Increased expression of the estrogen receptor alpha, ESR1, in the tumors of breast cancer patients treated with trastuzumab.

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Hormones function as growth factors, and estrogen provides growth signals to support and induce the proliferation of breast cancers¹⁻³. This is the basis of the use of endocrine therapies^{4,5} including tamoxifen and letrozole as first-line treatment for patients with breast cancer. We found through mining published microarray and multiplexed gene expression profiling datasets that the estrogen receptor α (ESR1) was among the genes most differentially expressed in the primary tumors and fine needle aspiration-isolated tumor cells of patients with breast cancer treated with trastuzumab. However, estrogen receptor α was expressed at higher rather than lower levels in the tumors of trastuzumab-treated patients. These data, obtained through blind, systems-level analysis of published microarray data⁶⁻⁸, suggest that trastuzumab administration in patients with breast cancer is associated with transcriptional induction of the estrogen receptor or selection of tumor clones with high expression of ESR1.

Keywords: breast cancer, ESR1, estrogen receptor alpha, ER α , systems biology of breast cancer, targeted therapeutics in breast cancer.

Endocrine therapy is a cornerstone of treatment for breast cancer. Trastuzumab, a monoclonal antibody targeted against the human epidermal growth factor receptor 2, HER2, it also widely utilized for the treatment of breast cancer. Endocrine therapy works by impairing binding of estrogen ligands like estradiol to hormone receptor like the estrogen receptor alpha, encoded by ESR1. We found, and present here, using published microarray and multiplexed gene expression datasets, concerning evidence arguing that trastuzumab either transcriptionally activates the estrogen receptor alpha in the primary tumors of patients with breast cancer, or supports clonal selection of tumor cells with high expression of ESR1.

Methods

We used datasets GSE38057⁶, GSE20194⁷, and GSE58644⁸ for this global differential gene expression analysis of the effects of trastuzumab treatment on primary tumors of the breast in conjunction with GEO2R. 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. 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 ESR1 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 whole transcriptome and multiplexed gene expression analysis of the primary tumors of patients with breast cancer across three independent datasets⁶⁻⁸ to discover genes associated with trastuzumab treatment.

ESR1 is differentially expressed in the breast tumors of patients treated with trastuzumab.

We found that the gene encoding the estrogen receptor alpha, ESR1, was among the genes whose expression was most significantly different in the primary tumors of patients with breast cancer treated with trastuzumab. 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, ESR1 ranked 69 out of 1506 total transcripts, ranking at 95.4% statistical significance (Table 1). Differential expression of ESR1 in the primary tumors of breast cancer patients treated with trastuzumab trended towards statistical significance (Table 1; $p=0.058124$).

We sought to validate our findings of ESR1 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 estrogen receptor alpha, ESR1, was among the genes whose expression was most significantly different transcriptome-wide when comparing the global gene expression profiles of fine needle aspiration-isolated breast cancer cells from patients treated with trastuzumab as compared to those not treated with trastuzumab. When sorting each of the genes expressed in primary tumor cells of the breast from patients treated with trastuzumab based on significance of change as compared to cells of primary tumor cells of the breast from patients not treated with trastuzumab, ESR1 ranked 1030 out of 22283 total transcripts, equating to 95.4% differential expression. Differential expression of ESR1 in fine needle aspiration-isolated primary tumor cells of the breast based on history of trastuzumab administration was statistically significant (Table 2; $p=2.4E-02$).

In a third microarray dataset analyzing gene expression of patients treated with trastuzumab, we again found that the gene encoding the estrogen receptor alpha, ESR1, was among the genes whose expression was most 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, ESR1 ranked 2200 out of 32321 total transcripts, equating to 93.2% differential expression. Differential expression of ESR1 approached statistical significance (Table 3; $p=5.32E-02$).

ESR1 is expressed at significantly higher levels in the primary tumors of patients treated with trastuzumab.

We obtained exact mRNA expression levels for ESR1 from the tumors of patients treated with trastuzumab and from the tumors of patients not receiving trastuzumab to determine magnitude and direction of ESR1 change in the tumors of patients treated with trastuzumab. We were alarmed to find that ESR1 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 ESR1 approached statistical significance (Figure 1: $p=0.0814$). It is difficult to determine at this point whether ESR1 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 ESR1. We calculated a mean fold change of 2.25 ± 3.00 when comparing the expression of ESR1 in the tumors of patients treated with trastuzumab as compared to the tumors of patients treated with trastuzumab.

Discussion

We provide evidence here that ESR1 is expressed at significantly higher levels in the primary tumors of breast cancer patients treated with trastuzumab, and among the genes whose expression is most significantly different when comparing the primary tumors and fine needle aspiration-isolated primary tumor cells of patients treated with trastuzumab with that of patients not treated with trastuzumab. Mutations in the estrogen receptor that render breast cancers unable to respond to hormone therapies are associated with tumor growth and enhanced metastatic capacity⁹. Use of trastuzumab and its ability to potentially transcriptionally activate the estrogen receptor must be further evaluated.

References

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Rank	ID	p-value	t	B	FC	Gene
69	GI_4503602-S-3	0.058124	1.94057	-4.03	2.25 ± 3.00	ESR1

Table 1: ESR1 is differentially expressed in the breast tumors of patients treated with trastuzumab.

The rank of differential expression, probe ID, p-value with respect to differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the two groups compared, fold change of expression in ESR1 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	Gene	Gene name
1030	205225_at	2.4E-02	-2.2702209	-3.5752	ESR1	estrogen receptor 1

Table 2: ESR1 is differentially expressed in the primary tumor cells of patients treated with trastuzumab.

The rank of differential expression, probe ID, p-value with respect to 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	Gene name
2200	8122843	5.32E-02	-1.9446288	-4.0465	ESR1	estrogen receptor 1

Table 3: ESR1 is differentially expressed in the breast tumors of patients treated with trastuzumab.

The rank of differential expression, probe ID, p-value with respect to 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: ESR1 is expressed at higher levels in the primary tumors of breast cancer patients treated with trastuzumab.

The mRNA expression level of ESR1 is graphically depicted in this chart in the primary tumors of breast cancer patients not treated with trastuzumab (“No Tras”) and in the primary tumors of breast cancer patients treated with trastuzumab (Tras), with mean mRNA expression level marked and the result of a statistical test evaluating significance of difference between ESR1 expression in the primary tumors of patients treated with trastuzumab or not, a p -value, listed above. Left: ESR1 tumor expression scaled normally. Right: ESR1 tumor expression scaled in natural logarithm (ln).