Protocadherin 11, Y-linked (PCDH11Y) is differentially expressed in the tumors of breast cancer patients treated with trastuzumab.

Shahan Mamoor, MS¹

¹shahanmamoor@gmail.com

East Islip, NY USA

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 significant transcriptional changes associated with trastuzumab treatment. We identified the protocadherin 11, Y-linked, PCDH11Y, 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 PCDH11Y messenger RNA than did patients not treated with trastuzumab, demonstrating increased expression of a neuronal cell adhesion molecule^{5,6} as a transcriptional consequence of treatment with trastuzumab.

Keywords: trastuzumab, breast cancer, PCDH11Y, 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 PCDH11Y is a differentially expressed gene in the tumors of trastuzumab-treated patients: patients treated with trastuzumab expressed significantly higher levels of PCDH11Y 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 PCDH11Y 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 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).

PCDH11Y is differentially expressed in the tumors of patients treated with trastuzumab.

We identified the protocadherin 11, Y-linked, encoded by PCDH11Y 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, PCDH11Y ranked 17 out of 32321 total transcripts (Table 1), equating to 99.95% differential expression. PCDH11Y differential expression in the tumors of patients treated with trastuzumab trended towards statistical significance (Table 1; *p*=5.15E-81).

We turned to a second microarray dataset to validate our findings of PCDH11Y differential expression in the patients treated with trastuzumab. We found again that PCDH11Y 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, PCDH11Y ranked 1240 out of 44290 total transcripts (Table 2), equating to 97.2% differential expression. Differential expression of PCDH11Y in the primary tumors of breast cancer patients following treatment with trastuzumab was statistically significant (Table 2; *p*=1.23E-02).

PCDH11Y 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 PCDH11Y 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 PCDH11Y based on history of trastuzumab treatment. PCDH11Y 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; $p < 0.0001$).

Thus, we found using published multiplexed gene expression and microarray data^{3,4} that PCDH11Y was among the genes most differentially expressed in the tumors of patients treated with trastuzumab, that PCDH11Y 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 the protocadherin 11, Y-linked, encoded by PCDH11Y 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 increased primary tumor expression of a cell adhesion molecule expressed in regions of the developing brain, including in the cerebral cortex, as a transcriptional consequence of treatment with trastuzumab in patients with breast cancer, either as a direct transcriptional change effected by trastuzumab or an alteration of gene expression acquired over the course of treatment due to tumor evolution following trastuzumab administration.

References

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Rank	ID	p-value	t	B	Gene	Gene name
17	A_32_P137980	5.15E-81	32.232478	174.141309	PCDH11Y	protocadherin 11 Y-linked

Table 1: PCDH11Y 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 PCDH11Y 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
1240	3666	1.23E-02	-2.54	-2.87531	PCDH11Y	protocadherin 11 Y-linked

Table 2: PCDH11Y 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: PCDH11Y is expressed at significantly higher levels in the primary tumors of patients treated with trastuzumab.

The mRNA expression level of PCDH11Y 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 PCDH11Y expression in the primary tumors of patients treated with trastuzumab or not, a *p*-value, listed above.