Transcriptional induction of the nerve growth factor receptor in the primary tumors of breast cancer patients treated with trastuzumab.

Shahan Mamoor, MS¹

¹shahanmamoor@gmail.com

East Islip, NY USA

The incidence of central nervous system metastases in breast cancer patients treated with the human epidermal growth factor receptor 2 (HER2) monoclonal antibody trastuzumab has been reported at 34%¹. To understand the most significant transcriptional changes associated with trastuzumab treatment in patients with breast cancer, we mined published microarray and multiplexed gene expression data^{2,3}, comparing the primary tumors of patients treated with trastuzumab or not. We identified significant differential expression and transcriptional induction of the nerve growth factor receptor, NGFR, in the primary tumors and tumor cells of breast cancer patients treated with trastuzumab. We suggest up-regulation of a receptor for a neurotrophic growth factor is a highly undesirable consequence of trastuzumab treatment in a population prone to develop metastases to the brain.

Keyword: breast cancer, trastuzumab, central nervous system metastases, brain metastases, systems biology of breast cancer, targeted therapeutics in breast cancer.

Central nervous system metastases, including metastases to the brain are a clinical problem for patients with breast cancer; in patients treated with the monoclonal antibody targeted HER2, trastuzumab, the incidence of CNS metastasis was reported at 34%^{1, 4-6}. To facilitate an enhanced understanding of the most significant transcriptional consequences of trastuzumab administration in patients with breast cancer, we mined published microarray and multiplexed gene expression data^{2,3} to discover genes whose expression was most significantly perturbed by trastuzumab treatment in an unbiased fashion and at the systems level. We identified the nerve growth factor receptor as among the genes whose expression was most significantly different in the primary tumors of breast cancer patients treated with trastuzumab. Transcriptional induction of NGFR is a potentially alarming molecular consequence of a treatment used for patients with HER2-overexpressing breast cancers, both as first-line treatment and for the treatment of metastatic breast cancer^{7,8}.

Methods

We used datasets GSE38057² and GSE20194³ for this global and multiplexed differential gene expression analysis in conjunction with GEO2R. 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. GSE20194 was generated using Affymetrix Human Genome U133A Array technology with $n=256$ samples of fine needle aspiration isolated breast cancer cells of patients not treated with trastuzumab and $n=12$ samples of fine needle aspiration isolated breast cancer cells of patients treated with trastuzumab, analysis performed using platform GPL96. 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 NGFR gene expression was significantly between primary breast tumors of patients receiving trastuzumab or not, 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

The gene encoding the nerve growth factor receptor, NGFR, is among the genes most differentially expressed in the primary tumors and tumor cells of patients treated with trastuzumab.

We identified NGFR as among the genes whose expression was most significantly different when comparing multiplexed gene expression profiles of primary tumors from patients with breast cancer treated with trastuzumab or not². When sorting each of the genes measured in primary breast tumors from patients treated with trastuzumab based on significance of change in expression as compared to primary breast tumors from patients not treated with trastuzumab, NGFR ranked 202 out of 1506 total transcripts (Table 1). Differential expression of NGFR in the primary tumors of patients with breast cancer treated with trastuzumab ranked at the 86.6% percentile but not deemed statistically significant (Table 1; $p=0.150938$).

We queried a second microarray dataset³ to validate transcriptome-wide differential expression of NGFR upon trastuzumab administration. We again found that NGFR was among the genes whose expression changed most significantly in the tumors of patients treated with trastuzumab or not, here

when comparing fine needle aspiration-isolated primary tumor cells of the breast from patients treated with trastuzumab to that of patients not treated with trastuzumab (Table 2). When sorting each of the genes expressed in primary tumor cells of patients treated with trastuzumab based on significance of change in expression as compared to that of patients not treated with trastuzumab, NGFR ranked 178 out of 22283 total transcripts (Table 2). Differential expression of NGFR in the primary tumor cells of breast cancer patients treated with trastuzumab was statistically significant (Table 2; $p=9.03E-04$).

NGFR is expressed at significantly higher levels in the primary tumors and tumor cells of patients treated with trastuzumab as compared to those not treated with trastuzumab.

