Neuropeptide Y receptor Y1 and Neuropeptide Y receptor Y5 are 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 data^{3,4} to discover in an unbiased manner the most significant transcriptional changes associated with trastuzumab treatment. We identified both neuropeptide Y receptor Y1 and neuropeptide Y receptor Y5, encoded by NPY1R and NPY5R 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 NPY1R and NPY5R messenger RNA than did patients not treated with trastuzumab; a single administration of trastuzumab was sufficient to result in differential expression of NPY1R in primary tumors of the breast, demonstrating increased expression of neuropeptide receptors, the expression of one of which in the peripheral blood is correlated with metastasis in patients with breast cancer⁵, as a transcriptional consequence of treatment with trastuzumab.

Keywords: trastuzumab, breast cancer, NPY1R, NPY5R, neuropeptide Y receptor Y1, neuropeptide Y receptor Y5, 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 data^{3,4}, we report here that NPY1R and NPY5R are differentially expressed genes in the tumors of trastuzumab-treated patients: patients treated with trastuzumab expressed significantly higher levels of NPY1R and NPY5R 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 NPY1R or NPY5R gene expression was significantly between primary breast tumors of patients receiving trastuzumab or not 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).

NPY1R is differentially expressed in the tumors of patients treated with trastuzumab.

We identified neuropeptide Y receptor Y1, encoded by NPY1R 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, NPY1R ranked 47 out of 41000 total transcripts (Table 1), equating to 99.9% differential expression. NPY1R differential expression in the tumors of patients treated with trastuzumab trended towards statistical significance (Table 1; $p=1.29E-67$).

We turned to a second microarray dataset to validate our findings of NPY1R 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

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 NPY1R 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, NPY1R ranked 4826 out of 29377 total transcripts (Table 2), equating to 83.6% differential expression. Differential expression of NPY1R in the primary tumors of breast cancer patients following treatment with trastuzumab was statistically significant (Table 2; $p=5.83E-03$).

NPY1R 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 NPY1R 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 NPY1R based on history of trastuzumab treatment. NPY1R 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 statistically significant (Figure 1; $p<0.0001$).

NPY5R is differentially expressed in the tumors of patients treated with trastuzumab.

We also identified neuropeptide Y receptor Y5, encoded by NPY5R 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, NPY5R ranked 48 out of 41000 total transcripts (Table 3), equating to 99.9% differential expression. NPY5R differential expression in the tumors of patients treated with trastuzumab trended towards statistical significance (Table 3; $p=3.25E-67$).

We turned to a second microarray dataset to validate our findings of NPY5R differential expression in the patients treated with trastuzumab. We again compared tumors from the same patient before and after a single, brief exposure to trastuzumab, rather than comparing tumors from different patients treated with a course of trastuzumab or not. We found that NPY5R was again among the genes most differentially expressed in the tumors of patients administered a single treatment of trastuzumab (Table 4). When sorting each of the genes expressed in the tumors of patients administered a single treatment of trastuzumab, NPY5R ranked 548 out of 32321 total transcripts (Table 4), equating to 98.3% differential expression. Differential expression of NPY5R in the primary tumors of breast cancer patients following treatment with trastuzumab approached statistical significance (Table 4; $p=1.7E-05$).

NPY5R 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 NPY5R 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 NPY5R based on history of trastuzumab treatment. NPY5R 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 statistically significant (Figure 2; $p<0.0001$).

1 Thus, we found using published microarray data^{3,4} that both NPY1R and NPY5R was among the
2 genes most differentially expressed in the tumors of patients treated with trastuzumab, that NPY1R and
3 NPY5R were both expressed at higher levels in the tumors of patients treated with trastuzumab as
4 compared to those not treated with trastuzumab, and that this transcriptional change, and that for both
5 NPY1R and NPY5R, this was likely a direct result of trastuzumab treatment as opposed to a gene
6 expression change associated with clonal selection and evolution of the tumor across the course of
7 treatment.

8 **Discussion**

9 We provided evidence here that the neuropeptide receptors NPY1R and NPY5R are differentially
10 expressed genes in the tumors of patients treated with trastuzumab, and expressed at significantly higher
11 levels in the primary tumors of breast cancer patients treated with trastuzumab as compared to patients not
12 treated with trastuzumab. Thus, trastuzumab administration in patients with breast cancer results in
13 increased expression of a neuropeptide receptor whose expression is correlated with metastasis in patients
14 with breast cancer⁵. It remains unclear how transcriptional induction of two neuropeptide receptors
15 relates to a reported 34% frequency of central nervous system metastasis in patients treated with
16 trastuzumab⁶.

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Rank	ID	p-value	t	B	Gene	Gene name
47	A_23_P69699	1.29E-67	26.767901	143.558733	NPY1R	neuropeptide Y receptor Y1

Table 1: NPY1R 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
4826	ILMN_1667947	5.83E-03	2.9320211	-2.48501	NPY1R

Table 2: A single exposure to trastuzumab is sufficient to drive differential expression of NPY1R 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: NPY1R is expressed at significantly higher levels in the primary tumors of patients treated with trastuzumab.

The mRNA expression level of NPY1R 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 NPY1R expression in the primary tumors of patients treated with trastuzumab or not, a p -value, listed above.

Rank	ID	p-value	t	B	Gene	Gene name
48	A_23_P251836	3.25E-67	26.48251	142.64458	NPY5R	neuropeptide Y receptor Y5

Table 1: NPY5R 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
548	ILMN_1718198	1.7E-05	4.9623452	2.92082	NPY5R

Table 2: NPY5R 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.

Figure 2: NPY5R is expressed at significantly higher levels in the primary tumors of patients treated with trastuzumab.

The mRNA expression level of NPY5R 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 NPY5R expression in the primary tumors of patients treated with trastuzumab or not, a *p*-value, listed above.