MAP9 is differentially expressed in lymph node metastasis in human breast cancer.

Shahan Mamoor, MS¹

¹shahanmamoor@gmail.com

East Islip, NY USA

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 microtubule associated protein 9, encoded by MAP9, 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 MAP9 was also differentially expressed in brain metastatic tissues⁵. MAP9 mRNA was present at increased quantities in lymph node metastatic tissues as compared to primary tumors of the breast. Expression of MAP9 in primary tumors was correlated with patient overall survival in lymph node positive patients but not in lymph node negative patients. Modulation of MAP9 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, microtubule associated protein 9, MAP9, 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 microtubule associated protein 9, MAP9, 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 MAP9 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 MAP9 mRNA expression levels with overall survival in $n=726$ lymph node negative patients and $n=452$ 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.

MAP9 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 microtubule associated protein 9, encoded by MAP9, 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, MAP9 ranked 165 out of 18034 total transcripts (Chart 1), equating to 99.1% differential expression. Differential expression of MAP9 in the lymph node metastases of patients with metastatic breast cancer was statistically significant (Chart 1; $p=1.75e-03$).

To attempt to validate differential transcriptome-wide differential expression of MAP9 in human metastatic breast cancer, we queried a second microarray dataset⁵; here, we identified MAP9 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, MAP9 ranked 421 out of 61359 total transcripts (Chart 2), equating to 99.3% differential expression. Differential expression of MAP9 in the brain metastases of patients with metastatic breast cancer trended towards statistical significance (Chart 2; $p=0.00216908$). Thus, transcriptome-wide differential expression of MAP9 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.

MAP9 is expressed at higher levels in the lymph node metastases of patients with metastatic breast cancer.

We obtained exact mRNA expression levels for MAP9, 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 MAP9 expression in lymph node metastatic tissues. We observed significantly higher expression of MAP9 in the lymph node metastases of patients with breast cancer as compared to primary tumors of the breast: MAP9 was expressed at 1.13 ± 0.85 units (AU) in primary tumors of the breast, while it was expressed at 0.08 ± 0.56 AU in lymph node metastatic tissues. The difference in MAP9 mRNA levels between primary tumors of the breast and lymph node metastatic tissues was statistically significant (Figure 1; $p=0.002048$).

MAP9 expression is significantly correlated with survival outcomes in human breast cancer.

We performed Kaplan-Meier survival analysis⁸ in $n=452$ lymph node positive patients and $n=726$ lymph node negative patients, to evaluate whether MAP9 tumor expression was correlated with survival outcomes in breast cancer. We observed a correlation between primary tumor expression of MAP9 and overall survival (OS) in lymph node positive patients with breast cancer, in the lower survival quartile (Figure 2). Lymph node positive patients whose primary tumors expressed low levels of MAP9 possessed median OS of 60 months, as compared to 40.68 months for lymph node positive patients whose tumors expressed high levels of MAP9. This difference in OS based on MAP9 tumor expression in lymph node positive patients with breast cancer trended towards statistical significance (Figure 2, Chart 3; logrank p -value: 0.094; hazard ratio: 1.41 (0.94-2.13)). Primary tumor expression of MAP9 was not correlated with MAP9 expression in lymph node negative breast cancer patients (Figure 2, Chart 3; logrank p -value: 0.44; hazard ratio: 0.86 (0.59-1.26)).

Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified microtubule associated protein 9, encoded by MAP9, 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 MAP9 in lymph node metastases as compared to primary tumors of the breast. Further, we observed a correlation between MAP9 expression and patient survival outcomes, as overall survival was greater in patients whose tumors expressed low levels of MAP9 as compared to patients whose tumors expressed high levels of MAP9, in lymph node positive patients but not in lymph node negative patients.

Discussion

We provided evidence here that microtubule associated protein 9, encoded by MAP9, is among the genes whose expression is most different in the lymph node metastases of patients with lymph node metastatic breast cancer, that MAP9 mRNA is present at significantly increased quantities in lymph node metastatic tissues as compared to primary tumors of the breast, and that primary tumor MAP9 expression is correlated with patient survival outcomes in lymph node positive human breast cancer. Evaluation of the effects of genetic depletion of MAP9 in mouse models of metastatic breast cancer on metastasis to the lymph nodes and to the central nervous system is merited. Modulation of MAP9 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.

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Rank: 165
Probe ID: 17128
p-value: 1.75e-03
t: 3.2392508
B: -1.2858
Gene: MAP9
Gene name: microtubule associated protein 9

Chart 1: MAP9 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: 421
probe ID: g13376225_3p_x_at
p-value: 0.00216908
t: 3.2629028
B: -1.45291
Gene: MAP9
Gene name: microtubule associated protein 9

Chart 2: MAP9 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: MAP9 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 MAP9 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 MAP9 expression between primary tumors of the breast and lymph node metastases is $p=0.002048$.

Figure 2: MAP9 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 $n=452$ patients with lymph node positive breast cancer (**left**) and $n=726$ patients with lymph node negative breast cancer (**right**), stratified into two groups, based on low or high expression of MAP9 in patient primary tumors, in the lower 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 MAP9 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 MAP9 expression: 60 months

High MAP9 expression: 40.68 months

Chart 3: Median overall survival is inferior in lymph node positive breast cancer patients with high primary tumor expression of MAP9.

The median OS (overall survival) of patients with low primary tumor expression of MAP9 and high primary tumor expression of MAP9 is listed in this chart, for $n=452$ lymph node positive breast cancer patients, in the lower survival quartile.