AKT1 is differentially expressed in primary tumors and brain metastases in humans with 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 report here the differential expression of the protein kinase AKT1 in the primary tumors and brain metastases of humans with breast cancer. AKT1 mRNA was present at significantly increased quantities in brain metastatic tissues as compared to primary tumors of the breast. These data combined suggest that up-regulation of AKT1 is a conserved event, both during transformation of breast tissues and progression to central nervous system metastasis and further point to potential importance of AKT1 modulation during progression of human breast cancer.

Keywords: breast cancer, metastasis, brain metastases, central nervous system metastases, AKT1, 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 trial 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 the protein kinase AKT1 in brain metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE10893⁴ and GSE109169⁵ 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 $n=4$ brain metastases from patients with breast cancer; analysis performed using platform GPL885. GSE109169 was generated using Affymetrix Human Exon 1.0 ST Array technology with $n=25$ normal breast tissue and $n=25$ tumors of the breast and; analysis was performed using platform GPL5175. The tissues from this dataset are paired tissues (25 tumors matching 25 breast tissues from 25 patients). 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 AKT1 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 tissues from patients with metastatic breast cancer using published microarray data^{4,5} to describe the transcriptional landscape of primary and metastatic tumors in human metastatic breast cancer in an unbiased fashion and for the discovery of novel therapeutic targets.

AKT1 is differentially expressed in the brain metastases of patients with brain metastatic breast cancer.

Through analysis of published microarray data⁴, we identified the v-akt murine thymoma viral oncogene homolog 1, AKT1, as a differentially expressed gene in the breast metastatic tissues of humans with breast cancer. 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, AKT1 ranked 38 out of 17418 total transcripts (Table 1), equating to 99.8% differential expression. Differential expression of AKT1 in the brain metastases of patients with metastatic breast cancer was statistically significant (Table 1; $p=5.6E-05$).

AKT1 is differentially expressed in the primary tumors of patients with breast cancer.

Next, we queried a second microarray dataset⁵ generated using primary tumors and normal breast tissues from patients with breast cancer to determine whether differential expression of AKT1 was specific to metastatic tissues or could be found in primary tumors as well. Our analysis revealed significant differential expression of AKT1 in primary tumors of patients with breast cancer. When sorting each of the genes expressed in primary tumors of the breast based on significance of difference as compared to normal breast tissues in patients with breast cancer, AKT1 ranked 4252 out of 19076 total transcripts (Table 2), equating to 77.7% differential expression. Differential expression of AKT1 in the primary tumors of patients with breast cancer was statistically significant (Table 2; $p=1.61E-04$). Thus, differential expression of AKT1 was conserved in the brain metastases and primary tumors of patients with breast cancer.

AKT1 is expressed at higher levels in the brain metastases in humans with breast cancer.

We obtained exact mRNA expression levels for AKT1, in normal breast tissues and in brain metastasis of patients with brain metastatic breast cancer to determine direction and statistical significance of change in AKT1 expression in brain metastatic tissues. AKT1 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; $p=0.0027$).

Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified AKT1 as among the genes whose expression was most different, transcriptome-wide, in the brain metastases of patients with breast cancer when compared to primary tumors of the breast, and in the primary tumors of patients with breast cancer when compared to normal breast tissue. AKT1 expression, as compared to primary tumors of the breast, was significantly higher in metastasis to the brain.

Discussion

We provided evidence here that the protein kinase AKT1 is among the genes whose expression is most different in the primary and metastatic tumors of humans with breast cancer. Evaluation of the effects of genetic depletion of AKT1 in mouse models of metastatic breast cancer on metastasis to the central nervous system are merited. Differential expression and up-regulation of AKT1 in primary tumors of the breast and metastasis to the brain suggests that its induction may be important for the progression of human breast cancer, but this remains to be experimentally evaluated.

References

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Rank	ID	p-value	t	B	Gene	Gene name
38	19766	5.6E-05	-5.45	2.053627	AKT1	v-akt murine thymoma viral oncogene homolog 1

Table 1: AKT1 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
4252	3581132	1.61E-04	4.07	0.268985	AKT1	v-akt murine thymoma viral oncogene homolog 1

Table 2: AKT1 is differentially expressed in primary tumors of the breast 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.

Figure 1: AKT1 is expressed at higher levels in the brain metastases of patients with brain metastatic breast cancer as compared to primary tumors of the breast.

The mRNA expression level of AKT1 in primary tumors of the breast (left) and in the brain metastases of brain metastatic breast cancer patients (right) is graphically represented here with mean AKT1 levels marked and the result of a statistical test evaluating difference in mRNA expression level between brain metastases and primary tumors of the breast, a *p*-value, listed above.