Deleted in malignant brain tumors 1 (DMBT1) is differentially expressed in the tumors of breast cancer patients treated with trastuzumab.

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Trastuzumab (Herceptin) is a monoclonal antibody targeting the extracellular domain of the human epidermal growth factor receptor 2 (HER2)¹ utilized for the treatment of adjuvant and metastatic breast cancer² in the United States and worldwide. We mined published microarray and gene expression data^{3,4} to discover in an unbiased manner the most significant transcriptional changes associated with trastuzumab treatment. We identified deleted in malignant brain tumors 1, DMBT1, as among the genes most differentially expressed in the primary tumors of patients with breast cancer treated with trastuzumab. The primary tumors of breast cancer patients treated with trastuzumab expressed lower levels of DMBT1 messenger RNA than did patients not treated with trastuzumab, and a single administration of trastuzumab was sufficient to result in differential expression of DMBT1 in primary tumors of the breast, demonstrating decreased expression of a gene defined by its lack of expression in tumors of the brain⁵ and whose expression is lacking in multiple human cancers⁶⁻⁸ as a direct transcriptional result of treatment with trastuzumab.

Keywords: trastuzumab, breast cancer, DMBT1, deleted in malignant brain tumors 1, systems biology of breast cancer, targeted therapeutics in breast cancer.

1 The mechanism of action ascribed to trastuzumab function is thought to be binding of the F_{ab}
2 domain of trastuzumab to the extracellular region of the human epidermal growth factor receptor 2
3 (HER2)¹, but the transcriptional consequences resulting from signaling downstream of trastuzumab
4 binding to HER2 is less well understood. Through unbiased, systems-level comparative transcriptome
5 analysis of published microarray and gene expression data^{3,4}, we report here that DMBT1 is a
6 differentially expressed gene in the tumors of trastuzumab-treated patients: patients treated with
7 trastuzumab expressed significantly lower levels of DMBT1 than did those of patients not treated with
8 trastuzumab.

9 **Methods**

10 We used datasets GSE38057³ and GSE114082⁴ this differential gene expression analysis of the
11 transcriptional response to trastuzumab treatment in patients with breast cancer. GSE38057 was
12 generated using DASL Human Cancer Panel by Probe technology with $n=11$ primary breast tumors from
13 patients treated with induction chemotherapy and/or hormone therapy without trastuzumab and $n=36$
14 primary breast tumors from patients treated with induction chemotherapy and/or hormone therapy in
15 addition to trastuzumab; patients in both groups compared had not developed brain metastases at time of
16 data collection by investigators; analysis was performed using platform GPL5826. GSE114082 was
17 generated using Illumina HumanHT-12 WG-DASL V4.0 R2 expression beadchip technology with $n=17$
18 primary breast tumors from patients before trastuzumab treatment and $n=17$ primary tumors from patients
19 after trastuzumab treatment, analysis performed using platform GPL14951. The Benjamini and Hochberg
20 method of p -value adjustment was used for ranking of differential expression but raw p -values were used
21 to assess statistical significance of global or multiplexed differential expression. Log-transformation of
22 data was auto-detected, and the NCBI generated category of platform annotation was used. A statistical
23 test was performed to evaluate whether DMBT1 gene expression was significantly between primary
24 breast tumors of patients receiving trastuzumab using a two-tailed, unpaired t-test with Welch's
25 correction. We used PRISM for all statistical analyses of differential gene expression in human breast
26 cancer (Version 8.4.0)(455).

27 **Results**

28 We performed unbiased comparative transcriptome analysis using published microarray and gene
expression data^{3,4} to identify the most significant transcriptional features of the primary breast tumors of
patients treated with trastuzumab (Herceptin).

DMBT1 is differentially expressed in the tumors of patients treated with trastuzumab.

We identified the deleted in malignant brain tumors 1, DMBT1, as among the genes whose
expression changed most significantly in the tumors of breast cancer patients treated with trastuzumab in
a multiplexed gene expression dataset³ (Table 1). When sorting each of the genes expressed in the
primary tumors of breast cancer patients treated with trastuzumab based on significance of change as
compared to that of patients not treated with trastuzumab, DMBT1 ranked 32 out of 1506 total transcripts
(Table 1), equating to 97.9% differential expression. DMBT1 differential expression in the tumors of
patients treated with trastuzumab was statistically significant (Table 1; $p=0.020463$).

