ATG14 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 metastasis to the lymph nodes in humans with metastatic breast cancer. We found that autophagy related 14, ATG14, 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. ATG14 mRNA was present at decreased quantities in lymph node metastases as compared to primary tumors of the breast. Modulation of ATG14 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, autophagy related 14, ATG14, 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 autophagy related 14, ATG14, in lymph node metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE10893⁴ and GSE124648⁵ 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. GSE124648 was generated using Affymetrix Human Genome U133A array technology with $n=10$ normal breast tissues and $n=44$ lymph node metastases from patients with breast cancer; analysis was performed using platform GPL96. 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 ATG14 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.

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.

ATG14 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 autophagy related 14, encoded by ATG14, 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, ATG14 ranked 215 out of 18034 total transcripts (Chart 1), equating to 98.8% differential expression. Differential expression of ATG14 in the lymph node metastases of patients with metastatic breast cancer was statistically significant (Chart 1; $p=2.65e-03$).

We queried a second microarray dataset⁵ to attempt to validate transcriptome-wide differential expression of ATG14 in the lymph node metastases of patients with metastatic breast cancer. We again observed ATG14 to be among the genes expressed most quantitatively differently in the lymph node metastases of patients with metastatic breast cancer as compared to primary tumors of the breast (Chart 2). When sorting each of the genes expressed in lymph node metastases based on significance of difference as compared to normal breast tissues, ATG14 ranked 1316 out of 22283 total transcripts (Chart 2), equating to 94.1% differential expression. Differential expression of ATG14 in the lymph node metastases of patients with metastatic breast cancer was statistically significant (Chart 2; $p=6.02e-06$).

ATG14 is expressed at lower levels in the lymph node metastases of patients with metastatic breast cancer.

We obtained exact mRNA expression levels for ATG14, 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 ATG14 expression in lymph node metastatic tissues. We observed significantly decreased expression of ATG14 in the lymph node metastases of patients with breast cancer as compared to primary tumors of the breast: ATG14 was expressed at 0.19 ± 0.44 arbitrary units (AU) in primary tumors of the breast, while it was expressed at -0.34 ± 0.33 AU in lymph node metastatic tissues (Figure 1). The difference in ATG14 mRNA levels between primary tumors of the breast and lymph node metastases was statistically significant (Figure 1; $p=0.002704$).

Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified ATG14 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 decreased expression of ATG14 in lymph node metastases as compared to primary tumors of the breast.

Discussion

We provided evidence here that autophagy related 14, encoded by ATG14, 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, and that ATG14 mRNA is present at significantly decreased quantities in lymph node metastatic tissues as compared to primary tumors of the breast. Evaluation of the effects of genetic depletion of ATG14 in mouse models of metastatic breast cancer on metastasis to the lymph nodes and to the central nervous system is merited. Modulation of ATG14 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: 215
Probe ID: 3460
p-value: 2.65e-03
t: 3.103784
B: -1.6328
Gene: ATG14
Gene name: autophagy related 14

Chart 1: ATG14 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: 1316
Probe ID: 204568_at
p-value: 6.02e-06
t: 5.003345
B: 3.437878
Gene: ATG14
Gene name: autophagy related 14

Chart 2: ATG14 is differentially expressed in lymph node metastatic breast cancer when comparing lymph node 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: ATG14 is expressed at significantly lower 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 ATG14 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 ATG14 expression between primary tumors of the breast and lymph node metastases, a p -value, is $p=0.002704$.