Trastuzumab treatment is associated with elevated transforming growth factor beta gene expression.

Shahan Mamoor, MS¹

¹shahanmamoor@gmail.com

East Islip, NY USA

The transforming growth factor beta superfamily, as its name implies, is critical for oncogenesis in multiple cellular contexts¹⁻⁸. We found, through mining published microarray and multiplexed gene expression data⁹⁻¹¹, that administration of trastuzumab was associated with increased expression of TGFBR1 and TGFBI. These data serve as one piece of evidence arguing against the use of trastuzumab in human breast cancer, a patient population already prone to difficult-to-treat metastases¹²⁻¹⁵.

Keywords: breast cancer, metastasis, trastuzumab, systems biology in breast cancer, targeted therapeutics in breast cancer, TGFBR1, TGFBI.

Trastuzumab, a monoclonal antibody targeting the extracellular domain of the human epidermal growth factor receptor 2 (HER2), is widely utilized in the United States and worldwide for the treatment of adjuvant and metastatic breast cancer¹⁶. While the mechanism of action underlying trastuzumab function is thought to involve trastuzumab F_{ab} binding to HER2¹⁷, the signals transduced following these binding events and the transcriptional output of these signals upon integration are less well understood. Patients treated with trastuzumab encounter a remarkable 34% frequency of central nervous system metastases. We mined published microarray and multiplexed mRNA gene expression datasets⁹⁻¹¹ to understand in an unbiased fashion and at the systems level genes most transcriptionally perturbed in the primary tumors of patients treated with trastuzumab. We report here concerning evidence that the breast tumors of patients treated with trastuzumab express significantly increased levels of the TGF beta receptor TGFBR1 and the TGF beta induced gene product TGFBI, both demonstrated to be closely linked to metastasis in human cancer¹⁻⁸.

Methods

We used datasets GSE38057⁹, GSE114082¹⁰ and GSE130788¹¹ for this global and multiplexed comparative gene expression analysis of brain metastatic breast cancer in conjunction with GEO2R. GSE38057 was generated using DASL Human Cancer Panel by Probe technology with either $n=11$ primary breast tumors from patients treated with induction chemotherapy and/or hormone therapy without trastuzumab who had not developed brain metastases at time of tissue sampling and $n=36$ primary breast tumors from patients treated with induction chemotherapy and/or hormone therapy, in addition to trastuzumab, in patients who had not developed brain metastases at time of tissue sampling; 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. GSE130788 was generated using Whole Human Genome Microarray 4x44K G4112F technology with $n=110$ primary breast tumors from patients before trastuzumab treatment and $n=89$ primary tumors from patients after trastuzumab treatment, analysis performed using platform GPL6480. 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 TGFBR1 or TGFBI gene expression was significantly between primary breast tumors in 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 mined published microarray and multiplexed gene expression datasets⁹⁻¹¹ to determine in an unbiased fashion genes most transcriptionally perturbed in the primary tumors of breast cancer patients treated with trastuzumab.

TGFBR1 is differentially expressed in the primary tumors of breast cancer patients treated with trastuzumab.

We identified the transforming growth factor beta receptor TGFBR1 as among the genes whose expression was most significantly different in primary tumors of patients treated with trastuzumab as compared to that of patients not treated with trastuzumab⁹. When sorting each of the genes expressed in

primary tumors from patients treated with trastuzumab based on significance of change in expression as compared to primary tumors from patients not treated with trastuzumab, TGFBR1 ranked 37 out of 1506 total transcripts (Table 1), equating to 97.5% differential expression. Differential expression of TGFBR1 in the primary tumors of breast cancer patients treated with trastuzumab was statistically significant (Table 1; $p=0.023245$).

We queried a second source of gene expression data¹⁰ from patients with human breast cancer, this a microarray dataset, to validate differential expression of TGFBR1 in the primary tumors of patients treated with trastuzumab. We again found that TGFBR1 was among the genes was most differentially expressed in the primary breast tumors of patients treated with trastuzumab (Table 2). However, in this case, the data analyzed were primary tumor tissues from the same patients before and after a brief, single exposure to trastuzumab. When sorting each of the genes expressed in primary tumors from patients treated with trastuzumab based on significance of change in expression as compared to primary tumors from patients before treatment with trastuzumab, TGFBR1 ranked 8612 out of 29377 total transcripts (Table 2). Differential expression of TGFBR1 in the primary tumors of breast cancer patients following treatment with trastuzumab was again statistically significant (Table 2; $p=3.36E-02$). Together, the data suggest that the expression of TGFBR1 is directly transcriptionally modulated by trastuzumab.

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

We identified a second TGF- β superfamily gene, TGFBI, among the genes whose expression was most significantly different in primary tumors of patients treated with trastuzumab as compared to that of patients not treated with trastuzumab⁹. When sorting each of the genes expressed in primary tumors from patients treated with trastuzumab based on significance of change in expression as compared to primary tumors from patients not treated with trastuzumab, TGFBI ranked 142 out of 1506 total transcripts (Table 3), equating to 90.6% differential expression. Differential expression of TGFBI in the primary tumors of breast cancer patients treated with trastuzumab was not statistically significant (Table 3; $p=0.112508$).

