The methyl CpG-binding protein MeCP2 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 data⁶⁻⁸ to discover genes associated with brain metastasis in patients with brain metastatic breast cancer. We found that the gene encoding the methyl CpG-binding protein MeCP2, a molecule with critical epigenetic functions in the brain^{9,10}, was among the genes most differentially expressed in the brain metastases of patients with brain metastatic breast cancer. MeCP2 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, MECP2, 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 metastases 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 cancer⁶⁻⁸. We discovered significant differential and over-expression of MeCP2, a methyl CpG-binding protein important for reading of epigenetic marks in brain metastases from patients with brain metastatic breast cancer.

Methods

We used datasets GSE125989⁶, GSE43837⁷ and GSE52604⁸ 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. 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 MECP2 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 for the discovery of novel therapeutic targets.

MECP2 is differentially expressed in the brain metastases of patients with brain metastatic breast cancer.

We identified the methyl CpG-binding protein MeCP2 as among the genes whose expression was most significantly different in the brain metastases of patients with brain metastatic breast cancer (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, MeCP2 ranked 104 out of 22297 total transcripts (Table 1). Differential expression of MeCP2 in the brain metastases of patients with brain metastatic breast cancer was statistically significant (Table 1; $p=4.23E-04$).

We mined a second microarray dataset to attempt to validate global differential expression of MeCP2 in brain metastatic breast cancer. Again, we found that MeCP2 was among the genes whose expression changed most in the brain metastases of patients with brain metastatic breast cancer when compared to primary tumors of the breast. 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, MeCP2 ranked 7395 out of 61359 total transcripts, equating to 87.9% differential expression (Table 2). Differential expression of MeCP2 in the brain metastases of patients with brain metastatic breast cancer approached the level of statistical significance (Table 2; $p=0.05828118$).

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 MeCP2 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 metastases revealed that MeCP2 was among the genes whose expression was most significantly different transcriptome-wide between the breast and brain metastases in patients with breast cancer (Table 3). When sorting each of the genes expressed in brain metastases based on significance of difference in expression between brain metastases and normal breast tissues, MeCP2 ranked 13193 out of 41093 total transcripts (Table 3). Differential expression of MeCP2 in brain metastases from patients with brain metastatic breast cancer in this dataset was statistically significant (Table 3; $p=1.65E-02$).

MeCP2 is expressed at significantly higher levels in the brain metastases of patients with brain metastatic breast cancer.

We obtained exact mRNA expression levels for MeCP2, 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 MeCP2 expression in brain metastatic tissues. MeCP2 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; $p=0.0005$). We calculated a mean fold change of 1.341 ± 0.145 in MeCP2 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 in an unbiased and systematic fashion, we identified the methyl CpG-binding protein MECP2 as among the genes whose expression was most different in the brain metastases of patients with breast cancer; MeCP2 was expressed at significantly higher levels in brain metastases as compared to primary tumors of the breast.

Discussion

We report here that the methyl CpG-binding protein and epigenetic reader MeCP2 is among the genes whose expression is most different in the brain metastases of patients with brain metastatic brain cancer. The genomic targets regulated by MeCP2 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
104	202616_s_at	4.23E-04	3.9004608	0.0212	1.341 ± 0.145	MECP2	methyl-CpG binding protein 2

Table 1: MeCP2 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 MeCP2 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
7395	Hs. 3239.0.S2_3p_a_at	0.05828118	1.9456036	-4.00186	MECP2	methyl-CpG binding protein 2

Table 2: MeCP2 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.

Rank	ID	p-value	t	B	Gene	Gene name
13193	A_23_P114361	1.65E-02	2.4866731	-3.803879	MECP2	methyl-CpG binding protein 2

Table 3: MeCP2 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: MeCP2 is expressed at significantly higher levels in the brain metastases of patients with brain metastatic breast cancer.

The mRNA expression level of MeCP2 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 MeCP2 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.