Succinate dehydrogenase B (SDHB) is differentially expressed in brain metastatic breast cancer.

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One study has reported a 34% incidence of central nervous system metastases, including metastasis to the brain, in breast cancer patients treated with trastuzumab¹. We mined published microarray data^{2,3} to discover genes associated with brain metastasis in breast cancer. We identified significant differential expression of the mitochondrial complex II subunit succinate dehydrogenase B, encoded by SDHB^{4,5}, in the brain metastases of patients with metastatic breast cancer. SDHB 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, SDHB, succinate dehydrogenase B, 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 limited⁶. 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 SDHB 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 SDHB 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.

SDHB is differentially expressed in brain metastatic breast cancer.

We identified succinate dehydrogenase B, encoded by SDHB, 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, SDHB ranked 76 out of 22277 total transcripts (Table 1), equating to 99.7% differential expression. Differential expression of SDHB in brain metastases in brain metastatic breast cancer was statistically significant (Table 1; $p=1.88E-04$).

We queried a second microarray dataset³ to validate transcriptome-wide differential expression of SDHB in brain metastatic breast cancer. We again found that SDHB 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, SDHB ranked 2088 out of 33297 total transcripts (Table 2), equating to 93.7% differential expression. Differential expression of SDHB in brain metastases in brain metastatic breast cancer was statistically significant (Table 2; $p=1.64E-03$).

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

We obtained exact mRNA expression values for SDHB from brain metastases and primary tumors of the breast to evaluate magnitude and direction of change in SDHB expression in brain metastases in patients with breast cancer. SDHB was expressed at lower levels in brain metastases in patients with breast cancer and this difference was statistically significant (Figure 1; $p=0.0001$). We calculated a mean fold change of 0.60 ± 0.17 in SDHB expression when comparing brain metastatic tissues to primary tumors of the breast (Table 1).

Thus, we found that SDHB 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, and present at significantly lower quantities at the mRNA level in brain metastatic tissues as compared to primary tumors of the breast.

Discussion

During cellular respiration, electron transport is performed by five complexes: Complex I-IV. Complex II of the mitochondria consists of four succinate dehydrogenase subunits: SdhA, SdhB, SdhC and SdhD, and effects transfer of electrons to ubiquinone, and functions in oxidation of succinate to produce fumarate; this involves Fe-S clusters in SdhB^{4,5}. In nematodes, the *mev-1* mutants are deficient in their ability to catalyze transport of electrons from succinate to ubiquinone; *mev-1* is an allele of succinate dehydrogenase cytochrome b560, or SdhB⁵. Under conditions of high oxygen (60%), the lifespan of *mev-1* mutants is markedly decreased as compared to wild-type N2 nematodes⁵. Mutation of SDHB is found in patients with familial pheochromocytoma and in familial paraganglioma, a disease featuring vascular tumors of the head and neck⁷. Mutation of SDHB is associated with paraganglioma syndrome type 4 (PGL-4), while mutation of SDHD is associated with paraganglioma syndrome type 1 (PGL-1); while 11/32 patients with SDHB mutation possessed malignant disease including pheochromocytoma and paraganglioma, 0/34 patients with SDHD mutation possessed malignant disease⁸. All 11 patients with SDHB mutation possessed distant metastases to the lung or bones⁸. Thus, mutation of SDHB is specifically associated with tumorigenesis and metastasis in humans. One study described suppression of SDHB expression in breast cancer cells by TGF- β produced by tumor associated macrophages, supporting tumor growth and angiogenesis through increased glycolysis⁹.

We report here that the gene encoding succinate dehydrogenase B, SDHB, was among those 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. SDHB 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
76	214166_at	1.88E-04	-4.181137	0.7356	0.60 ± 0.17	SDHB	succinate dehydrogenase complex iron sulfur subunit B

Table 1: SDHB 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 SDHB 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
2088	7912928	1.64E-03	3.6363787	-1.302699	SDHB	succinate dehydrogenase complex iron sulfur subunit B

Table 2: SDHB 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: SDHB is expressed at significantly lower levels in brain metastases as compared to primary tumors of the breast in patients with brain metastatic breast cancer.

The mRNA expression level of SDHB 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 SDHB 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.