Trastuzumab treatment in human breast cancer is associated with over-expression of the retinoic acid receptor alpha (RARA).

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Treatment with trastuzumab has been associated with an increased risk of central nervous system metastasis in patients with human breast cancer¹⁻⁵. We performed unbiased transcriptome profiling of primary tumors of patients treated with trastuzumab using published microarray and multiplexed gene expression datasets^{6,7} to understand the transcriptional makeup of breast tumors in the trastuzumab-treated patients. We found that the retinoic acid receptor alpha was among the genes most differentially expressed in the primary tumors of patients treated with trastuzumab; retinoic acid receptor alpha was expressed at significantly higher levels in tumors from patients with trastuzumab as compared to those not treated with trastuzumab. Increased retinoic acid receptor alpha expression is known to confer tamoxifen resistance to human breast cancers⁸, and RARA expression is higher in tumors with higher proliferative capacity⁹, suggesting that trastuzumab could contribute to resistance to endocrine therapy.

Keywords: breast cancer, trastuzumab, RARA, retinoic acid receptor alpha, systems biology of breast cancer, targeted therapeutics in breast cancer.

The incidence of central nervous system metastases in patients treated with trastuzumab for breast cancer treated was reported at 34% in one study³, and a randomized clinical trial demonstrated that the rate of central nervous system metastases was lower for patients treated with neratinib and paclitaxel as compared to patients treated with trastuzumab and paclitaxel¹. We mined published microarray and multiplexed gene expression data^{6,7} to discover genes most transcriptionally perturbed in the primary tumors of breast cancer patients treated with trastuzumab. We report here that the gene encoding the retinoic acid receptor alpha, RARA, was among those whose expression was most different in the primary tumors of patients treated with trastuzumab as compared to those not treated with trastuzumab, suggesting trastuzumab treatment induces the expression of or selects for clones with higher expression of RARA.

Methods

Datasets GSE38057⁶ and GSE114082⁷ were utilized for this study 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 not treated with trastuzumab and $n=36$ primary breast tumors from patients treated trastuzumab, both of which did not develop brain metastases, or $n=6$ primary breast tumors from patients treated not treated with trastuzumab and $n=35$ primary breast tumors from patients treated trastuzumab, both of which did develop brain metastases; 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 RARA gene expression was significantly different between primary breast tumors in patients receiving trastuzumab or not 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 comparative transcriptome analysis in patients with breast cancer, comparing primary tumors of patients treated with or without trastuzumab^{6,7}, to identify genes associated with trastuzumab treatment, a cancer therapeutic described, according to multiple studies, to result in an increased frequency of metastasis to the central nervous system.

RARA is differentially expressed in primary tumors of trastuzumab-treated patients with breast cancer.

We identified RARA, the gene encoding the retinoic acid receptor alpha, as among the genes most differentially expressed in primary tumors of trastuzumab-treated patients with breast cancer (Table 1). When sorting each of the genes expressed in primary tumors of the breast from patients treated with trastuzumab based on significance of change in expression, relative to all transcripts measured, as compared to the primary tumors of patients not treated with trastuzumab, RARA ranked 60 out of 1506 total transcripts, equating to 96% differential expression. Differential expression of RARA was statistically significant (Figure 1: 0.050392).

We mined a separate microarray dataset⁶ to validate differential expression of RARA in patients treated with trastuzumab. RARA was again among the genes most differentially expressed transcriptome-wide when in the primary tumors of patients treated with trastuzumab; however, in this study, patients with administered a single treatment with trastuzumab, and tumor samples compared were from before and after treatment in the same patients. When sorting each of the genes expressed in primary tumors of the breast based on significance of changes in expression when comparing tumors of the breast from patients treated with trastuzumab or not, RARA ranked 6554 out of 29377 total transcripts (Table 2), equating to 77.7% differential expression. Differential expression of RARA in the tumors of breast cancer patients treated with trastuzumab was statistically significant (Table 2: $p=1.46E-02$).

RARA is expressed at significantly higher levels in primary tumors of trastuzumab-treated breast cancer patients as compared to those not treated with trastuzumab.

We obtained exact mRNA expression levels for RARA to determine the magnitude and direction of change in RARA expression in the tumors of patients treated with trastuzumab. RARA was expressed at higher levels in the tumors of patients treated with trastuzumab as compared to the tumors of patients not treated with trastuzumab, and this difference was statistically significant (Figure 1; $p=0.0115$).

Thus, we found that the retinoic acid receptor alpha was among the genes most differentially expressed in the primary tumors of patients treated with trastuzumab; RARA was expressed at significantly higher levels in the primary tumors of trastuzumab-treated breast cancer patients.

Discussion

The retinoic acid receptor alpha (RARA) is described as a marker of tamoxifen resistance in breast cancer patients⁸. Increased expression of RARA was significantly correlated with inferior relapse-free survival in human breast cancer⁸. Importantly, increased expression of RARA is associated with tumors possessing increased proliferative activity⁹. Increased expression of RARA in the primary tumors of patients treated with trastuzumab is one piece of evidence arguing that despite its wide usage in adjuvant and metastatic breast cancer^{10,11}, the transcriptional responses to trastuzumab, tumor biology of trastuzumab treatment, and provided mechanism of action ascribed to trastuzumab - decreased proliferation resulting from F_{ab} engagement of the HER2 receptor¹² - are not yet fully understood.

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Rank	ID	p-value	t	B	FC	Gene
60	GI_4506418-S-1	0.050392	2.00643	-3.97	1.39 ± 0.69	RARA

Table 1: RARA 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 RARA 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
6554	ILMN_1677197	1.46E-02	-2.5659592	-3.30486	RARA

Table 2: RARA 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, and gene are listed in this chart.

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

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