TNFAIP1 is differentially expressed in the primary tumors of breast cancer patients treated with trastuzumab.

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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 tumor necrosis factor alpha-induced protein 1, TNFAIP1, was among the genes most differentially expressed in the primary tumors of patients treated with trastuzumab, and expressed at lower levels in the tumors of patients treated with trastuzumab. Thus, the use of trastuzumab in patients with breast cancer is associated with decreased expression of a gene⁵ that can induce apoptosis of cancer cells⁶.

Keywords: breast cancer, trastuzumab, TNFAIP1, tumor necrosis factor alpha-induced protein 1, 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 down-regulation of TNFAIP1 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 $n=11$ primary breast tumors from patients treated with induction chemotherapy and/or hormone therapy without trastuzumab and $n=36$ primary breast tumors from patients treated with induction chemotherapy and/or hormone therapy in addition to trastuzumab; 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 TNFAIP1 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 comparative transcriptome analysis of primary tumors from patients treated or not with trastuzumab (Herceptin), using published multiplexed gene expression data³ and a microarray dataset⁴, in order to identify the most significant transcriptional changes in the primary tumors of breast cancer patients following treatment with trastuzumab.

TNFAIP1 is differentially expressed in the primary tumors of patients treated with trastuzumab.

We found that the tumor necrosis factor alpha-induced protein 1, encoded by TNFAIP1, 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 the primary tumors of breast cancer patients treated with trastuzumab based on significance of difference in expression as compared to the primary tumors of breast cancer patients not treated with trastuzumab, TNFAIP1 ranked 40 out of 1506 total transcripts, equating to 97.3% statistical significance (Table 1). Differential expression of TNFAIP1 in the primary tumors of breast cancer patients treated with trastuzumab was statistically significant (Table 1; $p=0.026335$).

We queried a second dataset⁶ to validate our findings of TNFAIP1 differential expression upon trastuzumab treatment. In a second microarray dataset analyzing gene expression of patients treated with

trastuzumab, we again found that TNFAIP1 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, TNFAIP1 ranked 10466 out of 41000 total transcripts, equating to 74.5% differential expression. Differential expression of TNFAIP1 in the primary tumors of patients treated with trastuzumab was statistically significant (Table 2; $p=4.39E-07$).

TNFAIP1 is expressed at significantly lower levels in the tumors of patients treated with trastuzumab.

We obtained exact mRNA expression levels for TNFAIP1 from the tumors of patients treated with trastuzumab and from the tumors of patients not receiving trastuzumab to determine magnitude and direction of TNFAIP1 change in the tumors of patients treated with trastuzumab. TNFAIP1 expression was lower in the tumors of patients treated with trastuzumab as compared to the tumors of patients not treated with trastuzumab (Figure 1). Decreased expression of TNFAIP1 was statistically significant (Figure 1: $p=0.0370$). We calculated a mean fold change of 0.82 ± 0.22 when comparing the expression of TNFAIP1 in the tumors of patients treated with trastuzumab as compared to the tumors of patients treated with trastuzumab.

Thus, we found that tumor necrosis factor alpha-induced protein 1, encoded by TNFAIP1, was among the genes most differentially expressed in the primary tumors of patients with breast cancer and that TNFAIP1 expression was significantly lower in the primary tumors of patients treated with trastuzumab as compared to those not treated.

Discussion

We provided evidence here that the tumor necrosis factor alpha induced protein 1, encoded by TNFAIP1⁵ is expressed at significantly lower 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. Thus, administration of trastuzumab in patients with breast cancer, a therapeutic associated with a 34% rate of central nervous system metastasis⁷, results in decreased primary tumor expression of a molecule that can cooperate with RhoB to induce apoptosis in cancer cells *in vitro*⁶ and that has the capacity to control radial migration in the cerebral cortex⁸.

References

1. Slamon, D., Eiermann, W., Robert, N., Pienkowski, T., Martin, M., Press, M., Mackey, J., Glaspy, J., Chan, A., Pawlicki, M. and Pinter, T., 2011. Adjuvant trastuzumab in HER2-positive breast cancer. *New England Journal of Medicine*, 365(14), pp.1273-1283.
2. Hudis, C.A., 2007. Trastuzumab—mechanism of action and use in clinical practice. *New England Journal of Medicine*, 357(1), pp.39-51.
3. Duchnowska, R., Jassem, J., Goswami, C.P., Dundar, M., Gökmen-Polar, Y., Li, L., Woditschka, S., Biernat, W., Sosińska-Mielcarek, K., Czartoryska-Arłukowicz, B. and Radecka, B., 2015. Predicting early brain metastases based on clinicopathological factors and gene expression analysis in advanced HER2-positive breast cancer patients. *Journal of Neuro-oncology*, 122(1), pp.205-216.
4. 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](https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE130788). Hsiaowang Chen. UCLA, Department of Medicine. Santa Monica, CA.
5. Wolf, F.W., Marks, R.M., Sarma, V., Byers, M.G., Katz, R.W., Shows, T.B. and Dixit, V.M., 1992. Characterization of a novel tumor necrosis factor- α -induced endothelial primary response gene. *Journal of Biological Chemistry*, 267(2), pp.1317-1326.
6. Kim, D.M., Chung, K.S., Choi, S.J., Jung, Y.J., Park, S.K., Han, G.H., Ha, J.S., Song, K.B., Choi, N.S., Kim, H.M. and Won, M., 2009. RhoB induces apoptosis via direct interaction with TNFAIP1 in HeLa cells. *International journal of cancer*, 125(11), pp.2520-2527.
7. 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.
8. Gladwyn-Ng, I.E., Li, S.S., Qu, Z., Davis, J.M., Ngo, L., Haas, M., Singer, J. and Heng, J.I.T., 2015. Bacurd2 is a novel interacting partner to Rnd2 which controls radial migration within the developing mammalian cerebral cortex. *Neural development*, 10(1), pp.1-13.

Rank	ID	p-value	t	B	FC	Gene
40	GI_26051238-S-4	0.026335	-2.29109	-3.69	0.82 ± 0.22	TNFAIP1

Table 1: TNFAIP1 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 TNFAIP1 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
10466	A_23_P38446	4.39E-07	-5.224884	5.210559	TNFAIP1	TNF alpha induced protein 1

Table 2: TNFAIP1 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: TNFAIP1 is expressed at lower levels in the primary tumors of patients treated with trastuzumab as compared to those not treated with trastuzumab.

The mRNA expression level of TNFAIP1 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 TNFAIP1 expression in the primary tumors of patients treated with trastuzumab or not, a *p*-value, listed above.