Differential expression of the mutS homolog 6, MSH6, in the brain and lymph node 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 of the DNA mismatch repair gene MSH6 in both the brain metastases and lymph node metastases of patients with metastatic breast cancer as compared to primary tumors of the breast. MSH6 and its function in DNA mismatch repair which confers upon it cancer susceptibility properties⁶ may be relevant to the underlying biology by which tumor cells of the breast spread to the lymph nodes and to the brain.

Keywords: MSH6, 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 trastuzumab⁷. 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 cancer^{4,5}. We discovered significant differential expression of the eukaryotic translation initiation factor MSH6 in brain metastases from patients with brain metastatic breast cancer.

Methods

We used datasets GSE125989⁴ and GSE124648⁵ 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. GSE124648, a microarray dataset, was generated using Affymetrix Human Genome U133A Array technology with $n=256$ primary breast tumors from patients with breast cancer and $n=67$ lymph node metastases from patients with breast cancer; platform GPL96 was used for this analysis. 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 MSH6 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.

MSH6 is differentially expressed in the brain metastases of patients with brain metastatic breast cancer.

We identified the gene encoding the mutS homolog 6, MSH6, 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, MSH6 ranked 231 out of 22277 total transcripts (Table 1), equating to 99.0% differential expression. Differential expression of MSH6 in brain metastases in patients with breast cancer was statistically significant (Table 1; $p=1.95E-03$).

MSH6 is differentially expressed in the lymph node metastases of patients with metastatic breast cancer.

We queried a second microarray dataset⁵ to attempt to validate transcriptome-wide differential expression of MSH6 in metastatic breast cancer. We again found that MSH6 was among the genes whose expression was most significantly different in metastatic breast cancer, here when comparing primary tumors of the breast to lymph node metastases from patients with metastatic breast cancer. When sorting

each of the genes expressed in lymph node metastases based on significance of difference in expression as compared to primary tumors of the breast, MSH6 ranked 3239 out of 22283 total transcripts (Table 2), equating to 85.5% differential expression. Differential expression of MSH6 in lymph node metastases in patients with breast cancer approached the level of statistical significance (Table 2; $p=5.54E-09$).

MSH6 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 MSH6 in primary tumors of the breast and in brain metastases to evaluate magnitude and direction of change in MSH6 gene expression in brain metastatic tissues. MSH6 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 MSH6 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 1.36 ± 0.20 in MSH6 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 MSH6 was among the genes most differentially expressed in metastatic breast cancer, with significantly increased expression in brain metastatic tissues and in lymph node metastases 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 patients with metastatic breast cancer. We discovered the mutS homolog 6, MSH6, as among the genes most differentially expressed in both the lymph node and brain metastases of patients with metastatic breast cancer.

MSH6 is a DNA mismatch repair gene that can function to repair mismatches at basepair resolution with similarity to the bacterial MutHLS system⁷. Females with germline mutation of MSH6 are predisposed to endometrial cancer⁸, and mutation of MSH6 is the cause of hereditary nonpolyposis colorectal cancer (HNPCC)⁹. Proof that these mutations are drivers of oncogenesis is that simultaneous loss of MSH3 and MSH6 in mice results in the development of HNPCC-like cancer¹⁰. An interesting function of MSH6 is in somatic hypermutation and immunoglobulin class-switching¹¹.

We found here using published microarray data^{4,5} that MSH6 was among the genes whose expression changed most significantly in the brain and lymph node metastases of patients with metastatic breast cancer, and present at significantly higher quantities at the mRNA level in brain metastatic tissue. MSH6 and its DNA mismatch repair function may be relevant to the process by which metastatic breast cancer cells spread to the brain, or it may be a reflection of an extraordinarily high mutational burden present in metastatic clones that are capable of colonizing the brain.

References

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Rank	ID	p-value	t	B	FC	Gene	Gene name
231	211449_at	1.95E-03	3.3538154	-1.3179	1.36 ± 0.20	MSH6	mutS homolog 6

Table 1: Differential expression of MSH6 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 MSH6 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
3239	211450_s_at	5.54E-09	-5.991244	9.49102	MSH6	mutS homolog 6

Table 2: Differential expression of MSH6 in lymph node 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: MSH6 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 MSH6 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 MSH6 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.