Trastuzumab treatment in patients with breast cancer is associated with differential expression of the neurotrophic receptor tyrosine kinase receptor NTRK2.

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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 data^{2,3} 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 neurotrophic receptor tyrosine kinase receptor 2, NTRK2^{4,5}.

Keywords: trastuzumab, breast cancer, NTRK2, TRK-B, 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^{1,6}. 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 datasets^{2,3} 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 neurotrophic receptor tyrosine kinase receptor 2, NTRK2^{4,5}.

Methods

We used datasets GSE38057² and GSE114082³ 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. 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 NTRK2 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 data^{2,3}, 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.

NTRK2 is differentially expressed in the primary tumors of patients treated with trastuzumab.

We found that the gene encoding the neurotrophic receptor tyrosine kinase receptor 2, NTRK2 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, NTRK2 ranked 62 out of 1506 total transcripts, equating to 95.9% statistical significance (Table 1). Differential expression of NTRK2 in the primary tumors of breast cancer patients treated with trastuzumab was statistically significant (Table 1; $p=0.051105$).

We queried a second microarray dataset³ which utilized tumor tissue samples of the same patient before and after a single treatment with trastuzumab, as if NTRK2 was differentially expressed in this it would likely be a direct transcriptional consequence of trastuzumab treatment. NTRK2 was again among

the genes most differentially expressed, albeit to a lesser degree in this dataset (Table 1). 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, NTRK2 ranked 3045 out of 29377 total transcripts (Table 2), equating to 89.6% differential expression. Differential expression of NTRK2 was statistically significant (Table 2; $p=1.57E-03$). 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 NTRK2 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 identity⁷.

NTRK2 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 NTRK2 from the tumors of patients treated with trastuzumab and from the tumors of patients not receiving trastuzumab to determine magnitude and direction of NTRK2 change in the tumors of patients treated with trastuzumab. NTRK2 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 NTRK2 was statistically significant (Figure 1: $p=0.0045$). We calculated a mean fold change of 1.58 ± 0.91 when comparing the expression of NTRK2 in the tumors of patients treated with trastuzumab as compared to the tumors of patients treated with trastuzumab.

Thus, we found through unbiased transcriptome analysis of primary tumors of patients with breast cancer that NTRK2 was among the most significant transcriptional features of primary tumors in patients treated with trastuzumab; NTRK2 was expressed at significantly increased levels in the primary tumors of patients treated with trastuzumab, and this may likely be a direct result of transcriptional modulation downstream of trastuzumab binding events.

Discussion

NTRK2, also known as the tropomyosin kinase receptor B (TRK-B) is a receptor tyrosine kinase containing, like other RTKs, a transmembrane domain, an extracellular domain that engages ligand, and an intracellular tail region that transducer signals imparted by ligand binding via tyrosine phosphorylation and recruitment of signal transducing components to phosphotyrosines of the NTRK2 intracellular domain. The ligands for NTRK2 are the brain derived neurotrophic factor (BDNF) and neurotrophin-4⁸. Estradiol stimulation of astrocytes results in production of BDNF and subsequent up-regulation of NTRK2⁸. Expression of NTRK2 was documented in patient-derived breast cancer murine xenografts, in brain-trophic triple negative breast cancer cell lines, and in brain metastases from patients with breast cancer⁸. Mutations of NTRK2 have been found in pilocytic astrocytomas⁹, and splice variants of NTRK2 deficits in kinase activity have been discovered in gliomas, indicating importance of NTRK2 in human cancers of the brain¹⁰. Interestingly, one study suggested that HER2 and NTRK2 may be capable of heterodimerizing; phosphorylated NTRK2 was observed at relatively higher levels than total NTRK2 in brain metastatic tissues, and phosphorylated HER2 and phosphorylated NTRK2 were observed at significantly higher levels in HER2+ breast cancers as compared to triple negative breast cancers in a small subset of patient tumors¹¹. BDNF stimulation increased the proliferation of HER2+ brain metastatic cells and genetic depletion of NTRK2 in HER2+ brain metastatic cell lines grafted in NOD-SCID mice resulted in significantly increased overall survival following intracranial delivery of the BBM1 and BBM2 cell lines¹¹.

In summary, we found here that NTRK2 (TRK-B), a receptor whose ligand can support the proliferation of brain metastatic breast cancer cell lines, is among the genes whose expression most significantly distinguishes the primary tumors of patients treated with trastuzumab; NTRK2 is likely directly transcriptionally induced by treatment with trastuzumab.

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Rank	ID	p-value	t	B	FC	Gene
62	GI_21361305-S-2	0.051105	2.00001	-3.97	1.58 ± 0.91	NTRK2

Table 1: NTRK2 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 NTRK2 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
3045	ILMN_1714067	1.57E-03	3.421612	-1.29093	NTRK2

Table 2: NTRK2 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: NTRK2 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 NTRK2 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 NTRK2 expression in the primary tumors of patients treated with trastuzumab or not, a *p*-value, listed above.