IGF2 is differentially expressed in the tumors of breast cancer patients treated with trastuzumab.

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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 and gene expression data^{3,4} to discover in an unbiased manner the most significant transcriptional changes associated with trastuzumab treatment. We identified the growth factor IGF2 as 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 significantly higher levels of IGF2 messenger RNA than did patients not treated with trastuzumab, and a single administration of trastuzumab was sufficient to result in differential expression of IGF2 in primary tumors of the breast, demonstrating increased expression of a growth factor encoded at a paternally imprinted locus⁵ as a direct transcriptional result of treatment with trastuzumab.

Keywords: trastuzumab, breast cancer, IGF2, insulin-like growth factor 2, systems biology of breast cancer, targeted therapeutics in breast cancer.

1 The mechanism of action ascribed to trastuzumab function is thought to be binding of the F_{ab}
2 domain of trastuzumab to the extracellular region of the human epidermal growth factor receptor 2
3 (HER2)¹, but the transcriptional consequences resulting from signaling downstream of trastuzumab
4 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 IGF2 is a differentially
expressed gene in the tumors of trastuzumab-treated patients: patients treated with trastuzumab expressed
significantly higher levels of IGF2 than did those of patients not treated with trastuzumab.

5 **Methods**

6 We used datasets GSE38057³ and GSE114082⁴ this differential gene expression analysis of the
7 transcriptional response to trastuzumab treatment in patients with breast cancer. GSE38057 was
8 generated using DASL Human Cancer Panel by Probe technology with $n=11$ primary breast tumors from
9 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; patients in both groups compared had not developed brain metastases at time of
10 data collection by investigators; analysis was performed using platform GPL5826. GSE114082 was
11 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
12 after trastuzumab treatment, analysis performed using platform GPL14951. The Benjamini and Hochberg
13 method of p -value adjustment was used for ranking of differential expression but raw p -values were used
14 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
15 test was performed to evaluate whether IGF2 gene expression was significantly between primary breast
tumors of patients receiving trastuzumab using a two-tailed, unpaired t-test with Welch's correction. We
16 used PRISM for all statistical analyses of differential gene expression in human breast cancer (Version
8.4.0)(455).

17 **Results**

18 We performed unbiased comparative transcriptome analysis using published microarray and gene
19 expression data^{3,4} to identify the most significant transcriptional features of the primary breast tumors of
patients treated with trastuzumab (Herceptin).

20 **IGF2 is differentially expressed in the tumors of patients treated with trastuzumab.**

21 We identified the cell adhesion molecule IGF2 as among the genes whose expression changed
22 most significantly in the tumors of breast cancer patients treated with trastuzumab in a multiplexed gene
expression dataset³ (Table 1). When sorting each of the genes expressed in the primary tumors of breast
23 cancer patients treated with trastuzumab based on significance of change as compared to that of patients
not treated with trastuzumab, IGF2 ranked 25 out of 1506 total transcripts (Table 1), equating to 98.3%
24 differential expression. IGF2 differential expression in the tumors of patients treated with trastuzumab
was statistically significant (Table 1; $p=0.01773$).

25 The first dataset, although measuring the expression of over 1500 genes, was still limited. We
26 turned to a second microarray dataset⁴, this measuring the expression of genes across the transcriptome -
29377 genes in total. The tumors compared in this dataset were sampled from the same patient before
27 and after a single, brief exposure to trastuzumab, not comparing tumors from different patients treated
28 with a course of trastuzumab or not. Thus, while differentially expressed genes when comparing tumors

1 based on trastuzumab treatment can result from a variety of sources, including gene expression changes
2 resulting from tumor evolution over the course of treatment, changes identified in this dataset, occurring
3 after only a single treatment of trastuzumab, are more likely to be a direct transcriptional result of
4 trastuzumab binding events. We found again that IGF2 was among the genes most differentially
5 expressed in the tumors of patients administered a single treatment of trastuzumab (Table 2). When
6 sorting each of the genes expressed in the tumors of patients administered a single treatment of
7 trastuzumab, IGF2 ranked 7607 out of 29377 total transcripts (Table 2), equating to 74.1% differential
8 expression. Differential expression of IGF2 in the primary tumors of breast cancer patients following
9 treatment with trastuzumab was statistically significant (Table 2; $p=2.26E-02$).

