The fibroblast growth factor 1 (FGF1) 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 fibroblast growth factor FGF1 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 FGF1 messenger RNA than did patients not treated with trastuzumab, and a single administration of trastuzumab was sufficient to result in differential expression of FGF1 in primary tumors of the breast, demonstrating that increased expression of a growth factor⁵ whose therapeutic blockade results in decreased primary tumor volume and impairment of lung metastasis⁶ as a direct transcriptional result of treatment with trastuzumab.

Keywords: trastuzumab, breast cancer, FGF1, 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 FGF1 is a differentially expressed gene in the tumors of trastuzumab-treated patients: patients treated with trastuzumab expressed significantly higher levels of FGF1 than did those of patients not treated with trastuzumab.

Methods

We used datasets GSE38057³ and GSE114082⁴ this differential gene expression analysis of the transcriptional response to trastuzumab treatment in patients with breast cancer. GSE38057 was generated using DASL Human Cancer Panel by Probe technology with $n=11$ primary breast tumors from 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 data collection by investigators; analysis was performed using platform GPL5826. 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 FGF1 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).

FGF1 is differentially expressed in the tumors of patients treated with trastuzumab.

We identified the fibroblast growth factor FGF1 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, FGF1 ranked 89 out of 1506 total transcripts (Table 1), equating to 94.1% differential expression. FGF1 differential expression in the tumors of patients treated with trastuzumab trended towards statistical significance (Table 1; $p=0.07077$).

The first dataset, although measuring the expression of over 1500 genes, was still limited. We 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 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

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 FGF1 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, FGF1 ranked 5125 out of 29377 total transcripts (Table 2), equating to 82.6% differential
8 expression. Differential expression of FGF1 in the primary tumors of breast cancer patients following
9 treatment with trastuzumab was statistically significant (Table 2; $p=6.95E-03$).

7 **FGF1 is expressed at significantly higher levels in the tumors of breast cancer patients treated with**
8 **trastuzumab than in the tumors of those not treated with trastuzumab.**

9 We obtained exact mRNA expression levels for FGF1 from tumors of patients treated with
10 trastuzumab and from the tumors of patients not treated with trastuzumab to directly evaluate magnitude
11 and change of expression in FGF1 based on history of trastuzumab treatment. FGF1 was expressed at
12 higher levels in the tumors of patients treated with trastuzumab as compared to those of patients not
13 treated with trastuzumab, and this difference was deemed statistically significant (Figure 1; $p=0.0073$).
14 We calculated a mean fold change of 1.55 ± 0.91 in FGF1 expression in the primary tumors of patients
15 treated with trastuzumab relative to that of patients not treated with trastuzumab.

13 Thus, we found using published multiplexed gene expression and microarray data^{3,4} that FGF1
14 was among the genes most differentially expressed in the tumors of patients treated with trastuzumab, that
15 FGF1 was expressed at higher levels in the tumors of patients treated with trastuzumab as compared to
16 those not treated with trastuzumab, and that this transcriptional change was likely a direct result of
17 trastuzumab treatment as opposed to a gene expression change associated with clonal selection and
18 evolution of the tumor across the course of treatment.

17 **Discussion**

18 We provided evidence here that the fibroblast growth factor FGF1 is a differentially expressed
19 gene in the tumors of patients treated with trastuzumab, and expressed at significantly higher levels in the
20 primary tumors of breast cancer patients treated with trastuzumab as compared to patients not treated with
21 trastuzumab. These data demonstrate that increased primary tumor expression of a growth factor⁵ whose
22 inhibition results in decreased primary tumor volume and impairment of lung metastasis in an
23 experimental breast cancer model *in vivo*⁶ is a direct transcriptional consequence of treatment with
24 trastuzumab in patients with breast cancer.
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Rank	ID	p-value	t	B	FC	Gene
89	GI_15055544-A-2	0.07077	1.84748	-4.11	1.55 ± 0.91	FGF1

Table 1: FGF1 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 FGF1 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
5125	ILMN_1699735	6.95E-03	-2.8640484	-2.64242	FGF1

Table 2: A single exposure to trastuzumab is sufficient to drive differential expression of FGF1 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: FGF1 is expressed at significantly higher levels in the primary tumors of patients treated with trastuzumab.

The mRNA expression level of FGF1 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 FGF1 expression in the primary tumors of patients treated with trastuzumab or not, a *p*-value, listed above.