encoding histone lysine acetyltransferase 2B, KAT2B, in lymph node metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE10893⁴ and GSE100534⁵ 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. GSE100534 was generated using Affymetrix Human Gene 1.0 ST Array with $n=16$ primary breast tumors and $n=3$ brain metastases from patients metastatic breast cancer; analysis was performed using platform GPL6244. 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 KAT2B 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 KAT2B mRNA expression levels with recurrence-free survival in $n=1133$ lymph node positive breast cancer patients and $n=2020$ lymph node negative 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.

KAT2B 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 the histone lysine acetyltransferase 2B, encoded by KAT2B, 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, KAT2B ranked 224 out of 18034 total transcripts (Chart 1), equating to 98.8% differential expression. Differential expression of KAT2B in the lymph node metastases

of patients with metastatic breast cancer was statistically significant (Chart 1; $p=2.73\text{e-}03$).

To attempt to validate transcriptome-wide differential expression of KAT2B in metastatic tissues of patients with metastatic breast cancer, we queried a second microarray dataset⁵, here comparing primary tumors from patients with breast cancer to brain metastases. Again, we identified KAT2B as a differentially expressed gene in human brain metastatic 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, KAT2B ranked 5561 out of 41093 total transcripts (Chart 2), equating to 86.5% differential expression. Differential expression of KAT2B in the brain metastases of patients with metastatic breast cancer was statistically significant (Chart 2; $p=2.11\text{e-}02$). Thus, differential expression of KAT2B, transcriptome-wide, in the metastatic tissues of women with metastatic breast cancer was conserved across two independent microarray datasets. This suggests that KAT2B differential expression in human metastatic breast cancer is a *bona fide* biological process.

KAT2B is expressed at higher levels in the lymph node metastases of patients with metastatic breast cancer.

We obtained exact mRNA expression levels for KAT2B, 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 KAT2B expression in lymph node metastatic tissues. We observed significantly increased expression of KAT2B in the lymph node metastases of patients with breast cancer as compared to primary tumors of the breast: KAT2B was expressed at 0.16 ± 0.26 arbitrary units (AU) in primary tumors of the breast, while it was expressed at 0.66 ± 0.63 AU in lymph node metastatic tissues (Figure 1). The difference in KAT2B mRNA levels between primary tumors of the breast and lymph node metastases was statistically significant (Figure 1; $p=0.000104$).

KAT2B expression is correlated with survival outcomes in human breast cancer.

We performed Kaplan-Meier survival analysis⁷ in $n=1133$ lymph node positive breast cancer patients and $n=2020$ lymph node negative breast cancer patients, to evaluate whether KAT2B tumor expression was correlated with survival outcomes in breast cancer, and whether lymph node status of the patient influenced this correlation. We observed a correlation between primary tumor expression of KAT2B and recurrence-free survival (RFS) in lymph node positive breast cancer, in the lower survival tertile (Figure 2). Lymph node positive patients whose primary tumors expressed low levels of KAT2B possessed median RFS of 32.6 months, while lymph node positive patients whose tumors expressed high levels of KAT2B possessed median RFS of 43 months. This difference in RFS based on KAT2B tumor expression in lymph node positive patients with breast cancer approached statistical significance (Figure 2, Chart 3; logrank p -value: 0.053; hazard ratio: 0.82 (0.67-1)). KAT2B primary tumor expression was not significantly correlated with recurrence-free survival outcomes in lymph node negative patients (Figure 2, Chart 3; logrank p -value: 0.48; hazard ratio: 1.07 (0.89-1.28)).

Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified KAT2B 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; we observed significantly increased expression of KAT2B in lymph node metastases as compared to primary tumors of the breast. KAT2B was also differentially expressed in brain metastatic tissues. Further, we found a correlation between KAT2B expression and patient survival outcomes, as KAT2B primary tumor expression was correlated with recurrence-free survival in lymph node positive patients but not in lymph node negative patients.

Discussion

We provided evidence here that the histone lysine acetyltransferase 2B, KAT2B, 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 KAT2B is also differentially expressed in brain metastatic tissues in human breast cancer, that KAT2B mRNA is present at significantly increased quantities in lymph node metastatic tissues as compared to primary tumors of the breast, and that primary tumor KAT2B expression is correlated with patient survival outcomes in human breast cancer, in lymph node positive patients but not in lymph node negative patients. Evaluation of the effects of genetic depletion of KAT2B in mouse models of metastatic breast cancer on metastasis to the central nervous system is merited. Modulation of KAT2B 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

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Rank: 224
Probe ID: 10697
p-value: 2.73e-03
t: -3.0936802
B: -1.6582
Gene: KAT2B
Gene name: histone lysine acetyltransferase 2B

Chart 1: KAT2B 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: 5561
Probe ID: 8078227
p-value: 2.11e-02
t: 2.5020013
B: -3.690563
Gene: KAT2B
Gene name: histone lysine acetyltransferase 2B

Chart 2: KAT2B 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.

Figure 1: KAT2B 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 KAT2B 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 KAT2B expression between primary tumors of the breast and lymph node metastases, a p -value, is $p=0.000104$.

Figure 2: KAT2B primary tumor expression is correlated with recurrence-free survival in lymph node positive breast cancer patients but not in lymph node negative patients, nor in all patients.

Depicted in these Kaplan-Meier plots is the probability of recurrence-free survival in patients with breast cancer based on low or high expression of KAT2B (also known as GCN5L) in patient primary tumors, in the lower survival tertile. 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 KAT2B 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. Probability of recurrence-free survival in *n*=1133 lymph node positive patients is shown on the **left** and probability of recurrence-free survival in *n*=2020 lymph node negative patients is shown on the **right**.

Lymph node positive patients

Low KAT2B expression: 32.6 months

High KAT2B expression: 43 months

Chart 3: Median recurrence-free survival is inferior in lymph node positive patients with low primary tumor expression of KAT2B.

The median RFS (recurrence-free survival) of patients with low primary tumor expression of KAT2B and high primary tumor expression of KAT2B is listed in this chart for $n=1133$ lymph node positive patients, in the lower survival tertile.