The first dataset, although measuring the expression of over 1500 genes, was still limited. We
turned to a second microarray dataset⁴, this measuring the expression of genes across the transcriptome -
29377 genes in total. The tumors compared in this dataset were sampled from the same patient before
and after a single, brief exposure to trastuzumab, not comparing tumors from different patients treated
with a course of trastuzumab or not. Thus, while differentially expressed genes when comparing tumors

1 based on trastuzumab treatment can result from a variety of sources, including gene expression changes
2 resulting from tumor evolution over the course of treatment, changes identified in this dataset, occurring
3 after only a single treatment of trastuzumab, are more likely to be a direct transcriptional result of
4 trastuzumab binding events. We found again that DMBT1 was among the genes most differentially
5 expressed in the tumors of patients administered a single treatment of trastuzumab (Table 2). When
6 sorting each of the genes expressed in the tumors of patients administered a single treatment of
7 trastuzumab, DMBT1 ranked 7115 out of 29377 total transcripts (Table 2), equating to 75.8% differential
8 expression. Differential expression of DMBT1 in the primary tumors of breast cancer patients following
9 treatment with trastuzumab was statistically significant (Table 2; $p=1.86E-02$).

10 **DMBT1 is expressed at significantly lower levels in the tumors of breast cancer patients treated**
11 **with trastuzumab than in the tumors of those not treated with trastuzumab.**

12 We obtained exact mRNA expression levels for DMBT1 from tumors of patients treated with
13 trastuzumab and from the tumors of patients not treated with trastuzumab to directly evaluate magnitude
14 and change of expression in DMBT1 based on history of trastuzumab treatment. DMBT1 was expressed
15 at lower levels in the tumors of patients treated with trastuzumab as compared to those of patients not
16 treated with trastuzumab, and this difference was deemed statistically significant (Figure 1; $p=0.1035$).
17 We calculated a mean fold change of 0.58 ± 0.30 in DMBT1 expression in the primary tumors of patients
18 treated with trastuzumab relative to that of patients not treated with trastuzumab.

19 Thus, we found using published multiplexed gene expression and microarray data^{3,4} that DMBT1
20 was among the genes most differentially expressed in the tumors of patients treated with trastuzumab, that
21 DMBT1 was expressed at lower levels in the tumors of patients treated with trastuzumab as compared to
22 those not treated with trastuzumab, and that this transcriptional change was likely a direct result of
23 trastuzumab treatment as opposed to a gene expression change associated with clonal selection and
24 evolution of the tumor across the course of treatment.

25 **Discussion**

26 We provided evidence here that deleted in malignant brain tumors 1, DMBT1, is a differentially
27 expressed gene in the tumors of patients treated with trastuzumab, and that the primary tumors of breast
28 cancer patients treated with trastuzumab expressed lower levels of DMBT1 than did patients not treated
29 with trastuzumab.

30 These data demonstrate that a locus defined by its deletion in malignant brain tumors⁵ and one
31 whose expression is lost or down-regulated in cancers of the colon, lung and breast⁶⁻⁸ is substantially
32 decreased in the tumors of breast cancer patients treated with trastuzumab and that this decreased
33 expression is likely a direct transcriptional consequence of treatment with trastuzumab, a treatment that
34 has been associated with a 34% rate of central nervous system metastasis⁹.

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Rank	ID	p-value	t	B	FC	Gene
32	GI_4758169-A-4	0.020463	-2.39617	-3.58	0.58 ± 0.30	DMBT1

Table 1: DMBT1 is differentially expressed in the primary tumors of breast cancer patients treated with trastuzumab.

The rank of differential expression, probe ID, p-value with respect to global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the two groups compared, fold change of expression in DMBT1 between the tumors of patients treated with trastuzumab and the tumors of patients not treated with trastuzumab, and gene are listed in this chart.

Rank	ID	p-value	t	B	Gene
7115	ILMN_1681612	1.86E-02	-2.4644528	-3.5193	DMBT1

Table 2: A single exposure to trastuzumab is sufficient to drive differential expression of DMBT1 in the primary tumors of patients with breast cancer.

The rank of differential expression, probe ID, p-value with respect to global 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: DMBT1 is expressed at significantly higher levels in the primary tumors of patients treated with trastuzumab.

The mRNA expression level of DMBT1 is graphically depicted in this chart in the primary tumors of breast cancer patients not treated with trastuzumab (left) and in the primary tumors of breast cancer patients treated with trastuzumab (right), with mean mRNA expression level marked and the result of a statistical test evaluating significance of difference between DMBT1 expression in the primary tumors of patients treated with trastuzumab or not, a *p*-value, listed above.