Trastuzumab treatment in female breast cancer is associated with over-expression of the androgen receptor (AR).

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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 more clearly understand the transcriptional responses of primary tumors of the breast to trastuzumab treatment. We found that the androgen receptor was among the genes most differentially expressed in the primary tumors of patients treated with trastuzumab; androgen receptor was expressed at significantly higher levels in tumors from patients with trastuzumab as compared to those not treated with trastuzumab. Thus, trastuzumab treatment in females with breast cancer is associated with the expression of a receptor for a male sex hormone.

Keywords: breast cancer, trastuzumab, AR receptor, androgen receptor, 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 androgen receptor, AR receptor, 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 AR.

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 AR receptor 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.

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

We identified AR receptor, the gene encoding the androgen receptor, 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, AR receptor ranked 93 out of 1506 total transcripts, equating to 93.8% differential expression. Differential expression of AR receptor trended towards statistical significance (Figure 1: 0.073208).

We mined a separate microarray dataset⁶ to validate differential expression of AR receptor in patients treated with trastuzumab. AR receptor 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, AR receptor ranked 6830 out of 29377 total transcripts (Table 2), equating to 76.8% differential expression. Differential expression of AR receptor in the tumors of breast cancer patients treated with trastuzumab was statistically significant (Table 2: $p=1.64E-02$).

AR receptor 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 AR receptor to determine the magnitude and direction of change in AR receptor expression in the tumors of patients treated with trastuzumab. AR receptor 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; $p=0.0256$).

Thus, we found that the androgen receptor was among the genes most differentially expressed in the primary tumors of patients treated with trastuzumab; AR receptor was expressed at significantly higher levels in the primary tumors of trastuzumab-treated breast cancer patients. Since AR was not only differentially expressed in tumors of patients treated or not with trastuzumab, but also in tumors from the same patients before and after a brief, single exposure to trastuzumab, AR is likely transcriptionally induced as a direct result of trastuzumab administration.

Discussion

AR expression is enriched in HER2-over-expressing, ER- breast cancers, where up-regulation of WNT7B through AR signaling results in activation of beta-catenin⁸. Enzalutamide and bicalutamide, both silent antagonists of the androgen receptor, can impair proliferation of breast cancer cell lines *in vitro*⁹. Ectopic expression of AR in MCF-7 breast cancer cell lines results in loss of responsiveness to tamoxifen in xenograft tumor growth assay in nude mice¹⁰. One study showed that estrogen response elements were enriched for in estradiol-induced AR binding sites, and could overlap with estrogen receptor binding sites, demonstrating that AR and ER could cooperate in breast cancer¹¹. Phase II trials of AR antagonists enzalutamide¹³ in AR+ triple negative breast cancer and bicalutamide¹² in AR+ ER- metastatic breast cancer demonstrated partial clinical benefit in a fraction of patients, arguing that endogenous expression levels of AR - likely independent of the over-expression of AR resulting from trastuzumab treatment - are sufficient to drive some degree of clinical response to AR blockade in a portion of the patient population.

Increased expression of the androgen receptor 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^{14,15}, the transcriptional responses to trastuzumab, tumor biology of trastuzumab treatment, and provided mechanism of action ascribed to trastuzumab are not yet fully understood¹⁶.

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Rank	ID	p-value	t	B	FC	Gene
93	GI_21322251-S-2	0.073208	1.83118	-4.12	1.53 ± 0.97	AR

Table 1: AR 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 AR 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
6830	ILMN_1659572	1.64E-02	-2.519043	-3.40472	AR

Table 2: AR 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: AR is expressed at significantly higher levels in the primary tumors of patients treated with trastuzumab.

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