IL-6 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 published microarray and gene expression data^{3,4} to discover in an unbiased manner the most striking transcriptional features of trastuzumab treatment. We identified the cytokine interleukin-6 (IL-6)⁵ 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 IL-6 messenger RNA than did patients not treated with trastuzumab, and a single administration of trastuzumab was sufficient to result in differential expression of IL-6 in primary tumors of the breast, suggesting that increased primary tumor expression of IL-6 in the primary tumors of patients with breast cancer, a cytokine whose serum levels are correlated with worse patient survival outcomes in metastatic breast cancer, is a direct transcriptional result of treatment with trastuzumab.

Keywords: trastuzumab, breast cancer, IL-6, 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 and gene expression data^{3,4}, we report here that IL-6 is a differentially expressed gene in the tumors of trastuzumab-treated patients: patients treated with trastuzumab expressed significantly higher levels of IL-6 than did those of patients not treated with trastuzumab.

Methods

We used datasets GSE38057³ and GSE114082⁴ 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 $n=6$ primary breast tumors from patients treated with induction chemotherapy and/or hormone therapy without trastuzumab and $n=35$ primary breast tumors from patients treated with induction chemotherapy and/or hormone therapy in addition to trastuzumab, both of which developed 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 IL-6 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 comparative transcriptome analysis using published microarray and gene expression data^{3,4} to identify the most significant transcriptional features of the primary breast tumors of patients treated with trastuzumab (Herceptin).

IL-6 is differentially expressed in the tumors of patients treated with trastuzumab.

We identified the cytokine IL-6 as among the genes whose expression changed most significantly in the tumors of breast cancer patients treated with trastuzumab (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, IL-6 ranked 7 out of 1506 total transcripts (Table 1), equating to 99.5% differential expression. IL-6 differential expression in the tumors of patients treated with trastuzumab was statistically significant (Table 1; $p=0.01092$).

The first dataset, although measuring the expression of over 1500 genes, was still limited. We turned to a second microarray dataset, this measuring the expression of genes across the transcriptome - 29377 genes in total - but with another advantage. The tumors compared in this dataset were sampled from the same patient before and after a single, brief exposure to trastuzumab, not comparing tumors from different patients treated with a course of trastuzumab or not. Thus, while differentially expressed genes when comparing tumors based on trastuzumab treatment can result from a variety of sources, including gene expression changes resulting from tumor evolution over the course of treatment, changes identified

in this dataset, occurring after only a single treatment of trastuzumab, are more likely to be a direct transcriptional result of trastuzumab binding events. We found again that IL-6 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, IL-6 ranked 6834 out of 29377 total transcripts (Table 2), equating to 76.7% differential expression. Differential expression of IL-6 in the primary tumors of breast cancer patients following treatment with trastuzumab was statistically significant (Table 2; $p=1.64E-02$).

IL-6 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 IL-6 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 IL-6 based on history of trastuzumab treatment. IL-6 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 deemed statistically significant (Figure 1; $p=0.0001$). We calculated a mean fold change of 2.34 ± 1.67 in IL-6 expression in the primary tumors of patients treated with trastuzumab relative to that of patients not treated with trastuzumab.

Thus, we found that IL-6 was among the genes most differentially expressed in the tumors of patients treated with trastuzumab, that IL-6 was expressed at higher levels in the tumors of patients treated with trastuzumab as compared to those not treated with trastuzumab, and that this transcriptional change was likely a direct result of trastuzumab treatment as opposed to a gene expression change associated with clonal selection and evolution of the tumor across the course of treatment.

Discussion

Survival of metastatic breast cancer patients with high levels of IL-6 in the serum is significantly worse than the survival of patients with low levels of IL-6⁶. It has been reported that although IL-6 is present in the serum of patients with breast cancer and that its expression is associated with worse patient outcomes, the source of IL-6 present in patients with breast cancer was unknown^{5,6}. These data suggest that one source of IL-6 in patients with breast cancer is from the primary tumors of patients treated with trastuzumab. Ectopic expression of IL-6 in MCF-7 breast cancer cells resulted in transcriptional induction of genes associated with the epithelial to mesenchymal transition (EMT), including Vimentin, N-cadherin, Snail and Twist⁷. In cells that ectopically express Twist, a gene whose product supports metastasis⁸, Stat3 is activated and IL-6 expression is increased; we recently demonstrated that STAT3 is differentially expressed and present at higher levels in the tumors of patients treated with trastuzumab⁹, thus suggesting similarities at the level of signal transduction in cells that express a promoter of metastasis and the primary tumors of patients treated with trastuzumab. Autocrine production of IL-6 - that is, production of IL-6 by breast cancer cells themselves - supports drug resistance through activation of the multi-drug resistance gene *mdr1* in MCF-7 cells and the multi-drug resistance MDR-7/ADR cell line¹⁰. Moreover, IL- β can support expression of IL-6 in conjunction with oncostatin M (OSM)¹¹, suggesting the presence of a signaling loop induced by trastuzumab involving IL-6, IL- β and STAT3, since IL-6 signals through STAT3 and since we have demonstrated increased expression of each of these components in the primary tumors of patients treated with trastuzumab¹². Src tyrosine kinase may also be implicated in such a signaling loop.

We provided evidence here that the cytokine IL-6 is a differentially expressed gene in the tumors of patients treated with trastuzumab, and that the primary tumors of breast cancer patients treated with trastuzumab expressed higher levels of IL-6 in their primary tumors than did patients not treated with trastuzumab, demonstrating that a cytokine whose expression is associated with inferior survival outcomes in patients with breast cancer is expressed at significantly higher levels in the primary tumors of patients treated with trastuzumab.

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Rank	ID	p-value	t	B	FC	Gene
7	GI_10834983-S-1	0.01092	2.660727	-4.14	2.34 ± 1.67	IL6

Table 1: IL-6 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 IL-6 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
6834	ILMN_1699651	1.64E-02	2.5183831	-3.40611	IL6

Table 2: A single exposure to trastuzumab is sufficient to drive differential expression of IL-6 in the primary tumors of patients with breast cancer.

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: IL-6 is expressed at significantly higher levels in the primary tumors of patients treated with trastuzumab.

The mRNA expression level of IL-6 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 IL-6 expression in the primary tumors of patients treated with trastuzumab or not, a *p*-value, listed above.