The slit guidance ligand 1, SLIT1, 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 the slit guidance ligand 1, encoded by SLIT1, 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 higher levels of SLIT1 messenger RNA than did patients not treated with trastuzumab, and a single administration of trastuzumab was sufficient to result in differential expression of SLIT1 in primary tumors of the breast, demonstrating increased expression of a ligand involved in guidance of axon migration in the central nervous system⁵⁻⁹ as a direct transcriptional consequence of treatment with trastuzumab.

Keywords: trastuzumab, breast cancer, SLIT1, slit guidance ligand 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
analysis of published microarray and gene expression data^{3,4}, we report here that SLIT1 is a differentially
expressed gene in the tumors of trastuzumab-treated patients: patients treated with trastuzumab expressed
significantly higher levels of SLIT1 than did those of patients not treated with trastuzumab.

5 **Methods**

6 We used datasets GSE130788³, and GSE114082⁴ for this differential gene expression analysis of
7 the transcriptional response to trastuzumab treatment in patients with breast cancer. GSE130788 was
8 generated using Agilent-014850 Whole Human Genome Microarray 4x44K G4112F technology with
9 $n=11$ primary breast tumors from patients treated with trastuzumab and $n=36$ primary tumors from
10 patients treated with trastuzumab, analysis performed using platform GPL6480. GSE114082 was
11 generated using Illumina HumanHT-12 WG-DASL V4.0 R2 expression beadchip technology with $n=17$
12 primary breast tumors from patients before trastuzumab treatment and $n=17$ primary tumors from patients
13 after trastuzumab treatment, analysis performed using platform GPL14951. The Benjamini and Hochberg
14 method of p -value adjustment was used for ranking of differential expression but raw p -values were used
15 to assess statistical significance of global or multiplexed differential expression. Log-transformation of
16 data was auto-detected, and the NCBI generated category of platform annotation was used. A statistical
17 test was performed to evaluate whether SLIT1 gene expression was significantly between primary breast
18 tumors of patients receiving trastuzumab or not using a two-tailed, unpaired t-test with Welch's
19 correction. We used PRISM for all statistical analyses of differential gene expression in human breast
20 cancer (Version 8.4.0)(455).

21 **Results**

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

25 **SLIT1 is differentially expressed in the tumors of patients treated with trastuzumab.**

26 We identified the slit guidance ligand 1, encoded by SLIT1 as among the genes whose expression
27 changed most significantly in the tumors of breast cancer patients treated with trastuzumab in a
28 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, SLIT1 ranked 236 out of 41000 total transcripts (Table 1),
equating to 99.4% differential expression. SLIT1 differential expression in the tumors of patients treated
with trastuzumab trended towards statistical significance (Table 1; $p=1.87E-44$).

29 We turned to a second microarray dataset to validate our findings of SLIT1 differential expression
30 in the patients treated with trastuzumab. The tumors compared in this dataset were sampled from the
31 same patient before and after a single, brief exposure to trastuzumab, not comparing tumors from
32 different patients treated with a course of trastuzumab or not. Thus, while differentially expressed genes
33 when comparing tumors based on trastuzumab treatment can result from a variety of sources, including
34 gene expression changes resulting from tumor evolution over the course of treatment, changes identified
35 in this dataset, occurring after only a single treatment of trastuzumab, are more likely to be a direct

transcriptional result of trastuzumab binding events. We found again that SLIT1 was among the genes most differentially expressed in the tumors of patients administered a single treatment of trastuzumab (Table 2). When sorting each of the genes expressed in the tumors of patients administered a single treatment of trastuzumab, SLIT1 ranked 7326 out of 29377 total transcripts (Table 2), equating to 75.1% differential expression. Differential expression of SLIT1 in the primary tumors of breast cancer patients following treatment with trastuzumab was statistically significant (Table 2; $p=2.02E-02$).

SLIT1 is expressed at significantly higher levels in the tumors of breast cancer patients treated with trastuzumab than in the tumors of those not treated with trastuzumab.

We obtained exact mRNA expression levels for SLIT1 from tumors of patients treated with trastuzumab and from the tumors of patients not treated with trastuzumab to directly evaluate direction and statistical significance of change in expression of SLIT1 based on history of trastuzumab treatment. SLIT1 was expressed at higher levels in the tumors of patients treated with trastuzumab as compared to those of patients not treated with trastuzumab, and this difference was deemed statistically significant (Figure 1; $p<0.0001$).

Thus, we found using published multiplexed gene expression and microarray data^{3,4} that SLIT1 was among the genes most differentially expressed in the tumors of patients treated with trastuzumab, that SLIT1 was expressed at higher levels in the tumors of patients treated with trastuzumab as compared to those not treated with trastuzumab, and that this transcriptional change was likely a direct result of trastuzumab treatment as opposed to a gene expression change associated with clonal selection and evolution of the tumor across the course of treatment.

Discussion

We provided evidence here that the slit guidance ligand 1, SLIT1, is a differentially expressed gene in the tumors of patients treated with trastuzumab, and expressed at significantly higher levels in the primary tumors of breast cancer patients treated with trastuzumab as compared to patients not treated with trastuzumab. These data demonstrate that increased primary tumor expression of a molecule expressed in cells of the central nervous system and involved in guidance of axon migration as a direct transcriptional consequence of treatment with trastuzumab in patients with breast cancer.

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Rank	ID	p-value	t	B	Gene	Gene name
236	A_24_P940644	1.87E-44	18.266584	90.520511	SLIT1	slit guidance ligand 1

Table 1: SLIT1 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, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene
7326	ILMN_1756959	2.02E-02	-2.4312648	-3.58811	SLIT1

Table 2: A single exposure to trastuzumab is sufficient to drive differential expression of SLIT1 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: SLIT1 is expressed at significantly higher levels in the primary tumors of patients treated with trastuzumab.

The mRNA expression level of SLIT1 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 SLIT1 expression in the primary tumors of patients treated with trastuzumab or not, a p -value, listed above.