Differential expression of TAX1BP1 in the brain metastases of patients with breast cancer.

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Metastasis to the brain is a complication of breast cancer^{1,2} with limited treatment options³. We mined published microarray data^{4,5} to discover genes associated with brain metastases in patients with metastatic breast cancer. We identified significant differential expression TAX1BP1 in the brain metastases of patients with breast cancer as compared to primary tumors of the breast. TAX1BP1, an HTLV-I Tax-interacting protein with the ability to bind the deubiquitinase A20 and inhibit cell death mediated by Fas and TNF- α pathways⁶⁻¹⁰, may be relevant to the underlying biology by which tumor cells of the breast spread to the brain.

Keywords: TAX1BP1, breast cancer, brain metastases, central nervous system metastases, systems biology of breast cancer, targeted therapeutics in breast cancer.

One study documented a 34% incidence of metastases to the central nervous system in breast cancer patients treated with trastuzumab¹¹. An unbiased and systems-level understanding of the transcriptional makeup of brain metastatic tissues can support identification of therapeutic targets. We performed global transcriptional profiling of brain metastases in patients with brain metastatic breast cancer⁶⁻⁸. We discovered significant differential expression of the eukaryotic translation initiation factor TAX1BP1 in brain metastases from patients with brain metastatic breast cancer.

Methods

We used datasets GSE125989⁴ and GSE43837⁵ for this global differential gene expression analysis of brain metastatic breast cancer in conjunction with GEO2R. GSE125989 was generated using Affymetrix Human Genome U133A 2.0 Array technology with $n=16$ primary tumors from patients with breast cancer and $n=16$ brain metastases from patients with brain metastatic breast cancer. GSE43837 was generated using Affymetrix Human X3P Array technology with $n=19$ primary breast tumors and $n=19$ brain metastases from patients with breast cancer, analysis performed using platform GPL1352. 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 TAX1BP1 gene expression was significantly between primary breast tumors 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 sought to discover genes associated with metastasis to the brain in patients with breast cancer by mining published microarray datasets^{4,5}, comparing global gene expression profiles of primary tumors of the breast to brain metastatic tissues from patients with breast cancer.

TAX1BP1 is differentially expressed in the brain metastases of patients with brain metastatic breast cancer.

We identified the gene encoding the Tax1 binding protein 1, TAX1BP1, as among the genes whose expression was most quantitatively different in brain metastases when compared to primary tumors of the breast in patients with breast cancer⁴. When sorting each of the genes expressed in brain metastases based on significance of difference in expression as compared to primary tumors of the breast, TAX1BP1 ranked 362 out of 22277 total transcripts (Table 1), equating to 98.4% differential expression. Differential expression of TAX1BP1 in brain metastases in patients with breast cancer was statistically significant (Table 1; $p=4.8E-03$).

We queried a second microarray dataset⁵ to attempt to validate transcriptome-wide differential expression of TAX1BP1 in brain metastatic breast cancer. We again found that TAX1BP1 was among the genes whose expression was most significantly different when comparing primary tumors of the breast to brain metastases from patients with metastatic breast cancer. When sorting each of the genes expressed in brain metastases based on significance of difference in expression as compared to primary tumors of the breast, TAX1BP1 ranked 5749 out of 61359 total transcripts (Table 2), equating to 90.6% differential expression. Differential expression of TAX1BP1 in brain metastases in patients with breast cancer approached the level of statistical significance (Table 2; $p=0.04340912$).

TAX1BP1 is expressed at significantly higher levels in the brain metastases of patients with brain metastatic breast cancer as compared to primary tumors of the breast.

We obtained exact mRNA expression levels for TAX1BP1 in primary tumors of the breast and in brain metastases to evaluate magnitude and direction of change in TAX1BP1 gene expression in brain metastatic tissues. TAX1BP1 was expressed at higher levels in brain metastases from patients with breast cancer as compared to primary tumors of the breast (Figure 1). Increased expression of TAX1BP1 in brain metastases as compared to primary tumors of the breast was statistically significant (Figure 1: $p=0.0009$). We calculated a mean fold change of 1.12 ± 0.07 in TAX1BP1 expression in brain metastases when compared to primary tumors of the breast (Table 1).

Thus, through comparative transcriptome analysis of tumor and metastatic tumor tissues of patients with breast cancer using published microarray data^{4,5}, we found that TAX1BP1 was among the genes most differentially expressed in brain metastases, with significantly increased expression in brain metastatic tissues as compared to primary tumors of the breast.

Discussion

To facilitate discovery of novel therapeutic targets in brain metastatic breast cancer, and to more thoroughly understand the biology of metastasis to the brain in breast cancer, we mined published microarray data^{4,5} to identify genes most differentially expressed in the brain metastases of patients with metastatic breast cancer. We discovered the Tax1-binding protein 1, TAX1BP1, as among the genes whose expression was most significantly different in the brain metastases of patients with metastatic breast cancer as compared to primary tumors of the breast.

TAX1BP1 was identified as an interaction partner of HTLV-I Tax through a yeast two-hybrid screen^{7,8}. TAX1BP1 contains an amino terminal SKICH or SKIP carboxyl homology domain, two carboxy-terminal zinc fingers and three coiled-coil domains⁹. TAX1BP1 can interact with TRAF6; this interaction is dependent on IRAK1 and inducible by the interleukin-1⁹. TAX1BP1 can interact with the deubiquitinase A20 and can inhibit cell death (apoptosis) mediated by Fas and tumor necrosis factor alpha (TNF- α)¹⁰. TAX1BP1 can negatively regulate NF- κ B signaling and control activation of IKK and JNK following stimulation with TNF- α and IL-1¹¹. TAX1BP1 can function in antiviral signaling, mediating functions of ABIN1 in conjunction with A20 through TBK1 and IKKi^{12,13}. In T-lymphocytes, Tax1BP1 can control cell size and progression through the cell cycle, and this is due to defects in formation of mTOR complexes¹⁴. TAX1BP1 can interact with autophagic machinery LC3 and GABARAP through an LC3-interacting region (LIR). TAX1BP1 also contains a ubiquitin-binding domain¹⁴. Interestingly, another viral interaction partner of TAX1BP1 is the human papillomavirus transactivating gene E2¹⁵.

We found here using published microarray data^{4,5} that TAX1BP1, a multi-functional molecule with a ubiquitin-binding domain, LC3-interacting region, and the ability to interact with both HTLV-I Tax and HPV E2, is among the genes whose expression changes most significantly in the brain metastases of patients with metastatic breast cancer, and present at significantly higher quantities at the mRNA level in brain metastatic tissue. TAX1BP1 may be relevant to the processes underlying metastasis to the brain in human breast cancer.

References

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Rank	ID	p-value	t	B	FC	Gene	Gene name
362	200977_s_at	4.8E-03	3.0148984	-2.1011	1.12 ± 0.07	TAX1BP1	Tax1 binding protein 1

Table 1: Differential expression of TAX1BP1 in brain metastases in patients with metastatic breast cancer.

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, fold change (FC) of TAX1BP1 expression between the brain metastases of patients with breast cancer and primary tumors of the breast, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
5749	Hs.5437.4.S1_3p_at	0.04340912	-2.081381	-3.78335	TAX1BP1	Tax1 binding protein 1

Table 2: Differential expression of TAX1BP1 in brain metastases in patients with metastatic breast cancer.

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: TAX1BP1 is expressed at significantly higher levels in brain metastases as compared to primary tumors of the breast in patients with brain metastatic breast cancer.

The mRNA expression level of TAX1BP1 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 TAX1BP1 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.