GRIA2 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 glutamate ionotropic receptor AMPA type subunit 2, GRIA2 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 GRIA2 messenger RNA than did patients not treated with trastuzumab, and a single administration of trastuzumab was sufficient to result in differential expression of GRIA2 in primary tumors of the breast, demonstrating increased primary tumor expression of a glutamate receptor subunit found to be among the genes most significantly up-regulated in the brain metastases of an experimental melanoma model⁵ as a direct transcriptional consequence of trastuzumab treatment in patients with breast cancer.

Keywords: trastuzumab, breast cancer, GRIA2, glutamate ionotropic receptor AMPA type subunit 2, 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 GRIA2 is a differentially expressed gene in the tumors of trastuzumab-treated patients: patients treated with trastuzumab expressed significantly higher levels of GRIA2 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 GRIA2 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 data^{3,4} to identify the most significant transcriptional features of the primary breast tumors of patients treated with trastuzumab (Herceptin).

GRIA2 is differentially expressed in the tumors of patients treated with trastuzumab.

We identified glutamate ionotropic receptor AMPA type subunit 2, encoded by GRIA2 as among the genes whose expression changed most significantly in the tumors of breast cancer patients treated with trastuzumab in a published 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, GRIA2 ranked 52 out of 41000 total transcripts (Table 1), equating to 99.8% differential expression. GRIA2 differential expression in the tumors of patients treated with trastuzumab trended towards statistical significance (Table 1; *p*=1.73E-65).

We turned to a second microarray dataset to validate our findings of GRIA2 differential expression in the patients treated with trastuzumab⁴. The tumors compared in this dataset were sampled from the same patient before and after a single, brief exposure to trastuzumab, not comparing tumors from different patients treated with a course of trastuzumab or not. Thus, while differentially expressed genes when comparing tumors based on trastuzumab treatment can result from a variety of sources, including gene expression changes resulting from tumor evolution over the course of treatment, changes identified

1 in this dataset, occurring after only a single treatment of trastuzumab, are more likely to be a direct
2 transcriptional result of trastuzumab binding events. We found again that GRIA2 was among the genes
3 most differentially expressed in the tumors of patients administered a single treatment of trastuzumab
4 (Table 2). When sorting each of the genes expressed in the tumors of patients administered a single
5 treatment of trastuzumab, GRIA2 ranked 78 out of 29377 total transcripts (Table 2), equating to 99.7%
6 differential expression. Differential expression of GRIA2 in the primary tumors of breast cancer patients
7 following treatment with trastuzumab was statistically significant (Table 2; $p=7.01E-07$).

8
9
10
11
12
13
14
15
16
17
18 **GRIA2 is expressed at significantly higher levels in the tumors of breast cancer patients treated
19 with trastuzumab than in the tumors of those not treated with trastuzumab.**

20 We obtained exact mRNA expression levels for GRIA2 from tumors of patients treated with
21 trastuzumab and from the tumors of patients not treated with trastuzumab to directly evaluate direction
22 and statistical significance of change in expression of GRIA2 based on history of trastuzumab treatment.
23 GRIA2 was expressed at higher levels in the tumors of patients treated with trastuzumab as compared to
24 those of patients not treated with trastuzumab, and this difference was statistically significant (Figure 1;
25 $p<0.0001$).

26 We also obtained exact mRNA expression levels for GRIA2 from the tumors of patients before
27 and after a brief exposure to trastuzumab to evaluate magnitude, direction and statistical significance of
28 change in expression of GRIA2 following a single administration of trastuzumab. GRIA2 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 2; $p=0.0027$).

Thus, we found using published microarray data^{3,4} that GRIA2 was among the genes most
differentially expressed in the tumors of patients treated with trastuzumab, that GRIA2 was expressed at
higher levels in the tumors of patients treated with trastuzumab as compared to those not treated with
trastuzumab, and that this transcriptional change was likely a direct result of trastuzumab treatment as
opposed to a gene expression change associated with clonal selection and evolution of the tumor across
the course of treatment.

18 **Discussion**

19 We provided evidence here that GRIA2 is a differentially expressed gene in the tumors of patients
20 treated with trastuzumab; a single administration of trastuzumab was sufficient to result in significant
21 transcriptional induction of GRIA2. Thus, a direct transcriptional consequence of trastuzumab
22 administration, a treatment that has been associated with a 34% rate of central nervous system
23 metastases⁶, is up-regulation of an ionotropic glutamate AMPA receptor subunit found to be among the
24 genes most significantly up-regulated in brain metastases generated from melanoma cell lines in an
25 experimental murine cancer model *in vivo*⁵.

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. Nygaard, V., Prasmickaite, L., Vasiliauskaite, K., Clancy, T. and Hovig, E., 2014. Melanoma brain colonization involves the emergence of a brain-adaptive phenotype. *Oncoscience*, 1(1), p.82.
6. 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.

Rank	ID	p-value	t	B	Gene	Gene name
52	A_23_P340617	1.73E-65	25.810363	138.697812	GRIA2	glutamate ionotropic receptor AMPA type subunit 2

Table 1: GRIA2 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.

Rank	ID	p-value	t	B	Gene
78	ILMN_1660372	7.01E-07	6.0018693	5.91405	GRIA2

Table 2: A single exposure to trastuzumab is sufficient to drive differential expression of GRIA2 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, gene and gene name are listed in this chart.

Figure 1: GRIA2 is expressed at significantly higher levels in the primary tumors of patients treated with trastuzumab.

The mRNA expression level of GRIA2 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 GRIA2 expression in the primary tumors of patients treated with trastuzumab or not, a *p*-value, listed above.

Figure 2: GRIA2 is transcriptionally induced following a single treatment with trastuzumab.

The mRNA expression level of GRIA2 is graphically depicted in this chart in the primary tumors of breast cancer patients before trastuzumab administration (left) and after a single administration of trastuzumab (right), with mean mRNA expression level marked and the result of a statistical test evaluating significance of difference between GRIA2 expression in the primary tumors of patients before and after treatment with trastuzumab, a *p*-value, listed above.