We obtained exact mRNA expression levels for NGFR from the primary tumors of patients with breast cancer, in patients treated with trastuzumab and those not treated with trastuzumab, to determine magnitude and direction of change in NGFR expression following administration of trastuzumab and determine if this change was statistically significant. NGFR was expressed at higher levels in the primary tumors and tumor cells of patients with breast cancer following treatment with trastuzumab (Figure 1 and Figure 2, respectively); in one case, this difference was statistically significant (Figure 1; $p=0.0227$) while in the other case, it was not (Figure 2; $p=0.2574$). We calculated a fold change in NGFR expression of 1.60 ± 1.34 in the primary tumors of breast cancer patients treated with trastuzumab as compared to that of patients not treated with trastuzumab (Table 1), but only 1.115 ± 0.331 in the fine needle aspiration-obtained tumor cells of patients treated with trastuzumab administration as compared to that of patients not treated with trastuzumab (Table 2).

In summary, we identified NGFR as among the genes whose expression was most significantly different in the primary tumors and tumor cells of patients with breast cancer upon administration of trastuzumab as compared to primary tumors of patients not receiving trastuzumab. NGFR expression was significantly higher in the tumors of breast cancer patients treated with trastuzumab. Thus, it appears that a receptor for a neurotrophic factor is transcriptionally induced by trastuzumab administration.

Discussion

Brain metastases are a clinical problem for patients with breast cancer^{1,4-6}. One study reported an alarming 34% incidence of central nervous system metastases in breast cancer patients treated with trastuzumab (Herceptin), a monoclonal antibody targeting the human epidermal growth factor receptor (HER2)¹. To facilitate an unbiased and systems level understanding of the transcriptional changes that accompany trastuzumab administration in patients with breast cancer, we mined published microarray and multiplexed gene expression data^{2,3} to describe the transcriptional landscape of trastuzumab-treated primary tumors of the breast. We identified the gene encoding the nerve growth factor receptor, NGFR, as among the genes whose expression changed most significantly when comparing the primary tumors and primary tumor-derived cells of the breast from patients receiving trastuzumab or not.

NGFR, as its name implies, is a receptor for the nerve growth factor, a central neurotrophic factor⁹⁻¹¹. Literature describes important functions for neurotrophic factors in metastasis to the brain¹². In melanoma and glioma, NGFR has been demonstrated to support invasion and metastasis¹³⁻¹⁶. We argue that transcriptional induction of NGFR is an undesirable and potentially dangerous transcriptional consequence of trastuzumab administration in patients with breast cancer.

References

1. 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.
2. Duchnowska, R., Jassem, J., Goswami, C.P., Dundar, M., Gökmen-Polar, Y., Li, L., Woditschka, S., Biernat, W., Sosińska-Mielcarek, K., Czartoryska-Arlukowicz, 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.
3. Popovici, V., Chen, W., Gallas, B.D., Hatzis, C., Shi, W., Samuelson, F.W., Nikolsky, Y., Tsyganova, M., Ishkin, A., Nikolskaya, T. and Hess, K.R., 2010. Effect of training-sample size and classification difficulty on the accuracy of genomic predictors. *Breast Cancer Research*, 12(1), p.R5.
4. Lin, N.U., Amiri-Kordestani, L., Palmieri, D., Liewehr, D.J. and Steeg, P.S., 2013. CNS metastases in breast cancer: old challenge, new frontiers.
5. Tsukada, Y., Fouad, A., Pickren, J.W. and Lane, W.W., 1983. Central nervous system metastasis from breast carcinoma autopsy study. *Cancer*, 52(12), pp.2349-2354.
6. Witzel, I., Laakmann, E., Weide, R., Neunhöffer, T., Park-Simon, T.J., Schmidt, M., Fasching, P.A., Hesse, T., Polasik, A., Mohrmann, S. and Würschmidt, F., 2018. Treatment and outcomes of patients in the Brain Metastases in Breast Cancer Network Registry. *European Journal of Cancer*, 102, pp. 1-9.
7. Herceptin (trastuzumab). Indications. <https://www.herceptin.com/hcp/indications.html>.
8. HERCEPTIN (trastuzumab) label. https://www.accessdata.fda.gov/drugsatfda_docs/label/2010/103792s5250lbl.pdf.
9. Lee, R., Kermani, P., Teng, K.K. and Hempstead, B.L., 2001. Regulation of cell survival by secreted proneurotrophins. *Science*, 294(5548), pp.1945-1948.
10. Rodriguez-Tebar, A., Dechant, G. and Barde, Y.A., 1990. Binding of brain-derived neurotrophic factor to the nerve growth factor receptor. *Neuron*, 4(4), pp.487-492.
11. Yamamoto, M., Sobue, G., Yamamoto, K. and Mitsuma, T., 1996. Expression of mRNAs for neurotrophic factors (NGF, BDNF, NT-3, and GDNF) and their receptors (p75 NGFR, TrkA, TrkB, and TrkC) in the adult human peripheral nervous system and nonneural tissues. *Neurochemical research*, 21(8), pp.929-938.

