The histone arginine demethylase JMJD6 is over-expressed in brain metastatic breast cancer.

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Control of gene expression includes regulation by *trans*-acting transcription factors and *cis*-acting epigenetic regulation by chemical modification of histones and the DNA^{1,2}. Brain metastases are a clinical problem in patients with breast cancer³⁻⁵. We mined published microarray data^{6,7} to discover genes associated with brain metastasis in patients with brain metastatic breast cancer. We found that the gene encoding the histone arginine demethylase JMJD6 was among the genes most differentially expressed in the brain metastases of patients with brain metastatic breast cancer. JMJD6 may be of relevance to the biology underlying metastasis to the brain, and it may be of relevance as a potential therapeutic target in patients with intractable disease.

Keywords: breast cancer, brain metastases, central nervous system metastases, JMJD6, systems biology of breast cancer, targeted therapeutics in breast cancer.

One report described a 34% incidence of central nervous system metastases in trastuzumab-treated patients with breast cancer⁴. This alarmingly high frequency of CNS metastasis events demands an enhanced understanding of the transcriptional makeup of brain metastatic tissues to support identification of therapeutic targets. We performed a global comparative analysis of transcription in the brain metastases of patients with brain metastatic breast cancer^{6,7}. We discovered significant differential and over-expression of JMJD6, a histone arginine demethylase in brain metastases from patients with brain metastatic breast cancer.

Methods

We used datasets GSE125989⁶ and GSE52604⁷ 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. GSE52604 was generated using Whole Human Genome Microarray 4x44K G4112F technology with $n=10$ normal breast tissues and $n=35$ brain metastases from patients with breast cancer, analysis performed using platform GPL6480. 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 JMJD6 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 performed global comparative transcriptome analysis of the primary and metastatic tumor tissues of patients with brain metastatic breast cancer using published microarray data^{6,7} for the discovery of novel therapeutic targets.

JMJD6 is differentially expressed in the brain metastases of patients with brain metastatic breast cancer.

We identified the histone arginine demethylase JMJD6 as among the genes whose expression was most significantly different in the brain metastases of patients with brain metastatic breast cancer⁶ (Table 1). When sorting each of the genes expressed in brain metastases based on significance of difference as compared to the primary tumors of patients with brain metastatic breast cancer, JMJD6 ranked 191 out of 22297 total transcripts (Table 1). Differential expression of JMJD6 in the brain metastases of patients with brain metastatic breast cancer was statistically significant (Table 1; $p=1.36E-03$).

Cross-validation of differentially expressed genes across datasets in cancer can be difficult and more challenging than when cross-validating across organs and sorted cell populations due to higher levels of heterogeneity in tumors, and differing methods of tumor sampling between laboratories. Thus, we asked whether we could validate differential expression of JMJD6 when comparing brain metastases from patients with breast cancer to normal breast tissue as opposed to primary tumors of the breast. Comparison of global gene expression profiles of 10 normal breast tissues to 35 brain metastases⁷ revealed that JMJD6 was among the genes whose expression was most significantly different transcriptome-wide between the breast and brain metastases in patients with breast cancer (Table 2). When sorting each of the genes expressed in brain metastases based on significance of difference in

expression between brain metastases and normal breast tissues, JMJD6 ranked 2477 out of 41093 total transcripts (Table 2), equating to 94% differential expression. Differential expression of JMJD6 in brain metastases from patients with brain metastatic breast cancer in this dataset was statistically significant (Table 2; $p=1.02E-05$).

JMJD6 is expressed at significantly higher levels in the brain metastases of patients with brain metastatic breast cancer.

We obtained exact mRNA expression levels for JMJD6, in primary tumors of the breast and in brain metastasis of patients with brain metastatic breast cancer to determine magnitude and direction of change in JMJD6 expression in brain metastatic tissues. JMJD6 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; $p=0.0011$). We calculated a mean fold change of 1.397 ± 0.115 in JMJD6 expression when comparing primary tumors of the breast to brain metastatic tissues in patients with breast cancer (Table 1).

Thus, by mining published microarray data^{6,7} in an unbiased and systematic fashion, we identified the histone arginine demethylase JMJD6 as among the genes whose expression was most different in the brain metastases of patients with breast cancer both when compared to primary tumors of the breast and to the normal breast; JMJD6 was expressed at significantly higher levels in brain metastases as compared to primary tumors of the breast.

Discussion

JMJD6 is a JmjC domain-containing iron- and 2-oxoglutarate-dependent dioxygenase that can function in the demethylation of arginine 2 of histone H3 (H3R2) and arginine 3 of histone H4 (H4R3)⁷. JMJD6 can also function in the lysyl-5-hydroxylation of the U2 small nuclear ribonucleoprotein auxiliary factor subunit of 65 kilodaltons, U2AF65⁸. One report also described binding of single-stranded RNA by JMJD6⁹. Importantly, through regulating the splicing of Flt1 via U2AF65, JMJD6 controls angiogenic sprouting, as the vascular endothelial growth factor (VEGF) is a ligand of Flt1 (VEGFR-1)¹⁰.

We report here that the histone arginine demethylase JMJD6 with critical functions in the promotion of angiogenic sprouting is among the genes whose expression is most different in the brain metastases of patients with brain metastatic brain cancer. JMJD6, genomic targets affected by both its demethylase function and functions through U2AF65 (e.g., Flt1) may be of relevance to the biology underlying metastasis to the brain in patients with brain metastatic breast cancer, a disease with limited treatment options.

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Rank	ID	p-value	t	B	FC	Gene	Gene name
191	215233_at	1.36E-03	3.4837042	-1.0073	1.397 ± 0.115	JMJD6	arginine demethylase and lysine hydroxylase

Table 1: JMJD6 is differentially expressed in brain metastases 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 JMJD6 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
2477	A_23_P311616	1.02E-05	4.9427117	3.142649	JMJD6	arginine demethylase and lysine hydroxylase

Table 2: JMJD6 is differentially expressed in brain metastases 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: JMJD6 is expressed at significantly higher levels in the brain metastases of patients with brain metastatic breast cancer.

The mRNA expression level of JMJD6 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 JMJD6 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.