GAS7 is differentially expressed in the primary tumors of breast cancer patients treated with trastuzumab.

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Trastuzumab, a monoclonal antibody targeted against the human epidermal growth factor receptor 2 (HER2) is utilized for the treatment of human breast cancer^{1,2}, but a complete understanding of how tumor signal transduction is modulated by trastuzumab treatment is lacking. By mining published and public microarray and gene expression data^{3,4} from the primary tumors of patients treated with trastuzumab, we found that the growth arrest-specific gene 7, GAS7, was among the genes most differentially expressed in the primary tumors of patients treated with trastuzumab, and expressed at lower levels in the tumors of patients treated with trastuzumab. Thus, the use of trastuzumab in patients with breast cancer is associated with increased expression of a gene⁵ that can induce neurite outgrowth and support survival of acute lymphoblastic leukemias⁶.

Keywords: breast cancer, trastuzumab, GAS7, growth arrest-specific gene 7, systems biology of breast cancer, targeted therapeutics in breast cancer.

1 The mechanism of action by which trastuzumab exerts its therapeutic function in human breast
2 cancer is said to be binding of the F_{ab} domain of trastuzumab to the extracellular region of the human
3 epidermal growth factor receptor 2 (HER2)², possibly resulting in decreased cellular proliferation⁷.
4 Transcriptional modulation resulting from trastuzumab binding events in the primary tumors of treated
5 patients is less well understood. We mined published and public microarray and gene expression data^{3,4}
6 generated using primary tissues from patients treated with trastuzumab to compare tumor global gene
7 expression with that of patients not treated with trastuzumab. Using this unbiased, systems level
8 methodology, we identified differential expression and up-regulation of GAS7 in primary tumors of the
9 breast in patients treated with trastuzumab.

10 **Methods**

11 We used datasets GSE38057³ and GSE130788⁴ for this differential gene expression analysis of
12 the transcriptional response to trastuzumab treatment in patients with breast cancer. GSE38057 was
13 generated using DASL Human Cancer Panel by Probe technology with $n=11$ primary breast tumors from
14 patients treated with induction chemotherapy and/or hormone therapy without trastuzumab and $n=36$
15 primary breast tumors from patients treated with induction chemotherapy and/or hormone therapy in
16 addition to trastuzumab; analysis was performed using platform GPL5826. GSE130788 was generated
17 using Agilent-014850 Whole Human Genome Microarray 4x44K G4112F technology with $n=11$ primary
18 breast tumors from patients treated with trastuzumab and $n=36$ primary tumors from patients treated with
19 trastuzumab, analysis performed using platform GPL6480. The Benjamini and Hochberg method of p -
20 value adjustment was used for ranking of differential expression but raw p -values were used to assess
21 statistical significance of global or multiplexed differential expression. Log-transformation of data was
22 auto-detected, and the NCBI generated category of platform annotation was used. A statistical test was
23 performed to evaluate whether GAS7 gene expression was significantly between primary breast tumors of
24 patients receiving trastuzumab using a two-tailed, unpaired t-test with Welch's correction. We used
25 PRISM for all statistical analyses of differential gene expression in human breast cancer (Version 8.4.0)
26 (455).

27 **Results**

28 We performed comparative transcriptome analysis of primary tumors from patients treated or not
with trastuzumab (Herceptin), using published multiplexed gene expression data³ and a microarray
dataset⁴, in order to identify the most significant transcriptional changes in the primary tumors of breast
cancer patients following treatment with trastuzumab.

29 **GAS7 is differentially expressed in the primary tumors of patients treated with trastuzumab.**

30 We found that the growth arrest-specific gene 7, encoded by GAS7, was among the genes whose
31 expression was most significantly different in the primary tumors of patients with breast cancer treated
32 with trastuzumab³. When sorting each of the genes expressed in the primary tumors of breast cancer
33 patients treated with trastuzumab based on significance of difference in expression as compared to the
34 primary tumors of breast cancer patients not treated with trastuzumab, GAS7 ranked 50 out of 1506 total
35 transcripts, equating to 96.7% statistical significance (Table 1). Differential expression of GAS7 in the
36 primary tumors of breast cancer patients treated with trastuzumab was statistically significant (Table 1;
37 $p=0.039501$).

38 We queried a second dataset⁶ to validate our findings of GAS7 differential expression upon
trastuzumab treatment. In a second microarray dataset analyzing gene expression of patients treated with

trastuzumab, we again found that GAS7 was among the genes whose expression was most significantly different transcriptome-wide when comparing the global gene expression profiles of tumors from patients treated with trastuzumab as compared to those not treated with trastuzumab. When sorting each of the genes expressed in the tumors of patients treated with trastuzumab based on significance of change as compared to the tumors of patients not treated with trastuzumab, GAS7 ranked 7101 out of 41000 total transcripts, equating to 82.7% differential expression. Differential expression of GAS7 in the primary tumors of patients treated with trastuzumab was statistically significant (Table 2; $p=8.86E-10$).

GAS7 is expressed at higher levels in the tumors of patients treated with trastuzumab.

We obtained exact mRNA expression levels for GAS7 from the tumors of patients treated with trastuzumab and from the tumors of patients not receiving trastuzumab to determine magnitude and direction of GAS7 change in the tumors of patients treated with trastuzumab. GAS7 expression was higher in the tumors of patients treated with trastuzumab as compared to the tumors of patients not treated with trastuzumab (Figure 1). Increased expression of GAS7 was statistically significant (Figure 1: $p=0.0163$). We calculated a mean fold change of 1.30 ± 0.48 when comparing the expression of GAS7 in the tumors of patients treated with trastuzumab as compared to the tumors of patients treated with trastuzumab.

Thus, we found that the growth arrest-specific gene 7, encoded by GAS7, was among the genes most differentially expressed in the primary tumors of patients with breast cancer and that GAS7 expression was significantly higher in the primary tumors of patients treated with trastuzumab as compared to those not treated.

Discussion

We provided evidence here that the growth arrest-specific gene 7, encoded by GAS7 is expressed at higher levels in the tumors of patients treated with trastuzumab and among the genes most differentially expressed in the primary tumors of patients treated with trastuzumab. Thus, administration of trastuzumab in patients with breast cancer, a treatment that we recently demonstrated results in transcriptional induction of the nerve growth factor receptor⁷ and that has been associated with a 34% rate of central nervous system metastasis⁸, results in decreased primary tumor expression of a molecule that can induce proliferation of acute lymphoblastic leukemia cells⁶, and can cooperate with the nerve growth factor to induce neurite outgrowth⁹.

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Rank	ID	p-value	t	B	FC	Gene
50	GI_4503920-A-2	0.039501	2.11591	-3.87	1.30 ± 0.48	GAS7

Table 1: GAS7 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 GAS7 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	Gene name
7101	A_24_P395610	8.86E-10	6.437708	11.258973	GAS7	growth arrest specific 7

Table 2: GAS7 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 GAS7 between the tumors of patients treated with trastuzumab and the tumors of patients not treated with trastuzumab, and gene are listed in this chart.

Figure 1: GAS7 is expressed at higher levels in the primary tumors of patients treated with trastuzumab as compared to those not treated with trastuzumab.

The mRNA expression level of GAS7 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 GAS7 expression in the primary tumors of patients treated with trastuzumab or not, a *p*-value, listed above.