Similarly, we queried a second source of gene expression data from patients with human breast cancer, microarray dataset, to validate differential expression of TGFBI in the primary tumors of patients treated with trastuzumab¹¹. We again found that TGFBI was among the genes was most differentially expressed in the primary breast tumors of patients treated with trastuzumab (Table 4). When sorting each of the genes expressed in primary tumors from patients treated with trastuzumab based on significance of change in expression as compared to primary tumors from patients not treated with trastuzumab, TGFBI ranked 20992 out of 41000 total transcripts (Table 4). Differential expression of TGFBI in the primary tumors of breast cancer patients treated with trastuzumab was again statistically significant (Table 4; $p=4.74E-03$).

TGFBR1 is expressed at higher levels in the primary tumors of breast cancer patients treated with trastuzumab as compared to that of patients not treated with trastuzumab.

We obtained exact mRNA expression levels for TGFBR1 in primary tumors of the breast from patients treated with trastuzumab and from patients not treated with trastuzumab to determine the magnitude and direction of change in TGFBR1 expression in the breast tumors of trastuzumab-treated patients. TGFBR1 was expressed at higher levels in the primary tumors of breast cancer patients treated with trastuzumab as compared to that of patients not treated with trastuzumab; increased expression of TGFBR1 in trastuzumab-treated patient primary tumors was statistically significant (Figure 1; $p=0.0262$). We calculated a mean fold change of 1.40 ± 0.62 in TGFBR1 gene expression in the primary tumors of trastuzumab-treated breast cancer patients as compared to that of patients not treated with trastuzumab (Table 1).

TGFBI is expressed at higher levels in the primary tumors of breast cancer patients treated with trastuzumab as compared to that of patients not treated with trastuzumab.

We obtained exact mRNA expression levels for TGFBI in primary tumors of the breast from patients treated with trastuzumab and from patients not treated with trastuzumab to determine the magnitude and direction of change in TGFBI expression in the breast tumors of trastuzumab-treated patients. TGFBI was expressed at higher levels in the primary tumors of breast cancer patients treated with trastuzumab as compared to that of patients not treated with trastuzumab; increased expression of TGFBI in trastuzumab-treated patient primary tumors was not deemed statistically significant according to one measure (Figure 2; $p=0.1276$). We calculated a mean fold change of 1.21 ± 0.45 in TGFBI gene expression in the primary tumors of trastuzumab-treated breast cancer patients as compared to that of patients not treated with trastuzumab (Table 2).

Thus, through blind, comparative transcriptome analysis of primary tumor tissues from patients with breast cancer⁹⁻¹¹, we found that TGFBR1 and TGFBI were among the most distinguishing transcriptional features of the primary tumors of trastuzumab-treated patients.

Discussion

We report here that TGFBI and TGFBR1 are both among the genes most differentially expressed in the primary tumors of trastuzumab-treated breast cancer patients. Elevated expression of TGFBI is associated with high-grade colon cancers in humans⁸; we found that TGFBI was expressed at higher levels in the primary tumors of trastuzumab-treated patients as compared to that of patients not treated with trastuzumab. TGFBR1 and TGFBI may of particular relevance to understanding transcriptional consequences of trastuzumab treatment in patients with human breast cancer. Ectopic expression of TGFBI in SW480 colorectal adenocarcinoma cells *in vivo* was associated with a significant increase in lung metastases in xenografted mice⁸. Increased expression of TGFBI in primary tumors of trastuzumab-treated breast cancer patients may be an important factor to consider when evaluating a 34% incidence of central nervous system metastases in breast cancer patients treated with trastuzumab¹⁵.

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Rank	ID	p-value	t	B	FC	Gene
37	GI_4759225-S-4	0.023245	2.34341	-3.64	1.40 ± 0.62	TGFBR1

Table 1: TGFBR1 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 groups compared, fold change (FC) in TGFBR1 expression when comparing the tumors of patients treated with trastuzumab as to that of patients not treated with trastuzumab, and gene symbol are listed in this chart.

Rank	ID	p-value	t	B	Gene
8612	ILMN_1745927	3.36E-02	-2.209107	-4.03123	TGFBR1

Table 2: TGFBR1 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 groups compared, and gene symbol are listed in this chart.

Rank	ID	p-value	t	B	FC	Gene
142	GI_4507466-S-3	0.112508	1.61628	-4.3	1.21 ± 0.45	TGFBI

Table 3: TGFBI 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 groups compared, fold change (FC) in TGFBI expression when comparing the tumors of patients treated with trastuzumab as to that of patients not treated with trastuzumab, and gene symbol are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
20992	A_23_P156327	4.74E-03	2.856552	-3.6039	TGFBI	transforming growth factor beta induced

Table 4: TGFBI 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 groups compared, gene symbol and gene name are listed in this chart.

Figure 1: TGFB1 is expressed at significantly higher levels in the primary tumors of breast cancer patients treated with trastuzumab.

The mRNA expression level of TGFB1 is graphically depicted in the tumors of patients who had not developed brain metastases, not treated with trastuzumab (left) and in the tumors of patients treated with trastuzumab (right), with mean mRNA expression levels depicted and the result of a statistical test evaluating significance of difference in TGFB1 mRNA expression level between patients not treated with trastuzumab and patients with trastuzumab, a *p*-value, listed above.

Figure 2: TGFB1 is expressed at higher levels in the primary tumors of breast cancer patients treated with trastuzumab.

The mRNA expression level of TGFB1 is graphically depicted in the tumors of patients who had not developed brain metastases, not treated with trastuzumab (left) and in the tumors of patients treated with trastuzumab (right), with mean mRNA expression levels depicted and the result of a statistical test evaluating significance of difference in TGFB1 mRNA expression level between patients not treated with trastuzumab and patients with trastuzumab, a *p*-value, listed above.