CD36 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 data^{3,4} to discover in an unbiased manner the most significant transcriptional changes associated with trastuzumab treatment. We identified the scavenger receptor CD36 as among the genes most differentially expressed in the primary tumors of patients with breast cancer treated with trastuzumab. A single dose of trastuzumab was sufficient to result in differential expression of CD36 in the primary tumors of patients with breast cancer, suggesting that transcriptional induction of CD36 is a direct result of trastuzumab administration.

Keywords: trastuzumab, breast cancer, CD36, 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 are less well understood. Through unbiased, systems-level comparative transcriptome analysis of published microarray data^{3,4}, we report here that CD36 is a differentially expressed gene in the tumors of trastuzumab-treated patients: patients treated with a single dose of trastuzumab expressed significantly higher levels of CD36 than before treatment with trastuzumab.

Methods

We used datasets GSE114082³ and GSE22226⁴ for this differential gene expression analysis of the transcriptional response to trastuzumab treatment in patients with breast cancer. 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. 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 CD36 gene expression was significantly between primary breast tumors of patients receiving trastuzumab using a two-tailed t-test. 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 public microarray data^{3,4} to identify the most significant transcriptional features of the primary breast tumors of patients treated with trastuzumab (Herceptin).

CD36 is differentially expressed in the tumors of patients treated with trastuzumab.

We identified the scavenger receptor CD36, encoded by CD36 as among the genes whose expression changed most significantly in the tumors of breast cancer patients treated with trastuzumab in a microarray dataset³ (Table 1). The tumors compared in this dataset were sampled from the same patient before and after administration of a single dose of trastuzumab. 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 the same patients before a single administration of trastuzumab, CD36 ranked 38 out of 29377 total transcripts (Table 1), equating to 99.9% differential expression. CD36 differential expression in the tumors of patients following a single administration of trastuzumab was statistically significant (Chart 1; $p=3.21E-07$).

We queried a second microarray dataset to attempt to validate transcriptome-wide differential expression of CD36 in the primary tumors of patients treated with trastuzumab. We again found that CD36 was among the genes most differentially expressed in the tumors of patients treated with trastuzumab, but here, comparing patients based on treatment history (with or without trastuzumab) (Chart 2). When sorting each of the genes expressed in the tumors of patients treated with trastuzumab, CD36 ranked 5761 out of 28396 total transcripts (Chart 2), equating to 79.7% differential expression, when compared to patients not treated with trastuzumab. Differential expression of CD36 in the primary

tumors of breast cancer patients treated with trastuzumab was not deemed statistically significant (Chart 2; $p=1.45E-01$).

CD36 is expressed at significantly higher levels in the tumors of breast cancer patients following a single administration of trastuzumab.

We obtained exact mRNA expression levels for CD36 from tumors of the same patients, before and after a single administration of trastuzumab to directly evaluate direction and statistical significance of change in expression of CD36 following exposure to trastuzumab. CD36 was expressed at higher levels in the tumors of patients after treatment with trastuzumab as compared to before, and this difference was statistically significant (Figure 1; $p<0.0001$). We calculated a mean fold change of 3.16 in CD36 mRNA expression, as CD36 was expressed at 3907.33 ± 3363.22 arbitrary units (AU) before administration of trastuzumab, and at 12332.23 ± 5433.19 AU following administration of a single dose of trastuzumab.

Thus, we found using published microarray data^{3,4} that CD36 was among the genes most differentially expressed in the tumors of patients following a single exposure to trastuzumab, with significantly higher mRNA expression of CD36 in the tumors of patients following administration of a single dose of trastuzumab.

Discussion

We provided evidence here that the scavenger receptor CD36 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 a single dose of trastuzumab as compared to the same patient tumors before trastuzumab dosing. These data demonstrate that trastuzumab administration in patients with breast cancer, a treatment according to one report associated with a 34% rate of brain metastasis⁵, results in increased expression of a cell surface marker that we have observed to be a defining transcriptional feature of early-onset breast cancer⁶, a marker whose expression has been associated with enhanced metastasis in mouse models of cancer⁷, and that this increased expression is likely a direct result of treatment with trastuzumab³. Though it has been argued that CD36 is up-regulated during acquisition of resistance in response to HER2 inhibition by trastuzumab⁸, we find here that CD36 up-regulation is readily observable in patient tumor following a single administration of trastuzumab, arguing this reflects primary transcriptional effects of trastuzumab as opposed to changes in gene expression observed over a course of treatment (tumor evolution).

References

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Rank: 38
Probe ID: ILMN_1784863
p-value: 3.21E-07
t: -6.2567034
B: 6.64674
Gene: CD36
Gene name: cluster of differentiation 36

Chart 1: CD36 is differentially expressed in the primary tumors of breast cancer patients treated with trastuzumab, and a single dose of trastuzumab is sufficient to result in differential expression of CD36 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, fold change of expression in CD36 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: 5761
Probe ID: 20683
p-value: 1.45E-01
t: 1.465878
B: -4.69397
Gene: CD36

Chart 2: CD36 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 CD36 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: CD36 is expressed at significantly higher levels with trastuzumab in the primary tumors of patients following a single administration of trastuzumab.

The mRNA expression level of CD36 in the tumors of patients before treatment with trastuzumab (left) and in the primary tumors of patients after treatment with trastuzumab (right) is graphically depicted with mean mRNA level marked and the result of a statistical test evaluating significance of difference in mRNA expression between the groups, a *p*-value, is listed above.