ERBB2 is differentially expressed in the tumors of breast cancer patients treated with trastuzumab, a monoclonal antibody targeting HER2, but ERBB2 expression increases rather than decreasing.

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Trastuzumab (Herceptin), a monoclonal antibody targeting the human epidermal growth factor receptor 2 (HER2), encoded by the ERBB2 gene, is indicated for the treatment of adjuvant and metastatic breast cancer¹, and specifically for patients with HER2+ cancers, based on the rationale that over-expression of HER2 is an important factor in driving cell proliferation, and blocking HER2 will result in decreased cell proliferation². We used an unbiased comparative transcriptome approach, mining three independent microarray datasets³⁻⁵, to identify genes most transcriptionally perturbed in the tumors of patients treated with trastuzumab and found that the gene whose gene product is the target of trastuzumab, ERBB2, was among the genes most differentially expressed in primary breast tumors and primary tumor cells of patients treated with trastuzumab. Surprisingly, expression of the gene product of ERBB2, HER2, was higher rather lower in the tumors of patients treated with ERBB2. This puzzling trend was observed across three independent patient datasets. Our findings reveal a potentially alarming feedback loop wherein message for the target of a cancer therapeutic is induced through function of the therapeutic itself.

Keywords: breast cancer, trastuzumab, ERBB2, HER2, systems biology of breast cancer, targeted therapeutics in breast cancer.

If a cancer therapeutic is utilized on the basis that over-expression of the target makes a candidate suitable for treatment and over-expression supports increased tumor cell proliferation, then one should expect treatment specifically designed to block that target to reduce (or at the least control) levels of the target. We found recurrent evidence that trastuzumab treatment directly or indirectly results in increased levels of ERBB2 messenger RNA, prompting a critical evaluation of use of trastuzumab in patients with breast cancer following an unbiased investigation of the transcriptional consequences of trastuzumab treatment in the primary tumors of patients with breast cancer.

Methods

We used datasets GSE75685³, GSE20194⁴, and GSE58644⁵ for this global differential gene expression analysis of the effects of trastuzumab treatment on primary tumors of the breast in conjunction with GEO2R. GSE75685 was generated using Whole Human Genome Microarray 4x44K technology with $n=41$ primary breast tumors from patients not treated with trastuzumab and $n=11$ primary tumors from patients treated with trastuzumab, analysis performed using platform GPL4133. GSE20194 was generated using Affymetrix Human Genome U133A Array technology with $n=256$ samples of fine needle aspiration isolated breast cancer cells of patients not treated with trastuzumab and $n=12$ samples of fine needle aspiration isolated breast cancer cells of patients treated with trastuzumab, analysis performed using platform GPL96. 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. 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 ERBB2 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

ERBB2 is among the genes expressed most differently when comparing primary tumors of the breast from patients treated with trastuzumab as compared to those not treated with trastuzumab.

We found that the gene encoding the human epidermal growth factor receptor HER2, ERBB2, 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 using a public microarray dataset³. 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, ERBB2 ranked 2 out of 45220 total transcripts. Differential expression of ERBB2 was statistically significant (Table 1; $p=6.82E-08$).

We sought to validate our findings of ERBB2 differential expression upon trastuzumab treatment in an independent dataset. In a second microarray dataset analyzing gene expression of patients treated with trastuzumab⁴, we again found that the gene encoding the human epidermal growth factor receptor

HER2, ERBB2, was among the genes whose expression was most significantly different transcriptome-wide when comparing the global gene expression profiles of breast cancer cells from patients treated with trastuzumab as compared to those not treated with trastuzumab. When sorting each of the genes expressed in the fine needle aspirated-breast cancer cells of patients treated with trastuzumab based on significance of change as compared to the breast cancer cells of patients not treated with trastuzumab, ERBB2 ranked 84 out of 22283 total transcripts. Differential expression of ERBB2 was statistically significant (Table 2; $p=6.89E-05$).

In a third microarray dataset analyzing gene expression of patients treated with trastuzumab, we again found that the gene encoding the human epidermal growth factor receptor HER2, ERBB2, 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, ERBB2 ranked 3 out of 32321 total transcripts. Differential expression of ERBB2 was statistically significant (Table 3; $p=4.63E-14$).

ERBB2 is expressed at significantly higher levels in the primary tumors of breast cancer patients treated with trastuzumab.

