PHF20 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 and published microarray data^{3,4} to discover in an unbiased manner the most significant transcriptional changes associated with trastuzumab treatment. We identified the plant homeodomain (PHD) finger protein 20, PHF20, 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 lower levels of PHF20 messenger RNA than did patients not treated with trastuzumab, demonstrating decreased primary tumor expression of an epigenetic molecule which is described to stabilize and activate the tumor suppressor p53⁵ as a direct or indirect transcriptional consequence of treatment with trastuzumab.

Keywords: trastuzumab, breast cancer, PHF20, 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 public and published microarray data^{3,4}, we report here that PHF20 are differentially expressed genes in the tumors of trastuzumab-treated patients: patients treated with trastuzumab expressed significantly lower levels of PHF20 than did those of patients not treated with trastuzumab.

Methods

We used datasets GSE130788³ and GSE22226⁴ 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. GSE22226 was generated using Whole Human Genome Oligo Microarray G4112A technology with $n=118$ primary breast tumors from patients treated with chemotherapy and $n=11$ primary tumors from patients treated with chemotherapy and trastuzumab, analysis performed using platform GPL1708. 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 PHF20 gene expression was significantly between primary breast tumors of 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 unbiased 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).

PHF20 is differentially expressed in the tumors of patients treated with trastuzumab.

We also identified PHD finger protein 20, encoded by PHF20 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, PHF20 ranked 167 out of 41000 total transcripts (Table 3), equating to 99.6% differential expression. PHF20 differential expression in the tumors of patients treated with trastuzumab trended towards statistical significance (Table 1; $p=7.71E-49$).

We turned to a second microarray dataset to validate our findings of PHF20 differential expression in the patients treated with trastuzumab. We found again that PHF20 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, PHF20 ranked 3355 out of 44290 total transcripts (Table 2), equating to 92.4% differential expression. Differential expression of PHF20 in the primary tumors of breast cancer patients following treatment with trastuzumab approached statistical significance (Table 2; $p=4.5E-02$).

PHF20 is expressed at significantly lower 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 PHF20 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 PHF20 based on history of trastuzumab treatment. PHF20 was expressed at lower 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 2; $p<0.0001$).

Thus, we found using published microarray data^{3,4} that PHF20 was among the genes most differentially expressed in the tumors of patients treated with trastuzumab, that PHF20 was expressed at lower levels in the tumors of patients treated with trastuzumab as compared to those not treated with trastuzumab. At this point, we are not able to determine whether these changes are a direct transcriptional result of trastuzumab binding events or whether they are gene expression changes associated with tumor evolution and clonal selection over the course of the treatment.

Discussion

We provided evidence here from public and published microarray data^{3,4} that PHF20 is differentially expressed in the tumors of patients treated with trastuzumab, and expressed at significantly lower levels in the primary tumors of breast cancer patients treated with trastuzumab as compared to patients not treated with trastuzumab. These data demonstrate decreased primary tumor expression of an epigenetic molecule with described functions in stabilization of the tumor suppressor p53 as a direct or indirect transcriptional consequence of treatment with trastuzumab in patients with breast cancer.

References

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Rank	ID	p-value	t	B	Gene	Gene name
167	A_24_P375592	7.71E-49	-19.75659	100.573868	PHF20	PHD finger protein 20

Table 1: PHF20 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.

Rank	ID	p-value	t	B	Gene	Gene name
3355	15196	4.5E-02	2.02	-3.86902	PHF20	PHD finger protein 20

Table 2: PHF20 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: PHF20 is expressed at significantly lower levels in the primary tumors of patients treated with trastuzumab.

The mRNA expression level of PHF20 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 PHF20 expression in the primary tumors of patients treated with trastuzumab or not, a *p*-value, listed above.