The placental growth factor (PGF) is differentially expressed in the primary tumors of breast cancer patients treated with trastuzumab.

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Trastuzumab, a monoclonal antibody targeted against the human epidermal growth factor receptor 2 (HER2) is utilized for the treatment of human breast cancer^{1,2}, but a complete understanding of how tumor signal transduction is modulated by trastuzumab treatment is lacking. By mining published and public microarray and gene expression data^{3,4} from the primary tumors of patients treated with trastuzumab, we found that the placental growth factor, encoded by PGF was among the genes most differentially expressed in the primary tumors of patients treated with trastuzumab, and expressed at lower levels in the tumors of patients treated with trastuzumab. Thus, the use of trastuzumab in patients with breast cancer is associated with increased expression of a growth factor that is chemotactic, angiogenic⁵ and important for growth of blood vessels in the brain⁶.

Keywords: breast cancer, trastuzumab, PGF, PIGF, placental growth factor, systems biology of breast cancer, targeted therapeutics in breast cancer.

The mechanism of action by which trastuzumab exerts its therapeutic function in human breast cancer is said to be binding of the F_{ab} domain of trastuzumab to the extracellular region of the human epidermal growth factor receptor 2 (HER2)², possibly resulting in decreased cellular proliferation⁷. Transcriptional modulation resulting from trastuzumab binding events in the primary tumors of treated patients is less well understood. We mined published and public microarray and gene expression data^{3,4} generated using primary tissues from patients treated with trastuzumab to compare tumor global gene expression with that of patients not treated with trastuzumab. Using this unbiased, systems level methodology, we identified differential expression and up-regulation of the placental growth factor (PGF) in primary tumors of the breast in patients treated with trastuzumab.

Methods

We used datasets GSE38057³ and GSE130788⁴ for 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; analysis was performed using platform GPL5826. 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. 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 PGF 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 comparative transcriptome analysis of primary tumors from patients treated or not with trastuzumab (Herceptin), using published multiplexed gene expression data³ and a microarray dataset⁴, in order to identify the most significant transcriptional changes in the primary tumors of breast cancer patients following treatment with trastuzumab.

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

We found that the placental growth factor, encoded by PGF was among the genes whose expression was most significantly different in the primary tumors of patients with breast cancer treated with trastuzumab³. When sorting each of the genes expressed in the primary tumors of breast cancer patients treated with trastuzumab based on significance of difference in expression as compared to the primary tumors of breast cancer patients not treated with trastuzumab, PGF ranked 152 out of 1506 total transcripts, equating to 89.9% statistical significance (Table 1). Differential expression of PGF in the primary tumors of breast cancer patients treated with trastuzumab was not deemed statistically significant (Table 1; $p=0.121987$).

We queried a second dataset⁶ to validate our findings of PGF differential expression upon trastuzumab treatment. In a second microarray dataset analyzing gene expression of patients treated with

trastuzumab, we again found that PGF was among the genes whose expression was most significantly different transcriptome-wide when comparing the global gene expression profiles of tumors from patients treated with trastuzumab as compared to those not treated with trastuzumab. When sorting each of the genes expressed in the tumors of patients treated with trastuzumab based on significance of change as compared to the tumors of patients not treated with trastuzumab, PGF ranked 2391 out of 41000 total transcripts, equating to 94.2% differential expression. Differential expression of PGF in the primary tumors of patients treated with trastuzumab was statistically significant (Table 2; $p=3.77E-18$).

PGF is expressed at higher levels in the tumors of patients treated with trastuzumab.

We obtained exact mRNA expression levels for PGF from the tumors of patients treated with trastuzumab and from the tumors of patients not receiving trastuzumab to determine magnitude and direction of PGF change in the tumors of patients treated with trastuzumab. PGF expression was higher in the tumors of patients treated with trastuzumab as compared to the tumors of patients not treated with trastuzumab (Figure 1). Increased expression of PGF was statistically significant (Figure 1: $p=0.0115$). We calculated a mean fold change of 1.17 ± 0.67 when comparing the expression of PGF in the tumors of patients treated with trastuzumab as compared to the tumors of patients treated with trastuzumab.

Thus, we found that PGF was among the genes most differentially expressed in the primary tumors of patients with breast cancer and that PGF expression was significantly higher in the primary tumors of patients treated with trastuzumab as compared to those not treated.

Discussion

We provided evidence here that the placental growth factor among the genes most differentially expressed in the primary tumors of patients treated with trastuzumab and expressed at higher levels in the tumors of patients treated with trastuzumab as compared to those not treated with trastuzumab. Thus, the administration of trastuzumab, a treatment associated with a 34% rate of central nervous system metastasis in patients with breast cancer⁷, results in increased primary tumor expression of growth factor important for development of blood vessels in the brain⁶ with chemotactic and angiogenic properties⁵.

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Rank	ID	p-value	t	B	FC	Gene
152	GI_34147600-S-1	0.121987	1.57395	-4.33	1.17 ± 0.67	PGF

Table 1: PGF 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 PGF 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	Gene name
2391	A_23_P76992	3.77E-18	9.589583	30.277829	PGF	placental growth factor

Table 2: PGF 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 PGF between the tumors of patients treated with trastuzumab and the tumors of patients not treated with trastuzumab, and gene are listed in this chart.

Figure 1: PGF is expressed at higher levels in the primary tumors of patients treated with trastuzumab as compared to those not treated with trastuzumab.

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