12. Menter, D.G., Herrmann, J.L. and Nicolson, G.L., 1995. The role of trophic factors and autocrine/paracrine growth factors in brain metastasis. *Clinical & experimental metastasis*, 13(2), pp.67-88.
13. Johnston, A.L., Lun, X., Rahn, J.J., Liacini, A., Wang, L., Hamilton, M.G., Parney, I.F., Hempstead, B.L., Robbins, S.M., Forsyth, P.A. and Senger, D.L., 2007. The p75 neurotrophin receptor is a central regulator of glioma invasion. *PLoS Biol*, 5(8), p.e212.
14. Marchetti, D., Aucoin, R., Blust, J., Murry, B. and Greiter-Wilke, A., 2004. p75 neurotrophin receptor functions as a survival receptor in brain-metastatic melanoma cells. *Journal of cellular biochemistry*, 91(1), pp.206-215.
15. Fabricant, R.N., De Larco, J.E. and Todaro, G.J., 1977. Nerve growth factor receptors on human melanoma cells in culture. *Proceedings of the National Academy of Sciences*, 74(2), pp.565-569.
16. Herrmann, J.L., Menter, D.G., Hamada, J.I., Marchetti, D., Nakajima, M. and Nicolson, G.L., 1993. Mediation of NGF-stimulated extracellular matrix invasion by the human melanoma low-affinity p75 neurotrophin receptor: melanoma p75 functions independently of trkA. *Molecular biology of the cell*, 4(11), pp.1205-1216.

Rank	ID	p-value	t	B	FC	Gene
202	GI_4505392-S-3	0.150938	1.45922	-4.42	1.60 ± 1.34	NGFR

Table 1: Differential expression of NGFR in primary tumors of 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 when comparing brain metastases to primary tumors of the breast, fold change in NGFR expression between primary tumors of the breast from patients treated with trastuzumab as compared to those not treated with trastuzumab, and gene symbol are listed in this chart.

Rank	ID	p-value	t	B	FC	Gene	Gene name
178	205858_at	9.03E-04	3.3563905	-0.7929	1.115 ± 0.331	NGFR	nerve growth factor receptor

Table 2: Differential expression of NGFR in tumor cells isolated by fine needle aspiration in 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 when comparing the tumor cells of patients treated with trastuzumab or not, fold change in NGFR expression between primary tumor-derived cells of the breast from patients treated with trastuzumab as compared to those not treated with trastuzumab, gene symbol and gene name are listed in this chart.

Figure 1: Transcriptional induction of NGFR in primary tumors of patients treated with trastuzumab.

Expression of NGFR at the messenger RNA level is graphically depicted here in primary tumors of the breast from patients not treated with trastuzumab (left: “No Tras”) and in the primary tumors of patients with breast cancer treated with trastuzumab (right: “Tras”) with mean NGFR mRNA level marked and the result of a statistical test evaluating significance of difference in NGFR expression between primary tumors of the breast in patients treated with trastuzumab or not, a *p*-value, listed above.

Figure 2: Transcriptional induction of NGFR in fine needle aspiration-isolated primary tumor cells of patients treated with trastuzumab.

Expression of NGFR at the messenger RNA level is graphically depicted here in primary tumor cells of the breast from patients not treated with trastuzumab (left: “No Tras”) and in the primary tumor cells of patients with breast cancer treated with trastuzumab (right: “Tras”) with mean NGFR mRNA level marked and the result of a statistical test evaluating significance of difference in NGFR expression between primary tumor cells in breast cancer patients treated with trastuzumab or not, a *p*-value, listed above.