APOBEC3A is differentially expressed in lymph node metastases 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 metastasis to the lymph nodes in humans with metastatic breast cancer. We found that the apolipoprotein B mRNA editing enzyme catalytic subunit 3A, APOBEC3A, 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 APOBEC3A was also differentially expressed in brain metastatic tissues. APOBEC3A mRNA was present at increased quantities in lymph node metastases as compared to primary tumors of the breast. Importantly, expression of APOBEC3A in primary tumors of the breast was correlated with patient overall survival, more significantly in lymph node negative patients than in lymph node positive patients. Modulation of APOBEC3A 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. APOBEC3A differential expression in lymph node metastatic tissues raises the question of why a human papillomavirus restriction factor^{6,7} is up-regulated during metastatic progression in human breast cancer.

Keywords: breast cancer, metastasis, brain metastasis, central nervous system metastasis, lymph node metastasis, apolipoprotein B mRNA editing enzyme catalytic subunit 3A, APOBEC3A, 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 trastuzumab⁸. The alarmingly high rate of central nervous system metastasis described, as well as data, both anecdotal² and from a randomized, controlled setting⁸ 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 apolipoprotein B mRNA editing enzyme catalytic subunit 3A, APOBEC3A, in lymph node metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE10893⁴ and GSE52604⁵ 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. GSE52604 was generated using Agilent-014850 Whole Human Genome Microarray 4x44K G4112F with $n=10$ normal breast tissues and $n=35$ 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 APOBEC3A 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 tool⁹ for correlation of APOBEC3A mRNA expression levels with overall survival in $n=313$ lymph node positive patients and $n=594$ 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.

APOBEC3A 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 apolipoprotein B mRNA editing enzyme catalytic subunit 3A, encoded by APOBEC3A, 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, APOBEC3A ranked 105 out of 18034 total transcripts (Chart 1), equating to 99.4% differential expression. Differential expression of APOBEC3A in

the lymph node metastases of patients with metastatic breast cancer was statistically significant (Chart 1; $p=7.64e-04$).

Differential gene expression analyses that utilize primary and metastatic tumor tissues to discover genes associated with metastasis can be challenging due to the relatively heterogenous cellular composition and constitution of the tumor as compared to normal, untransformed (benign) tissues. Thus, to attempt to validate differential transcriptome-wide differential expression of APOBEC3A in metastatic tissues in human breast cancer, we queried a second microarray dataset⁵, here comparing normal breast tissues rather than primary tumors of the breast, to brain metastases. Again, we identified APOBEC3A as a differentially expressed gene in the metastatic tissues of humans with breast cancer, here, 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, APOBEC3A ranked 11652 out of 41093 total transcripts (Chart 2), equating to 71.6% differential expression. Differential expression of APOBEC3A in the brain metastases of patients with metastatic breast cancer was statistically significant (Chart 2; $p=9.82e-03$). Thus, differential expression of APOBEC3A, transcriptome-wide, in the metastatic tissues of women with metastatic breast cancer was conserved across two independent microarray datasets, both in lymph node and brain metastases. This suggests that APOBEC3A differential expression in human metastatic breast cancer is a *bona fide* biological process and that differential expression of APOBEC3A in metastatic breast cancer is shared between the brain and the lymph nodes.

APOBEC3A is expressed at higher levels in the lymph node metastases of patients with metastatic breast cancer.

We obtained exact mRNA expression levels for APOBEC3A, 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 APOBEC3A expression in lymph node metastatic tissues. We observed significantly increased expression of APOBEC3A in the lymph node metastases of patients with breast cancer as compared to primary tumors of the breast: APOBEC3A was expressed at -0.85 ± 0.95 arbitrary units (AU) in primary tumors of the breast, while it was expressed at 0.56 ± 1.72 AU in lymph node metastatic tissues (Figure 1). The difference in APOBEC3A mRNA levels between primary tumors of the breast and lymph node metastases was statistically significant (Figure 1; $p=0.000931$).

APOBEC3A expression is significantly correlated with survival outcomes in human breast cancer.

We performed Kaplan-Meier survival analysis⁷ in $n=1402$ breast cancer patients in total, to evaluate whether APOBEC3A tumor expression was correlated with survival outcomes in breast cancer. We observed a statistically significant correlation between primary tumor expression of APOBEC3A and overall survival (OS) in patients with breast cancer (Figure 2). Patients whose primary tumors expressed low levels of APOBEC3A possessed median OS of 108 months, while patients whose tumors expressed high levels of APOBEC3A possessed median OS of 66.12. This difference in OS based on APOBEC3A tumor expression in patients with breast cancer was statistically significant (Figure 2, Chart 3; logrank p -value: 0.032; hazard ratio: 1.3 (1.02-1.66)).

