SIAH1 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 E3 ubiquitin ligase SIAH1 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 SIAH1 messenger RNA than did patients not treated with trastuzumab, and a single administration of trastuzumab was sufficient to result in differential expression of SIAH1 in primary tumors of the breast, demonstrating increased expression of a molecule that functions in targeting an apoptotic effector (HIPK2) for proteasomal degradation⁵ as a direct transcriptional result of treatment with trastuzumab.

Keywords: trastuzumab, breast cancer, SIAH1, 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 SIAH1 is a differentially
expressed gene in the tumors of trastuzumab-treated patients: patients treated with trastuzumab expressed
significantly higher levels of SIAH1 than did those of patients not treated with trastuzumab.

5 **Methods**

6 We used datasets GSE38057³ and GSE114082⁴ this differential gene expression analysis of the
7 transcriptional response to trastuzumab treatment in patients with breast cancer. GSE38057 was
8 generated using DASL Human Cancer Panel by Probe technology with $n=11$ primary breast tumors from
9 patients treated with induction chemotherapy and/or hormone therapy without trastuzumab and $n=36$
primary breast tumors from patients treated with induction chemotherapy and/or hormone therapy in
addition to trastuzumab; patients in both groups compared had not developed brain metastases at time of
10 data collection by investigators; analysis was performed using platform GPL5826. GSE114082 was
11 generated using Illumina HumanHT-12 WG-DASL V4.0 R2 expression beadchip technology with $n=17$
primary breast tumors from patients before trastuzumab treatment and $n=17$ primary tumors from patients
after trastuzumab treatment, analysis performed using platform GPL14951. The Benjamini and Hochberg
12 method of p -value adjustment was used for ranking of differential expression but raw p -values were used
13 to assess statistical significance of global or multiplexed differential expression. Log-transformation of
14 data was auto-detected, and the NCBI generated category of platform annotation was used. A statistical
test was performed to evaluate whether SIAH1 gene expression was significantly between primary breast
15 tumors of patients receiving trastuzumab 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
16 8.4.0)(455).

17 **Results**

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

20 **SIAH1 is differentially expressed in the tumors of patients treated with trastuzumab.**

21 We identified the E3 ubiquitin ligase SIAH1 as among the genes whose expression changed most
22 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
23 cancer patients treated with trastuzumab based on significance of change as compared to that of patients
not treated with trastuzumab, SIAH1 ranked 71 out of 1506 total transcripts (Table 1), equating to 95.3%
24 differential expression. SIAH1 differential expression in the tumors of patients treated with trastuzumab
approached the level of statistical significance (Table 1; $p=0.059293$).

25 The first dataset, although measuring the expression of over 1500 genes, was still limited. We
26 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
27 and after a single, brief exposure to trastuzumab, not comparing tumors from different patients treated
28 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 SIAH1 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, SIAH1 ranked 1022 out of 29377 total transcripts (Table 2), equating to 96.5% differential
8 expression. Differential expression of SIAH1 in the primary tumors of breast cancer patients following
9 treatment with trastuzumab was statistically significant (Table 2; $p=7.3E-05$).

10 **SIAH1 is expressed at significantly higher levels in the tumors of breast cancer patients treated with**
11 **trastuzumab than in the tumors of those not treated with trastuzumab.**

12 We obtained exact mRNA expression levels for SIAH1 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 SIAH1 based on history of trastuzumab treatment. SIAH1 was expressed at
15 higher 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; $p=0.0022$).
17 We calculated a mean fold change of 1.54 ± 0.76 in SIAH1 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 SIAH1
20 was among the genes most differentially expressed in the tumors of patients treated with trastuzumab, that
21 SIAH1 was expressed at higher 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 Migration of MDA-MB-231 breast cancer cells is impaired by depletion of SIAH1 *in vitro*,
27 indicating a role for SIAH1 in breast cancer cell migration⁶. We provided evidence here that an E3
28 ubiquitin ligase that can function in migration of breast cancer cells, and by targeting an apoptotic effector
for proteasomal degradation limits a mechanism by which cells can execute death signaling, 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.

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Rank	ID	p-value	t	B	FC	Gene
71	GI_4506946-S-4	0.059293	1.93127	-4.04	1.54 ± 0.76	SIAH1

Table 1: SIAH1 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 SIAH1 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
1022	ILMN_2279413	7.3E-05	-4.4808903	1.55638	SIAH1

Table 2: A single exposure to trastuzumab is sufficient to drive differential expression of SIAH1 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: SIAH1 is expressed at significantly higher levels in the primary tumors of patients treated with trastuzumab.

The mRNA expression level of SIAH1 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 SIAH1 expression in the primary tumors of patients treated with trastuzumab or not, a *p*-value, listed above.