We obtained exact mRNA expression levels for ERBB2 from the tumors of patients treated with trastuzumab and from the tumors of patients not receiving trastuzumab to determine magnitude and direction of ERBB2 change in the tumors of patients treated with trastuzumab. We hypothesized, that like all inhibitors and targeted therapies directed toward a drug target, that trastuzumab treatment would result in decreased expression of ERBB2. We were alarmed to find that ERBB2 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). This was conserved across three independent datasets, demonstrating that this is a real consequence of treatment with trastuzumab (Figure 2 and Figure 3). Increased expression of ERBB2 was statistically significant (Figure 1: $p<0.0001$; Figure 2; $p=0.0075$; Figure 3: $p<0.0001$). It is difficult to determine at this point whether ERBB2 is being transcriptionally induced by trastuzumab treatment or whether trastuzumab in some manner selects and enriches for the survival of tumor clones with high expression of ERBB2. We calculated a mean fold change of 1.247 ± 0.119 , 1.178 ± 0.188 , and 1.264 ± 0.082 when comparing the expression of ERBB2 in the tumors of patients treated with trastuzumab as compared to the tumors of patients treated with trastuzumab.

Thus, we discovered using published microarray data that ERBB2 was among the genes whose expression was most significantly different transcriptome-wide when comparing the tumors and fine needle aspiration-obtained breast cancer cells of patients treated with trastuzumab as compared to the tumors of patients not treated with trastuzumab. However, ERBB2 expression was not lower in the tumors of patients treated with trastuzumab - it was higher.

Discussion

While the mechanism of action underlying trastuzumab function in breast cancer is thought to involve binding of the Fc domain of trastuzumab to the extracellular region of HER2, the gene product of ERBB2, the transcriptional consequences following signals transduced by such binding events are less well understood. We mined published and public microarray data to understand at the systems level and

1 in a blinded manner what were the most significant transcriptional changes resulting from trastuzumab
2 treatment of patients with breast cancer.

3 We found that the gene encoding the molecular target of trastuzumab, ERBB2, was among the
4 genes whose expression changed most significantly in the tumors of patients treated with trastuzumab.
5 However, ERBB2 was expressed at significantly higher, and not lower levels in the tumors of patients
6 treated with trastuzumab. One study demonstrated that over-expression of HER2 results in increased
7 metastasis of breast cancer cells in the brain⁶, suggesting one of several possible mechanisms for
8 increased metastasis to the brain in breast cancer patients treated with trastuzumab.

9 We provided evidence here that expression of ERBB2, the gene encoding HER2, is significantly
10 higher in the tumors and breast cancer cells of patients treated with trastuzumab, and that ERBB2
11 expression is stabilized or perhaps even transcriptionally induced in the tumors of patients with
12 trastuzumab. We argue that such data raise serious questions regarding suitability of trastuzumab given
13 the provided mechanism of action: specifically targeting a HER2+ over-expressing population using a
14 HER2 monoclonal antibody to block or inhibit HER2 function to impair proliferation. Future studies can
15 compare effects of trastuzumab to that of neratinib⁷⁻⁹, a ErbB tyrosine kinase inhibitor on ERBB2
16 expression levels following treatment, on tumor cell proliferation, clonal selection following treatment,
17 and incidence of metastasis to the brain.

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Rank	ID	p-value	t	B	FC	Gene	Gene name
2	4012	6.82E-08	6.2883188	7.844116	1.247 ± 0.119	ERBB2	erb-b2 receptor tyrosine kinase 2

Table 1: ERBB2 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 ERBB2 between the tumors of patients treated with trastuzumab and the tumors of patients not treated with trastuzumab, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	FC	Gene	Gene name
84	216836_s_at	6.89E-05	4.0428368	1.4709	1.178 ± 0.188	ERBB2	erb-b2 receptor tyrosine kinase 2

Table 2: ERBB2 is differentially expressed in primary tumor cells of breast cancer patients treated with trastuzumab obtained by fine needle aspiration.

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 ERBB2 between the tumor cells of patients treated with trastuzumab and the tumor cells of patients not treated with trastuzumab, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	FC	Gene	Gene name
3	8006906	4.63E-14	8.1107081	19.5861	1.264 ± 0.082	ERBB2	erb-b2 receptor tyrosine kinase 2

Table 3: ERBB2 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 ERBB2 between the tumors of patients treated with trastuzumab and the tumors of patients not treated with trastuzumab, gene and gene name are listed in this chart.

Figure 1: ERBB2 is expressed at significantly higher levels in the tumors of patients treated with trastuzumab.

The mRNA expression level of ERBB2 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 ERBB2 expression in the primary tumors of patients treated with trastuzumab or not, a *p*-value, listed above.

Figure 2: ERBB2 is expressed at significantly higher levels in the primary tumor cells of patients treated with trastuzumab.

The mRNA expression level of ERBB2 is graphically depicted in this chart in the primary tumor cells of breast cancer patients not treated with trastuzumab (left) and in the primary tumor cells 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 ERBB2 expression in the primary tumor cells of patients treated with trastuzumab or not, a *p*-value, listed above.

Figure 3: ERBB2 is expressed at significantly higher levels in the tumors of patients treated with trastuzumab.

The mRNA expression level of ERBB2 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 ERBB2 expression in the primary tumors of patients treated with trastuzumab or not, a *p*-value, listed above.