A second erb-B receptor tyrosine kinase, ERBB4, is differentially expressed in the tumors of breast cancer patients treated with trastuzumab.

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HER2, the human epidermal growth factor receptor 2, is encoded by the ERBB2 gene¹. Trastuzumab, a monoclonal antibody that targets HER2, is utilized for the treatment of breast cancer². We recently reported that trastuzumab treatment paradoxically increases HER2 expression in the primary tumors of patients with breast cancer³. We report here, using analysis of published microarray data^{4,5}, that a second erb-B receptor tyrosine kinase, ERBB4, is also differentially expressed in the tumors of patients with breast cancer treated with trastuzumab. Trastuzumab treatment appears to be associated with the up-regulation of two members of the erb-B receptor tyrosine kinase family in human breast cancer.

Keywords: breast cancer, trastuzumab, ERBB4, systems biology of breast cancer, targeted therapeutics in breast cancer.

Receptor tyrosine kinases are a broad family of plasma membrane cell surface receptors that in general possess an extracellular ligand-binding domain, a transmembrane domain and an intracellular domain often consisting of a tail with residues that can be phosphorylated to transduce signals across the plasma membrane to the nucleus⁶. The mechanism of action ascribed to trastuzumab is thought to be binding of trastuzumab to HER2⁷ but the transcriptional consequences resulting from HER2 binding are less well understood. We found that the erb-B receptor tyrosine kinase family member ERBB4 is among the genes most differentially expressed in the primary tumors of patients with breast cancer.

Methods

We used datasets GSE38057⁴ and GSE130788⁵ 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. 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 ERBB4 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 harnessed the power of blind, comparative transcriptome analysis of published microarray and gene expression data^{4,5} to identify the most significant transcriptional features of the primary breast tumors of patients treated with trastuzumab (Herceptin).

ERBB4 is differentially expressed in the tumors of patients treated with trastuzumab.

We identified ERBB4 as among the genes whose expression changed most significantly in the tumors of breast cancer patients treated with trastuzumab⁴ (Table 1). When sorting each of the genes expressed in the primary tumors of breast cancer patients treated with trastuzumab based on significance of change as compared to that of patients not treated with trastuzumab, ERBB4 ranked 155 out of 1506 total transcripts (Table 1), equating to 89.7% differential expression. ERBB4 differential expression in the tumors of patients treated with trastuzumab was not deemed statistically significant (Table 1; $p=0.123661$).

The first dataset, although measuring the expression of over 1500 genes, was still limited. We turned to a second microarray dataset⁵, this measuring the expression of genes across the transcriptome - 41000 genes in total. We found again that ERBB4 was among the genes most differentially expressed in the tumors of patients administered a single treatment of trastuzumab (Table 2). When sorting each of the genes expressed in the tumors of patients administered a single treatment of trastuzumab, ERBB4 ranked 5330 out of 41000 total transcripts (Table 2), equating to 87% differential expression. Differential expression of ERBB4 was statistically significant (Table 2; $p=7.5E-12$).

ERBB4 is expressed at significantly higher levels in the tumors of breast cancer patients treated with trastuzumab than in the tumors of those not treated with trastuzumab.

We obtained exact mRNA expression levels for ERBB4 from tumors of patients treated with trastuzumab and from the tumors of patients not treated with trastuzumab to directly evaluate magnitude and change of expression in ERBB4 based on history of trastuzumab treatment. ERBB4 was expressed at significantly higher levels in the tumors of patients treated with trastuzumab as compared to those of patients not treated with trastuzumab (Figure 1; $p=0.0180$). We calculated a mean fold change of 1.76 ± 1.58 in ERBB4 expression in the primary tumors of patients treated with trastuzumab relative to that of patients not treated with trastuzumab.

Thus, we found that ERBB4 was among the genes most differentially expressed in the tumors of patients treated with trastuzumab, that ERBB4 was expressed at significantly higher levels in the tumors of patients treated with trastuzumab as compared to those not treated with trastuzumab.

Discussion

The capacity of ERBB4 to transduce signals imparted by EGF-ligands can be enhanced by concomitant expression or co-expression of ERBB2⁸. We found here that ERBB4 was among the genes most differentially expressed in the tumors of patients treated with trastuzumab. The consequences of up-regulation of both ERBB2 and ERBB4 should be evaluated to assess whether proliferation of primary tumor cells from patients with breast cancer can be promoted rather than impaired by changes in erb-B tyrosine receptor signal resulting from trastuzumab treatment.

References

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Rank	ID	p-value	t	B	FC	Gene
155	GI_4885214-S-5	0.123661	1.56676	-4.34	1.76 ± 1.58	ERBB4

Table 1: ERBB4 is differentially expressed in the 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 ERBB4 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
5330	A_32_P183765	7.5E-12	7.283178	15.949082	ERBB4	erb-b receptor tyrosine kinase 4

Table 2: ERBB4 is differentially expressed in the 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: ERBB4 is expressed at significantly higher levels in the primary tumors of breast cancer patients treated with trastuzumab.

The mRNA expression level of ERBB4 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 ERBB4 expression in the primary tumors of patients treated with trastuzumab or not, a *p*-value, listed above.