Adropin, produced by the energy homeostasis associated gene *ENHO* 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 ENHO 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 ENHO messenger RNA than did patients not treated with trastuzumab, and a single administration of trastuzumab was sufficient to result in differential expression of ENHO in primary tumors of the breast, demonstrating increased expression of a molecule involved in regulation of glucose and lipid metabolism and whose expression is modulated by high-fat diet⁵ as a direct transcriptional consequence of treatment with trastuzumab.

Keywords: trastuzumab, breast cancer, ENHO, systems biology of breast cancer, targeted therapeutics in breast cancer.

The mechanism of action ascribed to trastuzumab function is thought to be binding of the F_{ab} domain of trastuzumab to the extracellular region of the human epidermal growth factor receptor 2 (HER2)¹, but the transcriptional consequences resulting from signaling downstream of trastuzumab 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 ENHO is a differentially expressed gene in the tumors of trastuzumab-treated patients: patients treated with trastuzumab expressed significantly higher levels of ENHO than did those of patients not treated with trastuzumab.

Methods

We used datasets GSE130788³, and GSE114082⁴ for this differential gene expression analysis of the transcriptional response to trastuzumab treatment in patients with breast cancer. GSE130788 was generated using Agilent-014850 Whole Human Genome Microarray 4x44K G4112F technology with *n*=11 primary breast tumors from patients treated with trastuzumab and *n*=36 primary tumors from patients treated with trastuzumab, analysis performed using platform GPL6480. GSE114082 was 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 method of *p*-value adjustment was used for ranking of differential expression but raw *p*-values were used to assess statistical significance of global or multiplexed 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 ENHO gene expression was significantly between primary breast 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 8.4.0)(455).

Results

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).

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

We identified neuronal differentiation 4, encoded by ENHO 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, ENHO ranked 176 out of 41000 total transcripts (Table 1), equating to 99.6% differential expression. ENHO differential expression in the tumors of patients treated with trastuzumab trended towards statistical significance (Table 1; *p*=5.13E-48).

We turned to a second microarray dataset to validate our findings of ENHO differential expression in the patients treated with trastuzumab. 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 based on trastuzumab treatment can result from a variety of sources, including gene expression changes resulting from tumor evolution over the course of treatment, changes identified

1 in this dataset, occurring after only a single treatment of trastuzumab, are more likely to be a direct
2 transcriptional result of trastuzumab binding events. We found again that ENHO was among the genes
3 most differentially expressed in the tumors of patients administered a single treatment of trastuzumab
4 (Table 2). When sorting each of the genes expressed in the tumors of patients administered a single
5 treatment of trastuzumab, ENHO ranked 4074 out of 29377 total transcripts (Table 2), equating to 86.1%
6 differential expression. Differential expression of ENHO in the primary tumors of breast cancer patients
7 following treatment with trastuzumab was statistically significant (Table 2; $p=3.47E-03$).

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

10 We obtained exact mRNA expression levels for ENHO from tumors of patients treated with
11 trastuzumab and from the tumors of patients not treated with trastuzumab to directly evaluate direction
12 and statistical significance of change in expression of ENHO based on history of trastuzumab treatment.
13 ENHO was expressed at higher levels in the tumors of patients treated with trastuzumab as compared to
14 those of patients not treated with trastuzumab, and this difference was statistically significant (Figure 1;
15 $p<0.0001$).

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

22 **Discussion**

23 We provided evidence here that adropin, expressed by the energy homeostasis associated gene
24 *ENHO* is differentially expressed in the tumors of patients treated with trastuzumab, at significantly
25 higher levels in the primary tumors of breast cancer patients treated with trastuzumab as compared to
26 patients not treated with trastuzumab. Thus, trastuzumab administration in patients with breast cancer
27 results in increased primary tumor expression of a protein whose expression is modulated by dietary
28 intake and regulates homeostasis of lipids and glucose⁵.

References

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Rank	ID	p-value	t	B	Gene	Gene name
176	A_24_P32560	5.13E-48	19.568177	98.695326	ENHO	energy homeostasis associated

Table 1: ENHO 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
4074	ILMN_1713835	3.47E-03	-3.1298755	-2.01459	ENHO

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

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