FGF10 is differentially expressed in the tumors of breast cancer patients treated with trastuzumab.

Shahan Mamoor, MS¹

¹shahanmamoor@gmail.com

East Islip, NY USA

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 FGF10 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 FGF10 messenger RNA than did patients not treated with trastuzumab, and a single administration of trastuzumab was sufficient to result in differential expression of FGF10 in primary tumors of the breast, demonstrating increased expression of a growth factor which provides signals to support development of the limbs and for the differentiation of radial glial cells from neuronal progenitor cells⁵⁻⁷ as a direct transcriptional consequence of treatment with trastuzumab.

Keywords: trastuzumab, breast cancer, FGF10, fibroblast growth factor 10, 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 FGF10 is a differentially
expressed gene in the tumors of trastuzumab-treated patients: patients treated with trastuzumab expressed
significantly higher levels of FGF10 than did those of patients not treated with trastuzumab.

5 **Methods**

6 We used datasets GSE130788³, and GSE114082⁴ for this differential gene expression analysis of
7 the transcriptional response to trastuzumab treatment in patients with breast cancer. GSE130788 was
8 generated using Agilent-014850 Whole Human Genome Microarray 4x44K G4112F technology with
9 $n=11$ primary breast tumors from patients treated with trastuzumab and $n=36$ primary tumors from
10 patients treated with trastuzumab, analysis performed using platform GPL6480. GSE114082 was
11 generated using Illumina HumanHT-12 WG-DASL V4.0 R2 expression beadchip technology with $n=17$
12 primary breast tumors from patients before trastuzumab treatment and $n=17$ primary tumors from patients
13 after trastuzumab treatment, analysis performed using platform GPL14951. The Benjamini and Hochberg
14 method of p -value adjustment was used for ranking of differential expression but raw p -values were used
15 to assess statistical significance of global or multiplexed differential expression. Log-transformation of
16 data was auto-detected, and the NCBI generated category of platform annotation was used. A statistical
17 test was performed to evaluate whether FGF10 gene expression was significantly between primary breast
18 tumors of patients receiving trastuzumab using a two-tailed, unpaired t-test with Welch's correction. We
19 used PRISM for all statistical analyses of differential gene expression in human breast cancer (Version
8.4.0)(455).

20 **Results**

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

24 **FGF10 is differentially expressed in the tumors of patients treated with trastuzumab.**

25 We identified neuronal differentiation 4, encoded by FGF10 as among the genes whose
26 expression changed most significantly in the tumors of breast cancer patients treated with trastuzumab in
27 a multiplexed gene expression dataset³ (Table 1). When sorting each of the genes expressed in the
28 primary tumors of breast cancer patients treated with trastuzumab based on significance of change as
compared to that of patients not treated with trastuzumab, FGF10 ranked 147 out of 41000 total
transcripts (Table 1), equating to 99.6% differential expression. FGF10 differential expression in the
tumors of patients treated with trastuzumab was statistically significant (Table 1; $p=1.91E-50$).

We turned to a second microarray dataset to validate our findings of FGF10 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 FGF10 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, FGF10 ranked 3108 out of 29377 total transcripts (Table 2), equating to 89.4%
6 differential expression. Differential expression of FGF10 in the primary tumors of breast cancer patients
7 following treatment with trastuzumab was statistically significant (Table 2; $p=1.65E-03$).

8 **FGF10 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 FGF10 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 FGF10 based on history of trastuzumab treatment.
13 FGF10 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 FGF10
17 was among the genes most differentially expressed in the tumors of patients treated with trastuzumab, that
18 FGF10 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 FGF10 is a differentially expressed gene in the tumors of patients
24 treated with trastuzumab, and expressed at significantly higher levels in the primary tumors of breast
25 cancer patients treated with trastuzumab as compared to patients not treated with trastuzumab. Thus,
26 trastuzumab, a treatment associated with a 34% rate of central nervous system metastases⁷, administration
27 in patients with breast cancer results in increased primary tumor expression of a growth factor with
28 functions in development of the limbs and of the differentiation of radial glial cells from neuronal stem
progenitors⁵⁻⁷.

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Rank	ID	p-value	t	B	Gene	Gene name
147	A_23_P377750	1.91E-50	20.31147	104.255638	FGF10	fibroblast growth factor 10

Table 1: FGF10 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
3108	ILMN_2110110	1.65E-03	3.4041421	-1.33519	FGF10

Table 2: A single exposure to trastuzumab is sufficient to drive differential expression of FGF10 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: FGF10 is expressed at significantly higher levels in the primary tumors of patients treated with trastuzumab.

The mRNA expression level of FGF10 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 FGF10 expression in the primary tumors of patients treated with trastuzumab or not, a *p*-value, listed above.