Differential expression of PTK7 in the primary tumors of breast cancer patients treated with trastuzumab.

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The mechanism of action underlying trastuzumab (Herceptin) function is ascribed to binding of the F_{ab} region of trastuzumab to the extracellular domain of the human epidermal growth factor receptor (HER2)¹. The transcriptional responses that follow signals transduced after trastuzumab binding of HER2 are less well understood. We mined published microarray and multiplexed gene expression data²⁻⁴ to understand in an unbiased fashion genes most differentially expressed in the primary tumors of breast cancer patients treated with trastuzumab. We observed significantly increased and differential expression of the protein tyrosine kinase 7, PTK7⁵⁻⁷.

Keywords: trastuzumab, breast cancer, PTK7, systems biology of breast cancer, targeted therapeutics in breast cancer.

Trastuzumab (Herceptin), a monoclonal antibody utilized for the treatment of adjuvant and metastatic breast cancer in the United States, targets the human epidermal growth factor receptor, or HER2^{1,8}. How transcription is most significantly different between the tumors of breast cancer patients treated with trastuzumab and those not treated with trastuzumab is not fully explored. We mined published microarray and multiplexed datasets²⁻⁴ to understand in an unbiased fashion and at the systems level genes most transcriptionally perturbed by treatment with trastuzumab in the primary tumors of patients with breast cancer. We found significant differential expression of the gene encoding the protein tyrosine kinase 7, PTK7⁵⁻⁷.

Methods

We used datasets GSE38057², GSE58644³ 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 either $n=11$ 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. GSE58644 was generated using Affymetrix Human Gene 1.0 ST Array technology with $n=191$ primary breast tumors from patients not treated with trastuzumab and $n=12$ primary tumors from patients treated with trastuzumab, analysis performed using platform GPL6244. 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 PTK7 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 systems-level transcriptome analysis using published microarray and multiplexed gene expression data²⁻⁴, comparing primary tumors of breast cancer patients who had been treated or not with trastuzumab to describe in an unbiased fashion the most significant features of the transcriptional response to trastuzumab treatment in primary tumors from humans with breast cancer.

PTK7 is differentially expressed in the primary tumors of patients treated with trastuzumab.

We found that PTK7 was among the genes whose expression was most significantly different in the primary tumors of patients with breast cancer treated with trastuzumab². When sorting each of the genes expressed in trastuzumab-treated breast tumors based on significance of difference in expression as compared to the primary breast tumors of patients not treated with trastuzumab, PTK7 ranked 41 out of 1506 total transcripts, equating to 97.3% statistical significance (Table 1). Differential expression of PTK7 in the primary tumors of breast cancer patients treated with trastuzumab was statistically significant (Table 1; $p=0.027522$).

We queried a second dataset³ to validate our findings of PTK7 differential expression upon trastuzumab treatment. In a second microarray dataset analyzing gene expression of patients treated with trastuzumab, we again found that PTK7 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, PTK7 ranked 3483 out of 32321 total transcripts, equating to 89.2% differential expression. Differential expression of PTK7 was statistically significant (Table 2; $p=8.84E-03$).

We queried a third microarray dataset which utilized tumor tissue samples of the same patient before and after a single treatment with trastuzumab, as if PTK7 was differentially expressed in this it would likely be a direct transcriptional consequence of trastuzumab treatment. PTK7 was again among the genes most differentially expressed, albeit to a lesser degree in this dataset. 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, PTK7 ranked 8421 out of 29377 total transcripts, equating to 71.3% differential expression. Differential expression of PTK7 was statistically significant (Table 3; $p=3.14E-02$). Since these tissue samples were not comparing tumors from patients receiving trastuzumab or not, but were comparing tumors from the same individuals before and after a single treatment with trastuzumab, this indicates that induction of PTK7 is likely a direct consequence of signals transduced downstream of trastuzumab binding to HER2, or of retrograde signal transduction transmitted when the F_c domain of trastuzumab engages one or more F_c receptors of unknown identity⁹.

PTK7 is expressed at significantly higher levels in the primary tumors of patients treated with trastuzumab as compared to that of patients not treated with trastuzumab.

We obtained exact mRNA expression levels for PTK7 from the tumors of patients treated with trastuzumab and from the tumors of patients not receiving trastuzumab to determine magnitude and direction of PTK7 change in the tumors of patients treated with trastuzumab. PTK7 expression was significantly 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 PTK7 was statistically significant (Figure 1; $p=0.0018$). We calculated a mean fold change of 1.95 ± 1.16 when comparing the expression of PTK7 in the tumors of patients treated with trastuzumab as compared to the tumors of patients treated with trastuzumab.

Thus, we found that PTK7 was among the most significant transcriptional features of primary tumors in breast cancer patients treated with trastuzumab; PTK7 was expressed at significantly increased levels in the primary tumors of patients treated with trastuzumab, and may likely be a direct result of transcriptional modulation downstream of trastuzumab binding events.

Discussion

PTK7, or protein tyrosine kinase 7 was suggested to be an RTK evolutionarily distinct from other RTKs; PTK7 contains a catalytically inactive tyrosine kinase domain⁵. PTK7 contains a transmembrane domain, and mutation of PTK7 in mice results in defects in neural tube closure as well as in the orientation of the stereociliary bundle, suggesting PTK7 functions in the planar cell polarity pathway⁶. In *Xenopus*, PTK7 functions in recruitment of dishevelled to the plasma membrane, and PTK7 can be found in a complex with frizzled 7 and dishevelled⁷. A loss-of-function mutant of PTK7 in *Xenopus* impairs migration of the neural crest⁷. PTK7 is a transcriptional target of the homeodomain transcription factors Cdx1/2¹⁰. Our analyses implicate induction of a gene involved in neurodevelopment as a transcriptional consequence of trastuzumab, a therapeutic associated with a 34% central nervous system metastasis rate¹¹.

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Rank	ID	p-value	t	B	FC	Gene
41	GI_27894369-S-3	0.027522	2.27243	-3.71	1.95 ± 1.16	PTK7

Table 1: PTK7 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 PTK7 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
3483	42893	8.84E-03	-2.7209643	-2.724998	PTK7

Table 2: PTK7 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
8421	ILMN_2353202	3.14E-02	-2.2403969	-3.97071	PTK7

Table 3: PTK7 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.

Figure 1: PTK7 is expressed at significantly higher levels in the primary tumors of patients treated with trastuzumab as compared to those not treated with trastuzumab.

The mRNA expression level of PTK7 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 PTK7 expression in the primary tumors of patients treated with trastuzumab or not, a *p*-value, listed above.