E2F5 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 striking transcriptional features of trastuzumab treatment. We identified the transcription factor E2F5⁵ 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 higher levels of E2F5 messenger RNA than did patients not treated with trastuzumab, and a single administration of trastuzumab was sufficient to result in differential expression of E2F5 in primary tumors of the breast, demonstrating that a transcription factor important for progression of the cell cycle into the S-phase is likely transcriptionally induced by trastuzumab.

Keywords: trastuzumab, breast cancer, E2F5, 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
5 analysis of published microarray and gene expression data^{3,4}, we report here that E2F5 is a differentially
6 expressed gene in the tumors of trastuzumab-treated patients: patients treated with trastuzumab expressed
7 significantly higher levels of E2F5 than did those of patients not treated with trastuzumab.

8 **Methods**

9 We used datasets GSE38057³ and GSE114082⁴ this differential gene expression analysis of the
10 transcriptional response to trastuzumab treatment in patients with breast cancer. GSE38057 was
11 generated using DASL Human Cancer Panel by Probe technology with *n*=6 primary breast tumors from
12 patients treated with induction chemotherapy and/or hormone therapy without trastuzumab and *n*=35
13 primary breast tumors from patients treated with induction chemotherapy and/or hormone therapy in
14 addition to trastuzumab, both of which developed brain metastases; analysis was performed using
15 platform GPL5826. GSE114082 was generated using Illumina HumanHT-12 WG-DASL V4.0 R2
16 expression beadchip technology with *n*=17 primary breast tumors from patients before trastuzumab
17 treatment and *n*=17 primary tumors from patients after trastuzumab treatment, analysis performed using
18 platform GPL14951. The Benjamini and Hochberg method of *p*-value adjustment was used for ranking
19 of differential expression but raw *p*-values were used to assess statistical significance of global or
20 multiplexed differential expression. Log-transformation of data was auto-detected, and the NCBI
21 generated category of platform annotation was used. A statistical test was performed to evaluate whether
22 E2F5 gene expression was significantly between primary breast tumors of patients receiving trastuzumab
23 using a two-tailed, unpaired t-test with Welch's correction. We used PRISM for all statistical analyses of
24 differential gene expression in human breast cancer (Version 8.4.0)(455).

25 **Results**

26 We harnessed the power of blind, comparative transcriptome analysis using published microarray
27 and gene expression data^{3,4} to identify the most significant transcriptional features of the primary breast
28 tumors of patients treated with trastuzumab (Herceptin).

29 **E2F5 is differentially expressed in the tumors of patients treated with trastuzumab.**

30 We identified the transcription factor E2F5 as among the genes whose expression changed most
31 significantly in the tumors of breast cancer patients treated with trastuzumab in a multiplexed gene
32 expression dataset³ (Table 1). When sorting each of the genes expressed in the primary tumors of breast
33 cancer patients treated with trastuzumab based on significance of change as compared to that of patients
34 not treated with trastuzumab, E2F5 ranked 26 out of 1506 total transcripts (Table 1), equating to 98.3%
35 differential expression. E2F5 differential expression in the tumors of patients treated with trastuzumab
36 was statistically significant (Table 1; *p*=0.02897).

37 The first dataset, although measuring the expression of over 1500 genes, was still limited. We
38 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 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 in this dataset, occurring after only a single treatment of trastuzumab, are more likely to be a direct transcriptional result of trastuzumab binding events. We found again that E2F5 was among the genes most differentially expressed in the tumors of patients administered a single treatment of trastuzumab (Table 2). When sorting each of the genes expressed in the tumors of patients administered a single treatment of trastuzumab, E2F5 ranked 6060 out of 29377 total transcripts (Table 2), equating to 79.4% differential expression. Differential expression of E2F5 in the primary tumors of breast cancer patients following treatment with trastuzumab was statistically significant (Table 2; $p=1.15E-02$).

E2F5 is expressed at higher levels in the tumors of breast cancer patients treated with trastuzumab than in the tumors of those not treated with trastuzumab.

We obtained exact mRNA expression levels for E2F5 from tumors of patients treated with trastuzumab and from the tumors of patients not treated with trastuzumab to directly evaluate magnitude and change of expression in E2F5 based on history of trastuzumab treatment. E2F5 was expressed at higher levels in the tumors of patients treated with trastuzumab as compared to those of patients not treated with trastuzumab, but this difference was not deemed statistically significant (Figure 1; $p=0.02897$). We calculated a mean fold change of 1.42 ± 1.29 in E2F5 expression in the primary tumors of patients treated with trastuzumab relative to that of patients not treated with trastuzumab.

Thus, we found using published multiplexed gene expression and microarray data^{3,4} that E2F5 was among the genes most differentially expressed in the tumors of patients treated with trastuzumab, that E2F5 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.

Discussion

Depletion of E2F5 induces cell death in breast cancer cells⁷. We provided evidence here that E2F5, a transcription factor with the capacity to transactivate the expression of a host of genes important for entry into the S-phase as cells progress through G1, is a differentially expressed gene in the tumors of patients treated with trastuzumab, and that the primary tumors of breast cancer patients treated with trastuzumab expressed higher levels of E2F5 in their primary tumors than did patients not treated with trastuzumab.

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. Gaubatz, S., Lindeman, G.J., Ishida, S., Jakoi, L., Nevins, J.R., Livingston, D.M. and Rempel, R.E., 2000. E2F4 and E2F5 play an essential role in pocket protein-mediated G1 control. *Molecular cell*, 6(3), pp.729-735.
6. Polanowska, J., Le Cam, L., Orsetti, B., Vallés, H., Fabbrizio, E., Fajas, L., Taviaux, S., Theillet, C. and Sardet, C., 2000. Human E2F5 gene is oncogenic in primary rodent cells and is amplified in human breast tumors. *Genes, Chromosomes and Cancer*, 28(1), pp.126-130.
7. Inagaki, Y., Wu, D., Fujiwara, K., Ishizuka, Y., Oguni, A., Tokunaga, T., Takayama, T., Soma, M., Fukuda, N., Ozaki, T. and Masuda, S., 2020. Knockdown of E2F5 induces cell death via the TP53-dependent pathway in breast cancer cells carrying wild-type TP53. *Oncology Reports*, 44(5), pp.2241-2252.

Rank	ID	p-value	t	B	FC	Gene
26	GI_12669916-S-3	0.02897	2.259685	-4.28	1.42 ± 1.29	E2F5

Table 1: E2F5 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 E2F5 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
6060	ILMN_1782551	1.15E-02	-2.662399	-3.09573	E2F5

Table 2: A single exposure to trastuzumab is sufficient to drive differential expression of E2F5 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: E2F5 is expressed at higher levels in the primary tumors of patients treated with trastuzumab.

The mRNA expression level of E2F5 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 E2F5 expression in the primary tumors of patients treated with trastuzumab or not, a *p*-value, listed above.