The tumors of breast cancer patients treated with trastuzumab express significantly increased quantities of CD34.

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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 found that the primary tumors of patients treated with trastuzumab express significantly higher levels of CD34 than do those of patients not treated with trastuzumab. CD34 is a differentially expressed gene that distinguishes breast cancer from the normal breast⁵, and these data demonstrate that trastuzumab enhances, not reverses, a marker of the breast tumor.

Keywords: trastuzumab, breast cancer, CD34, systems biology of breast cancer, targeted therapeutics in breast cancer.

We recently described differential expression of CD34 in tumors of the breast⁵, a transcriptional feature that distinguished primary tumors of the breast in patients with breast cancer from that of the normal breast. Through unbiased, systems-level comparative transcriptome analysis of published microarray and gene expression data^{3,4}, we report here that CD34 is also a differentially expressed gene in the tumors of trastuzumab-treated patients: patients treated with trastuzumab express significantly higher levels of CD34 than do those of patients not treated with trastuzumab. Thus, CD34 is a gene expression marker of the breast tumor, and trastuzumab enhances, not reverses expression of this marker at the mRNA level.

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 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. 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 CD34 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 harnessed the power of blind, comparative transcriptome analysis using published gene expression and microarray data^{3,4} to identify the most significant transcriptional features of the primary breast tumors of patients treated with trastuzumab (Herceptin).

CD34 is differentially expressed in the tumors of patients treated with trastuzumab.

We identified CD34 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, CD34 ranked 10 out of 1506 total transcripts (Table 1), equating to 99.3% differential expression. CD34 differential expression in the tumors of patients treated with trastuzumab was statistically significant (Table 1; $p=0.004992$).

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 CD34 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, CD34 ranked 3365 out of 29377 total transcripts (Table 2), equating to 88.5% differential expression. Differential expression of CD34 was statistically significant (Table 2; $p=2.01E-03$).

CD34 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 CD34 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 CD34 based on history of trastuzumab treatment. CD34 was expressed at significantly higher levels in the tumors of patients treated with trastuzumab as compared to those of patients not treated with trastuzumab (Figure 1; $p<0.0001$). We calculated a mean fold change of 1.82 ± 0.54 in CD34 expression in the primary tumors of patients treated with trastuzumab relative to that of patients not treated with trastuzumab.

Thus, we found that CD34 was among the genes most differentially expressed in the tumors of patients treated with trastuzumab, that CD34 was expressed at significantly 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 tumor evolution.

Discussion

While classically viewed as a marker of hematopoietic cell types⁶, CD34 can be used as a surrogate marker for the existence and growth of blood vessels in the tumor by measurement of microvessel density, including in breast cancer⁷⁻¹⁰. Aside from its role as a marker of the vasculature, CD34 has been described as a marker for a cancer type: the gastrointestinal stromal tumor (GIST)¹¹. We recently described CD34 as among the most significant transcriptional features of human breast cancers.

We provided evidence here that CD34 is a differentially expressed gene in the tumors of patients treated with trastuzumab, and that this is likely a direct transcriptional result of trastuzumab administration patients with breast cancer. It is possible that this reflects enhancement of tumor gene expression by trastuzumab; another possibility, not mutually exclusive, is that this is a reflection of increased tumor vasculature resulting from trastuzumab treatment. Nevertheless, we found that a marker used to measure microvessel density, and a marker of the GIST tumor is expressed at significantly higher levels in the tumors of patients treated with trastuzumab and following a single exposure to trastuzumab.

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Rank	ID	p-value	t	B	FC	Gene
10	GI_4502660-S-4	0.004992	2.9415	-2.97	1.82 ± 0.54	CD34

Table 1: CD34 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 CD34 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
3365	ILMN_1732799	2.01E-03	3.3310723	-1.51907	CD34

Table 2: A single, brief exposure to trastuzumab is sufficient to drive differential expression of CD34 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: CD34 is expressed at significantly higher levels in the primary tumors of patients treated with trastuzumab

The mRNA expression level of CD34 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 CD34 expression in the primary tumors of patients treated with trastuzumab or not, a *p*-value, listed above.