Trastuzumab administration in patients with breast cancer is associated with increased primary tumor expression of CEACAM1.

Shahan Mamoor, MS¹

¹shahanmamoor@gmail.com

East Islip, NY USA

Trastuzumab, a monoclonal antibody targeted against the human epidermal growth factor receptor 2 (HER2) is utilized for the treatment of human breast cancer^{1,2}, but a complete understanding of how tumor signal transduction is modulated by trastuzumab treatment is lacking. By mining published and public microarray and gene expression data^{3,4} from the primary tumors of patients treated with trastuzumab, we found that the cell adhesion molecule CEACAM1 was among the genes most differentially expressed in the primary tumors of patients treated with trastuzumab. Thus, a molecule whose expression is predictive of metastatic disease in patients with malignant melanoma⁵ and whose expression in melanoma cell lines can support invasive properties⁶ is expressed at significantly higher levels in the primary tumors of patients treated with trastuzumab for breast cancer.

Keywords: breast cancer, trastuzumab, CEACAM1, systems biology of breast cancer, targeted therapeutics in breast cancer.

The mechanism of action by which trastuzumab exerts its therapeutic function in human breast cancer is said to be binding of the F_{ab} domain of trastuzumab to the extracellular region of the human epidermal growth factor receptor 2 (HER2)², possibly resulting in decreased cellular proliferation⁷. Transcriptional modulation resulting from trastuzumab binding events in the primary tumors of treated patients is less well understood. We mined published and public microarray and gene expression data^{3,4} generated using primary tissues from patients treated with trastuzumab to compare tumor global gene expression with that of patients not treated with trastuzumab. Using this unbiased, systems level methodology, we identified differential expression and up-regulation the cell adhesion molecule CEACAM1 in primary tumors of the breast in patients treated with trastuzumab.

Methods

We used datasets GSE38057³ and GSE130788⁴ for 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. 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. 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 CEACAM1 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

CEACAM1 is differentially expressed in the primary tumors of patients treated with trastuzumab.

We found that CEACAM1, the carcinoembryonic antigen related cell adhesion molecule 1, was among the genes whose expression was most significantly different in the primary tumors of patients with breast cancer treated with trastuzumab³. When sorting each of the genes expressed in trastuzumab-treated breast tumors based on significance of difference in expression as compared to the primary breast tumors of patients not treated with trastuzumab, CEACAM1 ranked 15 out of 1506 total transcripts, equating to 99% statistical significance (Table 1). Differential expression of CEACAM1 in the primary tumors of breast cancer patients treated with trastuzumab was statistically significant (Table 1; $p=0.02346$).

We queried a second dataset⁴ to validate our findings of CEACAM1 differential expression upon trastuzumab treatment. In a second microarray dataset analyzing gene expression of patients treated with trastuzumab, we again found that CEACAM1 was among the genes whose expression was most significantly different transcriptome-wide when comparing the global gene expression profiles of tumors from patients treated with trastuzumab as compared to those not treated with trastuzumab. When sorting each of the genes expressed in the tumors of patients treated with trastuzumab based on significance of change as compared to the tumors of patients not treated with trastuzumab, CEACAM1 ranked 9953 out

of 41000 total transcripts, equating to 75.7% differential expression. Differential expression of CEACAM1 in the primary tumors of patients treated with trastuzumab was statistically significant (Table 2; $p=2.07E-07$).

CEACAM1 is expressed at higher levels in the tumors of patients treated with trastuzumab.

We obtained exact mRNA expression levels for CEACAM1 from the tumors of patients treated with trastuzumab and from the tumors of patients not receiving trastuzumab to determine magnitude and direction of CEACAM1 change in the tumors of patients treated with trastuzumab. CEACAM1 expression was significantly higher in the tumors of patients treated with trastuzumab as compared to the tumors of patients not treated with trastuzumab (Figure 1). Increased expression of CEACAM1 was statistically significant (Figure 1: $p=0.0136$). We calculated a mean fold change of 1.73 ± 0.87 when comparing the expression of CEACAM1 in the tumors of patients treated with trastuzumab as compared to the tumors of patients treated with trastuzumab.

Thus, we found using published microarray and gene expression data^{3,4} that the cell adhesion molecule CEACAM1 was among the genes most differentially expressed in the primary tumors of patients with breast cancer and that

Discussion

Multiple studies have described roles for the carcinoembryonic antigen related cell adhesion molecule 1 (CEACAM) in cancer. CEACAM can support migration and invasion of the melanoma cell lines MV3 and MEL6 *in vitro*⁵. CEACAM is expressed at higher levels in pancreatic adenocarcinomas as compared to benign pancreatic tissue⁸. Moreover, the expression CEACAM in patients with cutaneous malignant melanoma has been described as a predictor of the development of metastasis⁶. In urothelial carcinoma of the bladder (UCB), CEACAM1 was expressed at significantly higher levels in patients with UCB and its expression was described as a potential cancer biomarker⁹.

We provided evidence here that CEACAM1, a molecule over-expressed in the tumors of patients with pancreatic cancer⁸ and in the urine of patients with bladder cancer⁹, and whose expression in patients with malignant melanoma was described as a predictor of metastasis⁶, is expressed at significantly higher levels in the tumors of patients treated with trastuzumab and among the genes most differentially expressed in the primary tumors of patients treated with trastuzumab.

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Rank	ID	p-value	t	B	FC	Gene
15	GI_19923194-S-3	0.02346	2.349595	-4.25	1.73 ± 0.87	CEACAM1

Table 1: CEACAM1 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 CEACAM1 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
9953	A_23_P434118	2.07E-07	-5.380429	5.935803	CEACAM1	carcinoembryonic antigen related cell adhesion molecule 1

Table 2: CEACAM1 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 CEACAM1 between the tumors of patients treated with trastuzumab and the tumors of patients not treated with trastuzumab, and gene are listed in this chart.

Figure 1: CEACAM1 is expressed at significantly higher levels in the primary tumors of patients treated with trastuzumab as compared to those not treated with trastuzumab.

The mRNA expression level of CEACAM1 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 CEACAM1 expression in the primary tumors of patients treated with trastuzumab or not, a *p*-value, listed above.