DLK1 is differentially expressed in the lymph node and brain metastases of patients with metastatic breast cancer.

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Metastasis to the brain is a clinical problem in patients with breast cancer¹⁻³. We mined published microarray data^{4,5} to compare primary and metastatic tumor transcriptomes to discover genes associated with brain metastasis in patients with metastatic breast cancer. We found that the delta like non-canonical Notch ligand 1, encoded by DLK1 was among the genes whose expression was most different in the brain and lymph node metastases of patients with metastatic breast cancer as compared to primary tumors of the breast and normal breast tissues, respectively. DLK1 mRNA was present at higher quantities in brain metastatic tissues as compared to primary tumors of the breast. These data combined suggest some level of common origin for metastases that reside in the lymph nodes and colonize the brain. Up-regulation of Notch pathway signals may contribute to pathways influencing metastasis to the brain.

Keywords: breast cancer, metastasis, brain metastasis, central nervous system metastasis, lymph node metastasis, DLK1, delta like non-canonical Notch ligand 1, 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 trastuzumab. 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 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 brain metastatic breast cancer^{4,5}. We discovered significant differential and increased expression of DLK1, in brain metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE10893⁴ and GSE124648⁵ and 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 $n=11$ primary breast tumors and $n=3$ brain metastases from patients with breast cancer; analysis performed using platform GPL885. GSE124648, a microarray dataset, 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; platform GPL96 was used for this analysis. 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 DLK1 gene expression was significantly between normal breast tissues and brain metastases in humans with breast cancer using a two-tailed, unpaired t-test with Welch's correction. We used PRISM for all statistical analyses of differential gene expression in human breast cancer (Version 8.4.0)(455).

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 brain and lymph node metastasis in human breast cancer in an unbiased fashion and for the discovery of novel therapeutic targets.

DLK1 is differentially expressed in the brain metastases of patients with brain metastatic breast cancer.

Through analysis of published microarray data⁴, we identified the delta like non-canonical Notch ligand 1, encoded by DLK1, 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 brain metastases based on significance of difference as compared to primary tumors of the breast in patients with breast cancer, DLK1 ranked 1 out of 22575 total transcripts (Table 1), equating to 99.996% differential expression. DLK1 was the single most differentially expressed gene in this dataset, transcriptome-wide, when comparing primary tumors of the breast and metastases to the brain. Differential expression of DLK1 in the brain metastases of patients with metastatic breast cancer was statistically significant (Table 1; $p=1.26E-08$).

DLK1 is differentially expressed in the lymph node metastases of patients with metastatic breast cancer.

Next, we queried a second microarray dataset⁵ generated using lymph node metastases and normal breast tissues from patients with breast cancer to determine whether we could validate differential expression of DLK1 in metastatic tissues of humans with breast cancer, and whether differential expression of DLK1 was specific to brain metastases or could be observed in metastases to other sites in patients with metastatic breast cancer. We also observed significant differential expression of DLK1 in lymph node metastases of patients with metastatic breast cancer (Table 2). When sorting each of the genes expressed in lymph node metastases based on significance of difference as compared to normal breast tissues in patients with breast cancer, DLK1 ranked 84 out of 22283 total transcripts (Table 2), equating to 99.6% differential expression. Differential expression of DLK1 in the lymph node metastases of patients with metastatic breast cancer was statistically significant (Table 2; $p=3.53E-15$). Thus, differential expression of DLK1 was conserved in the brain and lymph node metastases of patients with metastatic breast cancer in two independent microarray datasets^{4,5}.

DLK1 is expressed at significantly higher levels in the brain metastases of patients with brain metastatic breast cancer.

We obtained exact mRNA expression levels for DLK1, in primary tumors of the breast and in brain metastasis of patients with brain metastatic breast cancer to determine direction and statistical significance of change in DLK1 expression in brain metastatic tissues. DLK1 was expressed at higher levels in the brain metastases of patients with breast cancer as compared to primary tumors of the breast, and this difference was statistically significant (Figure 1; $p=0.0349$).

Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified the DLK1 as among the genes whose expression was most different, transcriptome-wide, in the brain and lymph node metastases of patients with breast cancer when compared to tissues of the breast, transformed and benign; DLK1 expression, as compared to the transformed breast, was significantly higher in metastasis to the brain.

Discussion

We provided evidence here that the delta like non-canonical Notch ligand 1, DLK1, is among the genes whose expression is most different in the brain and lymph node metastases of patients with metastatic breast cancer. DLK1-deficient mice manifest defects in neurogenesis in the subventricular zone, demonstrating that DLK1 has a critical function in neural stem cell function in mammals⁷. Evaluation of the effects of depletion of DLK1 in mouse models of metastatic breast cancer on metastasis to the central nervous system are merited. Differential expression of DLK1 in metastasis to the lymph nodes and to the brain suggests some level of common origin for metastases that reside in the lymph nodes, successfully evade immune clearance, penetrate the central nervous system by unknown means and subsequently colonize the brain.

References

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Rank	ID	p-value	t	B	Gene	Gene name
1	13326	1.26E-08	1.13E+01	9.14213	DLK1	delta-like 1 homolog (Drosophila)

Table 1: DLK1 is differentially expressed in brain metastases in patients with metastatic breast cancer when compared to primary tumors of the breast.

Rank of differential expression, probe ID, *p*-value with respect to differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the two groups compared, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
84	209560_s_at	3.53E-15	10.747958	24.315854	DLK1	delta like non-canonical Notch ligand 1

Table 2: DLK1 is differentially expressed in lymph node metastases of patients with metastatic breast cancer when compared to normal breast tissue.

Rank of differential expression, probe ID, *p*-value with respect to differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the two groups compared, gene and gene name are listed in this chart.