Arachidonate 12-lipoxygenase (ALOX12) is differentially expressed in the tumors of breast cancer patients treated with trastuzumab.

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Trastuzumab (Herceptin) is a monoclonal antibody targeting the extracellular domain of the human epidermal growth factor receptor 2 (HER2)¹ utilized for the treatment of adjuvant and metastatic breast cancer² in the United States and worldwide. We mined public microarray data^{3,4} to discover in an unbiased manner the most significant transcriptional changes associated with trastuzumab treatment. We identified arachidonate 12-lipoxygenase, encoded by ALOX12, as among the genes most differentially expressed in the primary tumors of patients with breast cancer treated with trastuzumab. The primary tumors of breast cancer patients treated with trastuzumab expressed higher levels of ALOX12 messenger RNA than did patients not treated with trastuzumab, demonstrating that in patients with breast cancer, administration of trastuzumab is associated with increased expression of an enzyme⁵ whose inhibition results in enhanced responsiveness of breast cancer cells to chemotherapeutics *in vitro*⁶.

Keywords: trastuzumab, breast cancer, ALOX12, arachidonate 12-lipoxygenase, systems biology of breast cancer, targeted therapeutics in breast cancer.

The mechanism of action ascribed to trastuzumab function is thought to be binding of the F_{ab} domain of trastuzumab to the extracellular region of the human epidermal growth factor receptor 2 (HER2)¹, but the transcriptional consequences resulting from signaling downstream of trastuzumab binding to HER2 is less well understood. Through unbiased, systems-level comparative transcriptome analysis of published microarray data^{3,4}, we report here that ALOX12 is a differentially expressed gene in the tumors of trastuzumab-treated patients: patients treated with trastuzumab expressed significantly higher levels of ALOX12 than did those of patients not treated with trastuzumab.

Methods

We used datasets GSE130788³ and GSE75685⁴ for this differential gene expression analysis of the transcriptional response to trastuzumab treatment in patients with breast cancer. 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. 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. 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 ALOX12 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 unbiased comparative transcriptome analysis using public microarray data^{3,4} to identify the most significant transcriptional features of the primary breast tumors of patients treated with trastuzumab (Herceptin).

ALOX12 is differentially expressed in the tumors of patients treated with trastuzumab.

We identified the progesterone receptor, encoded by ALOX12 as among the genes whose expression changed most significantly in the tumors of breast cancer patients treated with trastuzumab in a multiplexed gene expression dataset³ (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, ALOX12 ranked 38 out of 32321 total transcripts (Table 1), equating to 99.9% differential expression. ALOX12 differential expression in the tumors of patients treated with trastuzumab trended towards statistical significance (Table 1; $p=2.79E-71$).

We turned to a second microarray dataset to validate our findings of ALOX12 differential expression in the patients treated with trastuzumab. We found again that ALOX12 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, ALOX12 ranked 1518 out of 45220 total transcripts (Table 2), equating to 96.6% differential expression. Differential expression of ALOX12 in the primary tumors of breast cancer patients following treatment with trastuzumab was statistically significant (Table 2; $p=1.92E-03$).

ALOX12 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 ALOX12 from tumors of patients treated with trastuzumab and from the tumors of patients not treated with trastuzumab to directly evaluate direction and statistical significance of change in expression of ALOX12 based on history of trastuzumab treatment. ALOX12 was expressed at higher levels in the tumors of patients treated with trastuzumab as compared to those of patients not treated with trastuzumab, and this difference was statistically significant (Figure 1; $p < 0.0001$).

Thus, we found using public microarray data^{3,4} that ALOX12 was among the genes most differentially expressed in the tumors of patients treated with trastuzumab, that ALOX12 was expressed at higher levels in the tumors of patients treated with trastuzumab as compared to those not treated with trastuzumab.

Discussion

We provided evidence here that arachidonate 12-lipoxygenase, encoded by ALOX12 is a differentially expressed gene in the tumors of patients treated with trastuzumab, and expressed at significantly higher levels in the primary tumors of breast cancer patients treated with trastuzumab as compared to patients not treated with trastuzumab. These data demonstrate trastuzumab treatment of patients with breast cancer results in increased expression of an enzyme⁵ whose inhibition leads to increased responsiveness of breast cancer cells to chemotherapy⁶.

References

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Rank	ID	p-value	t	B	Gene	Gene name
38	A_23_P152906	2.79E-71	28.104995	151.93752	ALOX12	arachidonate 12-lipoxygenase, 12S type

Table 1: ALOX12 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 ALOX12 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
1518	19674	1.92E-03	3.2697478	-1.389543	ALOX12	arachidonate 12-lipoxygenase, 12S type

Table 2: ALOX12 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, gene and gene name are listed in this chart.

Figure 1: ALOX12 is expressed at significantly higher levels in the primary tumors of patients treated with trastuzumab.

The mRNA expression level of ALOX12 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 ALOX12 expression in the primary tumors of patients treated with trastuzumab or not, a *p*-value, listed above.