We also performed Kaplan-Meier survival analysis⁷ in $n=313$ lymph node positive breast cancer patients and $n=594$ lymph node negative breast cancer patients, to evaluate whether lymph node status influenced correlation of APOBEC3A tumor expression with survival outcomes in human breast cancer. We observed a correlation between primary tumor expression of APOBEC3A and overall survival (OS) in lymph node negative breast cancer (Figure 2). Lymph node negative patients whose primary tumors expressed low levels of APOBEC3A possessed median OS of 175.46 months, while lymph node negative patients whose tumors expressed high levels of APOBEC3A possessed median OS of 115 months. This

1 difference in OS based on APOBEC3A tumor expression in lymph node negative patients with breast
2 cancer trended towards statistical significance (Figure 2, Chart 3; logrank *p*-value: 0.061; hazard ratio:
3 1.48 (0.98-2.25)). Thus, overall survival based on APOBEC3A primary tumor expression was markedly
4 longer in lymph node negative patients than in the general breast cancer patient population. APOBEC3A
5 primary tumor expression was not significantly correlated with overall survival outcomes in lymph node
6 positive patients (Figure 2, Chart 3; logrank *p*-value: 0.6; hazard ratio: 1.12 (0.72-1.76)).

7 Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified
8 APOBEC3A as among the genes whose expression was most different, transcriptome-wide, in the lymph
9 node metastases of patients with breast cancer, when compared to primary tumors; we observed
10 significantly increased expression of APOBEC3A in lymph node metastases as compared to primary
11 tumors of the breast. Further, we found a significant correlation between APOBEC3A expression and
12 patient survival outcomes, as overall survival was significantly greater in patients whose primary tumors
13 expressed higher levels of APOBEC3A as compared to patients whose primary tumors expressed lower
14 levels of APOBEC3A, and this correlation was markedly more significant in lymph node negative patients
15 than in lymph node positive patients.

16 **Discussion**

17 We provided evidence here that apolipoprotein B mRNA editing enzyme catalytic subunit 3A,
18 APOBEC3A, is among the genes whose expression is most different in the lymph node metastases of
19 patients with metastatic breast cancer as compared to primary tumors of the breast, that APOBEC3A is
20 also differentially expressed in brain metastatic tissues in human breast cancer, that APOBEC3A mRNA is
21 present at significantly increased quantities in lymph node metastatic tissues as compared to primary
22 tumors of the breast, and that primary tumor APOBEC3A expression is significantly correlated with
23 patient survival outcomes in human breast cancer, markedly more so in lymph node negative patients than
24 in lymph node negative patients. Evaluation of the effects of genetic depletion of APOBEC3A in mouse
25 models of metastatic breast cancer on metastasis to the central nervous system is merited. Modulation of
26 APOBEC3A expression may be relevant to the processes by which breast cancer cells exit the breast, enter
27 the vasculature and/or lymphatics, reside in the lymph nodes, evade immune clearance, breach the
28 blood-brain barrier and colonize the brain. APOBEC3A differential expression in lymph node metastatic
tissues raises the question of why a molecule described to function as a human papillomavirus restriction
factor^{6,7} is up-regulated during metastatic progression in human breast cancer

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Rank: 105
Probe ID: 100
p-value: 7.64e-04
t: -3.501491
B: -0.5835
Gene: APOBEC3A
Gene name: apolipoprotein B mRNA editing enzyme catalytic subunit 3A

Chart 1: APOBEC3A 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: 11652
Probe ID: A_32_P9543
p-value: 9.82e-03
t: -2.6917382
B: -3.337094
Gene: APOBEC3A
Gene name: apolipoprotein B mRNA editing enzyme catalytic subunit 3A

Chart 2: APOBEC3A is differentially expressed in brain metastatic breast cancer when comparing brain metastases 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: APOBEC3A 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 APOBEC3A 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 APOBEC3A expression between primary tumors of the breast and lymph node metastases, a p -value, is $p=0.000931$.

Figure 2: Significant correlation between APOBEC3A primary tumor expression and overall survival in patients with breast cancer, which is markedly more significant in lymph node negative patients than in lymph node positive patients.

Depicted in this Kaplan-Meier plot (**above**) is the probability of overall survival for $n=1402$ total patients stratified into two groups, based on low or high expression of APOBEC3A 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 APOBEC3A 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. Below, Kaplan-Meier plots for probability of overall survival in $n=313$ lymph node positive patients (**left**) and $n=594$ lymph node negative patients (**right**).

All patients

Low APOBEC3A expression: 108 months

High APOBEC3A expression: 66.12 months

Lymph node negative patients

Low APOBEC3A expression: 175.46 months

High APOBEC3A expression: 115 months

Chart 3: Median overall survival is inferior in patients with high primary tumor expression of APOBEC3A.

The median OS (overall survival) of patients with low primary tumor expression of APOBEC3A and high primary tumor expression of APOBEC3A is listed in this chart, for $n=1402$ breast cancer patients, and $n=594$ lymph node negative patients.