SIX6 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 public microarray data^{3,4} to discover in an unbiased manner the most significant transcriptional changes associated with trastuzumab treatment. We identified the sine oculis family homeobox transcription factor SIX6 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 SIX6 in the primary tumors of patients with breast cancer, suggesting that transcriptional induction of SIX6, a transcription factor with functions in specification of the ventral stalk from the neural retina⁵, is a direct result of trastuzumab administration.

Keywords: trastuzumab, breast cancer, SIX6, 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 SIX6 is a differentially expressed gene in the tumors of trastuzumab-treated patients: patients treated with trastuzumab expressed significantly higher levels of SIX6 than did those of patients not treated with trastuzumab.

Methods

We used datasets GSE130788³ and GSE114082⁴ 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. 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 SIX6 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).

SIX6 is differentially expressed in the tumors of patients treated with trastuzumab.

We identified the sine oculis homeobox homolog Six, encoded by SIX6, also known as OPTX2, 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). 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, SIX6 ranked 112 out of 32321 total transcripts (Table 1), equating to 99.7% differential expression. SIX6 differential expression in the tumors of patients treated with trastuzumab trended towards statistical significance (Chart 1; $p=9.45e-44$).

We queried a second microarray dataset to attempt to validate transcriptome-wide differential expression of SIX6 in the primary tumors of patients treated with trastuzumab. The tumors compared in this dataset were sampled from the same patient before and after administration of a single dose of trastuzumab, not comparing tumors from different patients treated with a course of trastuzumab or not. Thus, while differential expression of genes identified through comparison of tumors based on a history of trastuzumab treatment can arise due to a variety of reasons, 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 likely to be a direct transcriptional result of trastuzumab binding events. We found that SIX6 was among the genes most differentially expressed in the tumors of patients administered a single treatment of trastuzumab (Chart 2). When sorting each of the genes expressed in the tumors of patients administered a single treatment of trastuzumab, SIX6 ranked 5904 out of 29377 total transcripts (Chart 2), equating to 79.9% differential expression. Differential expression of SIX6 in the primary tumors of breast cancer patients following treatment with trastuzumab was statistically significant (Chart 2; $p=1.06e-02$).

SIX6 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 SIX6 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 SIX6 based on history of trastuzumab treatment. SIX6 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 statistically significant (Figure 1; $p<0.0001$).

Thus, we found using public microarray data^{3,4} that SIX6 was among the genes most differentially expressed in the tumors of patients treated with trastuzumab, that SIX6 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 sine oculis family homeobox transcription factor SIX6, also known as OPTX2, 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 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 transcription factor expressed in the optic primordium and important for development of the neural retina, and that this increased expression is likely a direct result of treatment with trastuzumab⁶.

References

1. Hudis, C.A., 2007. Trastuzumab—mechanism of action and use in clinical practice. *New England Journal of Medicine*, 357(1), pp.39-51.
2. Herceptin (Trastuzumab). Indications. <https://www.herceptin.com/hcp/indications.html>.
3. GSE130788. A Phase II Randomized Trial of Neoadjuvant Trastuzumab or Lapatinib or the Combination of Trastuzumab and Lapatinib, Followed by Docetaxel and Carboplatin with Trastuzumab and/or Lapatinib in Patients with HER2+ Breast Cancer. <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE130788>. Hsiaowang Chen. UCLA, Department of Medicine. Santa Monica, CA.
4. Triulzi, T., Regondi, V., De Cecco, L., Cappelletti, M.R., Di Modica, M., Paolini, B., Lollini, P.L., Di Cosimo, S., Sfondrini, L., Generali, D. and Tagliabue, E., 2018. Early immune modulation by single-agent trastuzumab as a marker of trastuzumab benefit. *British journal of cancer*, 119(12), pp.1487-1494.
5. Bendell, J.C., Domchek, S.M., Burstein, H.J., Harris, L., Younger, J., Kuter, I., Bunnell, C., Rue, M., Gelman, R. and Winer, E., 2003. Central nervous system metastases in women who receive trastuzumab-based therapy for metastatic breast carcinoma. *Cancer*, 97(12), pp.2972-2977.
6. Jean, D., Bernier, G. and Gruss, P., 1999. Six6 (Optx2) is a novel murine Six3-related homeobox gene that demarcates the presumptive pituitary/hypothalamic axis and the ventral optic stalk. *Mechanisms of development*, 84(1-2), pp.31-40.

Rank: 112
Probe ID: A_23_P106117
p-value: 9.45e-44
t: 4.68e-46
B: 18.8643
Gene: SIX6
Gene name: SIX homeobox 6

Chart 1: SIX6 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 SIX6 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: 5904
Probe ID: ILMN_1786631
p-value: 1.06e-02
t: -2.6951011
B: -3.02365
Gene: SIX6

Chart 2: A single dose of trastuzumab is sufficient to result in differential expression of SIX6 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 SIX6 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: SIX6 is expressed at significantly higher levels in the primary tumors of patients treated with trastuzumab than in the primary tumors of patients not treated with trastuzumab.

The mRNA expression level of SIX6 in the tumors of patients not treated with trastuzumab (left) and in the primary tumors of patients treated 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.