Trastuzumab administration in patients with breast cancer is associated with increased primary tumor expression of STAT3.

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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 cell signaling intermediate signal transducer and activator of transcription STAT3 was among the genes most differentially expressed in the primary tumors of patients treated with trastuzumab. STAT3 was expressed at significantly higher levels in the tumors of patients treated with trastuzumab, indicating that trastuzumab is potentially associated with activation of a signal transduction pathway important for survival of breast cancer cells, and demonstrating that a molecule described as an oncogene⁵ with constitutive expression in all cell lines transformed by the Src proto-oncogene⁶ is present at significantly higher quantities in the tumors of breast cancer patients treated with trastuzumab.

Keywords: breast cancer, trastuzumab, STAT3, 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 up-regulation of STAT3 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 $n=6$ primary breast tumors from patients treated with induction chemotherapy and/or hormone therapy without trastuzumab and $n=35$ primary breast tumors from patients treated with induction chemotherapy and/or hormone therapy in addition to trastuzumab, both of which developed brain metastases; 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 STAT3 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

STAT3 is differentially expressed in the primary tumors of patients treated with trastuzumab.

We found that STAT3 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 trastuzumab-treated breast tumors based on significance of difference in expression as compared to the primary breast tumors of patients not treated with trastuzumab, STAT3 ranked 39 out of 1506 total transcripts, equating to 97.4% statistical significance (Table 1). Differential expression of STAT3 in the primary tumors of breast cancer patients treated with trastuzumab was statistically significant (Table 1; $p=0.03848$).

We queried a second dataset⁴ to validate our findings of STAT3 differential expression upon trastuzumab treatment. In a second microarray dataset analyzing gene expression of patients treated with trastuzumab, we again found that STAT3 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, STAT3 ranked 1918 out

of 41000 total transcripts, equating to 95.3% differential expression. Differential expression of STAT3 in the primary tumors of patients treated with trastuzumab was statistically significant (Table 2; $p=5.71E-20$).

STAT3 is expressed at higher levels in the tumors of patients treated with trastuzumab.

We obtained exact mRNA expression levels for STAT3 from the tumors of patients treated with trastuzumab and from the tumors of patients not receiving trastuzumab to determine magnitude and direction of STAT3 change in the tumors of patients treated with trastuzumab. STAT3 expression was significantly higher in the tumors of patients treated with trastuzumab as compared to the tumors of patients not treated with trastuzumab (Figure 1). Increased expression of STAT3 was statistically significant (Figure 1: $p=0.0034$). We calculated a mean fold change of 1.30 ± 0.31 when comparing the expression of STAT3 in the tumors of patients treated with trastuzumab as compared to the tumors of patients treated with trastuzumab.

Thus, we found using published microarray and gene expression data^{3,4} that the signal transduction intermediate STAT3 was among the genes most differentially expressed in the primary tumors of patients with breast cancer and that STAT3 expression was significantly higher in the primary tumors of patients treated with trastuzumab as compared to those not treated.

Discussion

Signal transducers and activators of transcription, or STAT molecules, are pathway intermediates that relay signals transduced at the plasma membrane from receptors of multiple cytokines and growth factors⁸ including the epidermal growth factor⁹ and interleukin-6 (IL-6)¹⁰, resulting in dimerization upon phosphorylation and subsequent translocation to the nucleus where they can act as transcriptional activators of ligand-induced gene expression⁸. A STAT3 mutant that has the ability to remain as a constitutive dimer is capable of driving transformation, demonstrating that STAT3 can function as a proto-oncogene⁸. Depletion of either STAT3 or IL-6 significantly impaired growth of ALDH+ CD133+ HCT116 cells colon cancer cells in mouse xenografts *in vivo*, demonstrating that STAT3 is important for tumor growth in at least one animal model of cancer¹¹. Moreover, administration of a chemical inhibitor of the Janus kinase, AG 490, and expression of a dominant negative Stat3 mutant could both impair cell proliferation and induce apoptotic cell death in MDA-MB-468 breast cancer cells expressing constitutively active Stat3 *in vitro*¹². Aside from its role in transducing growth factor and cytokine signals from the plasma membrane to the nucleus, STAT3 also has a role in cellular metabolism, as it can be found localized to the mitochondria, with cells lacking STAT3 deficient in their capacity to carry out the electron transport chain in both complex I and complex II¹³.

