Ma1 antigen (PNMA1) is over-expressed in brain metastatic breast cancer.

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One study reported the incidence of central nervous system metastases in breast cancer patients treated with trastuzumab as 34%¹. We mined published microarray data^{2,3} to discover genes associated with brain metastasis in breast cancer. We identified significant differential expression of the paraneoplastic Ma1 antigen, encoded by the PNMA1 gene, in the brain metastases of patients with breast cancer. PNMA1 may be relevant to the biology underlying colonization of the brain with metastatic breast cancer clones.

Keyword: breast cancer, metastasis, brain metastasis, central nervous system metastasis, PNMA1, Ma1 antigen, systems biology of breast cancer, targeted therapeutics in breast cancer.

One study reported a 34% incidence of central nervous system metastases in patients with breast cancer treated with the monoclonal antibody trastuzumab (Herceptin)¹. Treatment options for patients with brain metastatic breast cancer are limited⁴. We mined published microarray datasets^{2,3} to understand in an unbiased and systems-level fashion genes whose expression most significantly distinguished brain metastases from primary tumors of the breast, the cells from which these metastatic clones are presumably derived. We found significant differential expression of PNMA1 in the brain metastases of patients with brain metastatic breast cancer.

Methods

We utilized microarray datasets GSE125989² and GSE100534³ for this differential gene expression analysis of brain metastatic breast cancer in conjunction with GEO2R. GSE100534 was generated using Affymetrix Human Gene 1.0 ST Array technology with $n=16$ primary tumors from patients with breast cancer and $n=3$ 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 PNMA1 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 mined published microarray data^{2,3} to discover genes associated with brain metastasis in breast cancer.

PNMA1 is differentially expressed in brain metastatic breast cancer.

We identified the paraneoplastic Ma antigen PNMA1 as among the genes whose expression changes most significantly when comparing brain metastases to primary tumors of the breast in patients with brain metastatic breast cancer² (Table 1). When sorting each of the genes expressed in brain metastases based on significance of change as compared to primary tumors of the breast, PNMA1 ranked 336 out of 22277 total transcripts (Table 1), equating to 98.5% differential expression. Differential expression of PNMA1 in brain metastases in brain metastatic breast cancer was statistically significant (Table 1; $p=4.24E-03$).

We queried a second microarray dataset³ to validate transcriptome-wide differential expression of PNMA1 in brain metastatic breast cancer. We again found that PNMA1 was among the genes whose expression changed most significantly when comparing metastases of the brain to primary tumors of the breast in patients with breast cancer (Table 2). When sorting each of the genes expressed in brain metastases based on significance of change as compared to primary tumors of the breast, PNMA1 ranked 5840 out of 33297 total transcripts (Table 2), equating to 82.5% differential expression. Differential expression of PNMA1 in brain metastases in brain metastatic breast cancer was statistically significant (Table 2; $p=2.38E-02$).

PNMA1 is expressed at significantly higher levels in brain metastases as compared to primary tumors of the breast in brain metastatic breast cancer.

We obtained exact mRNA expression values for PNMA1 from brain metastases and primary tumors of the breast to evaluate magnitude and direction of change in PNMA1 expression in brain metastases in patients with breast cancer. PNMA1 was expressed at higher levels in brain metastases in patients with breast cancer and this difference was statistically significant (Figure 1; $p=0.0011$). We calculated a mean fold change of 1.23 ± 0.15 in PNMA1 expression when comparing brain metastatic tissues to primary tumors of the breast (Table 1).

Thus, we found that PNMA1 was among the genes, globally, whose expression was most different when comparing primary tumors of the breast to brain metastases in patients with breast cancer, with significantly increased expression of PNMA1 in metastases to the brain as compared to primary tumors of the breast.

Discussion

Paraneoplastic Ma antigens (PNMA) were identified utilizing the sera of patients with paraneoplastic neurologic syndromes^{5,6}, thought to be an autoimmune reaction to antigen whose expression is common to both the nervous system and the tumor the patient is afflicted with⁷. Paraneoplastic neurologic syndromes are described to both inhibit growth of the tumor associated with the syndrome as well as result in damage to tissue in the nervous system⁸. Ma1 is a 37 kilodalton protein with expression was described as restricted to the brain and testis and possess putative phosphorylation sites for the casein kinase II and protein kinase C⁶. While Ma1 was localized primarily to the cytoplasm in cells of the primary tumor with which the paraneoplastic syndrome was associated with, Ma1 was primarily localized to the nucleus in neurons of these patients⁶. Described functional characteristics of Ma1/PNMA1 are limited but one study reported that ectopic expression of PNMA1 could induce cell death in cerebellar granule neurons and in cortical neurons through a BH3-like domain at its amino-terminal half, and that over-expression of Bcl2 could block cell death instigated by Ma1⁹. Expression of PNMA1 was reported as quantitatively increased in pancreatic ductal adenocarcinoma cell lines as compared to hTERT-HPNE, intermediary pancreatic duct cells, and qualitatively in patients tumors relative to normal pancreatic tissues¹⁰. PNMA1 expression was also found to be increased in hepatocellular carcinoma (HCC) primary tumors as compared to adjacent liver, with high PNMA1 expression significantly correlating with worse overall and recurrence-free survival after surgery¹¹. Significantly, PNMA1 was associated with metastasis in HCC as PNMA1 expression was significantly higher in portal vein tumor thrombus as compared to primary HCC tumors; gene signatures of the Wnt pathway and epithelial to mesenchymal transition were enriched for¹¹. Depletion of PNMA1 in SK-Hep1 and Huh7 cell lines resulted in impaired cell proliferation, as well as reduced in vitro migration and invasion. PNMA1 contained a putative microRNA miR-33a-5p binding site at its 3' untranslated region, and ectopic expression of miR-33a-5p resulted in decreased expression of PNMA1 in both SK-Hep1 and Huh7 cell lines. Thus, PNMA1 is an antigen expressed in paraneoplastic syndromes, with described functions in the induction of apoptosis and control of cell proliferation, and with expression controlled potentially controlled by miR-33a-5p.

We reported here that the gene encoding the Ma1 antigen PNMA1 was among the genes whose expression was most quantitatively different in metastases to the brain when compared to primary tumors of the breast in patients with metastatic breast cancer. PNMA1 may be of relevance to the biology underlying any number of steps important for metastasis to the brain: exit from the breast, traversing the vasculature and the blood brain barrier, and colonization of the brain.

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Rank	ID	p-value	t	B	FC	Gene	Gene name
336	218224_at	4.24E-03	3.0619379	-1.995	1.23 ± 0.15	PNMA1	paraneoplastic Ma antigen 1

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Table 1: PNMA1 is differentially expressed in brain 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 PNMA1 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
5840	7980044	2.38E-02	-2.4446507	-3.801245	PNMA1	paraneoplastic Ma antigen 1

Table 2: PNMA1 is differentially expressed in brain 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: PNMA1 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 PNMA1 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 PNMA1 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.