10 **IGF2 is expressed at significantly higher levels in the tumors of breast cancer patients**
11 **treated with trastuzumab than in the tumors of those not treated with trastuzumab.**

12 We obtained exact mRNA expression levels for IGF2 from tumors of patients treated with
13 trastuzumab and from the tumors of patients not treated with trastuzumab to directly evaluate magnitude
14 and change of expression in IGF2 based on history of trastuzumab treatment. IGF2 was expressed at
15 higher levels in the tumors of patients treated with trastuzumab as compared to those of patients not
16 treated with trastuzumab, and this difference was deemed statistically significant (Figure 1; $p=0.0372$).
17 We calculated a mean fold change of 1.57 ± 0.81 in IGF2 expression in the primary tumors of patients
18 treated with trastuzumab relative to that of patients not treated with trastuzumab.

19 Thus, we found using published multiplexed gene expression and microarray data^{3,4} that IGF2
20 was among the genes most differentially expressed in the tumors of patients treated with trastuzumab, that
21 IGF2 was expressed at higher levels in the tumors of patients treated with trastuzumab as compared to
22 those not treated with trastuzumab, and that this transcriptional change was likely a direct result of
23 trastuzumab treatment as opposed to a gene expression change associated with clonal selection and
24 evolution of the tumor across the course of treatment.

25 **Discussion**

26 We provided evidence here that the insulin-like growth factor IGF2 is a differentially expressed
27 gene in the tumors of patients treated with trastuzumab, and that the primary tumors of breast cancer
28 patients treated with trastuzumab expressed higher levels of IGF2 than did patients not treated with
trastuzumab. IGF2 is a paternally imprinted locus⁵, and in general, loss of imprinting results in increased
gene expression at that locus; in a murine model of colorectal cancer, loss of imprinting at the IGF2 locus
resulted in the development of twice as many tumors as control littermates⁶. Significantly, IGF2 serum
levels correlate with tumor size in patients with breast cancer⁷. These data demonstrate that a growth
factor whose increased expression results in marked increases in tumor development in animal models
and whose expression in the serum of humans with breast cancer is associated with size of the tumor is
likely directly transcriptionally induced by treatment with trastuzumab in patients with breast cancer.

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. 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. 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. Giannoukakis, N., Deal, C., Paquette, J., Goodyer, C.G. and Polychronakos, C., 1993. Parental genomic imprinting of the human IGF2 gene. *Nature genetics*, 4(1), pp.98-101.
6. Sakatani, T., Kaneda, A., Iacobuzio-Donahue, C.A., Carter, M.G., de Boor Witzel, S., Okano, H., Ko, M.S., Ohlsson, R., Longo, D.L. and Feinberg, A.P., 2005. Loss of imprinting of Igf2 alters intestinal maturation and tumorigenesis in mice. *Science*, 307(5717), pp.1976-1978.
7. Singer, C.F., Mogg, M., Koestler, W., Pacher, M., Marton, E., Kubista, E. and Schreiber, M., 2004. Insulin-like growth factor (IGF)-I and IGF-II serum concentrations in patients with benign and malignant breast lesions: free IGF-II is correlated with breast cancer size. *Clinical Cancer Research*, 10(12), pp.4003-4009.

Rank	ID	p-value	t	B	FC	Gene
25	GI_6453816-S-1	0.01773	2.4547	-3.52	1.57 ± 0.81	IGF2

Table 1: IGF2 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 IGF2 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
7607	ILMN_2413956	2.26E-02	2.3834723	-3.68602	IGF2

Table 2: A single exposure to trastuzumab is sufficient to drive differential expression of IGF2 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: IGF2 is expressed at significantly higher levels in the primary tumors of patients treated with trastuzumab.

The mRNA expression level of IGF2 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 IGF2 expression in the primary tumors of patients treated with trastuzumab or not, a *p*-value, listed above.