We provided evidence here that STAT3, a gene with oncogenic functions as a relay of multiple lines of growth factor and cytokine signals, is expressed at significantly higher 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. Increased primary tumor expression of STAT3 in patients with breast cancer following treatment with trastuzumab should be viewed as an undesirable consequence of the use of a therapeutic aimed at impairing⁷, and not supporting^{9,11,12} tumor cell proliferation.

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-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.
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>. Hsiaowang Chen. UCLA, Department of Medicine. Santa Monica, CA.
5. Bromberg, J.F., Wrzeszczynska, M.H., Devgan, G., Zhao, Y., Pestell, R.G., Albanese, C. and Darnell Jr, J.E., 1999. Stat3 as an oncogene. *Cell*, 98(3), pp.295-303.
6. Yu, C.L., Meyer, D.J., Campbell, G.S., Lerner, A.C., Carter-Su, C., Schwartz, J. and Jove, R., 1995. Enhanced DNA-binding activity of a Stat3-related protein in cells transformed by the Src oncoprotein. *Science*, 269(5220), pp.81-83.
7. Herceptin. Treating HER2+ cancer. <https://www.herceptin.com/hcp/treating-HER2-cancer.html>.
8. Darnell, J.E., 1997. STATs and gene regulation. *Science*, 277(5332), pp.1630-1635.
9. Grandis, J.R., Drenning, S.D., Chakraborty, A., Zhou, M.Y., Zeng, Q., Pitt, A.S. and Tweardy, D.J., 1998. Requirement of Stat3 but not Stat1 activation for epidermal growth factor receptor-mediated cell growth in vitro. *The Journal of clinical investigation*, 102(7), pp.1385-1392.
10. Wang, Y., van Boxel-Dezaire, A.H., Cheon, H., Yang, J. and Stark, G.R., 2013. STAT3 activation in response to IL-6 is prolonged by the binding of IL-6 receptor to EGF receptor. *Proceedings of the National Academy of Sciences*, 110(42), pp.16975-16980.
11. Lin, L., Liu, A., Peng, Z., Lin, H.J., Li, P.K., Li, C. and Lin, J., 2011. STAT3 is necessary for proliferation and survival in colon cancer-initiating cells. *Cancer research*, 71(23), pp.7226-7237.
12. Burke, W.M., Jin, X., Lin, H.J., Huang, M., Liu, R., Reynolds, R.K. and Lin, J., 2001. Inhibition of constitutively active Stat3 suppresses growth of human ovarian and breast cancer cells. *Oncogene*, 20(55), pp.7925-7934.
13. Wegrzyn, J., Potla, R., Chwae, Y.J., Sepuri, N.B., Zhang, Q., Koeck, T., Derecka, M., Szczepanek, K., Szlag, M., Gornicka, A. and Moh, A., 2009. Function of mitochondrial Stat3 in cellular respiration. *Science*, 323(5915), pp.793-797.

Rank	ID	p-value	t	B	FC	Gene
39	GI_21618337-A-3	0.03848	2.135346	-4.32	1.30 ± 0.31	STAT3

Table 1: STAT3 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 STAT3 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
1918	A_23_P107206	5.71E-20	10.214654	34.43235	STAT3	signal transducer and activator of transcription 3

Table 2: STAT3 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 STAT3 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: STAT3 is expressed at significantly higher levels in the primary tumors of patients treated with trastuzumab as compared to those not treated with trastuzumab.

The mRNA expression level of STAT3 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 STAT3 expression in the primary tumors of patients treated with trastuzumab or not, a *p*-value, listed above.