The EphB4 receptor 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 EphB4 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 lower levels of EphB4 messenger RNA than did patients not treated with trastuzumab, and a single administration of trastuzumab was sufficient to result in differential expression of EphB4 in primary tumors of the breast, demonstrating decreased expression of a receptor tyrosine kinase that can impair tumorigenicity of breast cancer cells⁵ as a direct transcriptional result of treatment with trastuzumab.

Keywords: trastuzumab, breast cancer, EphB4, 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 EphB4 is a differentially expressed gene in the tumors of trastuzumab-treated patients: patients treated with trastuzumab expressed lower quantities of EphB4 message 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 EphB4 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).

EphB4 is differentially expressed in the tumors of patients treated with trastuzumab.

We identified the receptor tyrosine kinase EphB4 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, EphB4 ranked 129 out of 1506 total transcripts (Table 1), equating to 91.4% differential expression. EphB4 differential expression in the tumors of patients treated with trastuzumab was not deemed statistically significant (Table 1; $p=0.10224$).

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 EphB4 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, EphB4 ranked 1060 out of 29377 total transcripts (Table 2), equating to 96.4% differential
8 expression. Differential expression of EphB4 in the primary tumors of breast cancer patients following
9 treatment with trastuzumab was statistically significant (Table 2; $p=8.14E-05$).

10 **EphB4 is expressed at lower levels in the tumors of breast cancer patients treated with trastuzumab**
11 **than in the tumors of those not treated with trastuzumab.**

12 We obtained exact mRNA expression levels for EphB4 from tumors of patients treated with
13 trastuzumab and from the tumors of patients not treated with trastuzumab to directly evaluate magnitude
14 and change of expression in EphB4 based on history of trastuzumab treatment. EphB4 was expressed at
15 lower levels in the tumors of patients treated with trastuzumab as compared to those of patients not
16 treated with trastuzumab, but this difference was not deemed statistically significant (Figure 1; $p=0.3155$).
17 We calculated a mean fold change of 0.81 ± 0.70 in EphB4 expression in the primary tumors of patients
18 treated with trastuzumab relative to that of patients not treated with trastuzumab.

19 Thus, we found using published multiplexed gene expression and microarray data^{3,4} that EphB4
20 was among the genes most differentially expressed in the tumors of patients treated with trastuzumab, that
21 EphB4 was expressed at lower levels in the tumors of patients treated with trastuzumab as compared to
22 those not treated with trastuzumab, and that this transcriptional change was likely a direct result of
23 trastuzumab treatment as opposed to a gene expression change associated with clonal selection and
24 evolution of the tumor across the course of treatment.

25 **Discussion**

26 We provided evidence here that EphB4 is a differentially expressed gene in the tumors of patients
27 treated with trastuzumab, and expressed at significantly higher levels in the primary tumors of breast
28 cancer patients treated with trastuzumab as compared to patients not treated with trastuzumab. These data
demonstrate that decreased primary tumor expression of a receptor tyrosine kinase that has the capacity to
suppress oncogenicity of breast cancer cells⁵ is a direct transcriptional consequence of treatment with
trastuzumab in patients with breast cancer.

References

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Rank	ID	p-value	t	B	FC	Gene
129	GI_32528300-S-6	0.10224	-1.66554	-4.26	0.81 ± 0.70	EPHB4

Table 1: EphB4 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 EphB4 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
1060	ILMN_2214910	8.14E-05	-4.4446999	1.45504	EPHB4

Table 2: A single exposure to trastuzumab is sufficient to drive differential expression of EphB4 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: EphB4 is expressed at lower levels in the primary tumors of patients treated with trastuzumab.

The mRNA expression level of EphB4 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 EphB4 expression in the primary tumors of patients treated with trastuzumab or not, a *p*-value, listed above.