YES1 tyrosine kinase is differentially expressed in the primary tumors of breast cancer patients treated with trastuzumab.

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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 tyrosine kinase YES1 was among the genes most differentially expressed in the primary tumors of patients treated with trastuzumab, and expressed at lower levels in the tumors of patients treated with trastuzumab. Thus, the use of trastuzumab in patients with breast cancer is associated with increased expression of a proto-oncogenic tyrosine kinase⁵ that can support growth and metastasis of lung cancer cells *in vitro* and *in vivo*⁶.

Keywords: breast cancer, trastuzumab, YES1, systems biology of breast cancer, targeted therapeutics in breast cancer.

1 The mechanism of action by which trastuzumab exerts its therapeutic function in human breast
2 cancer is said to be binding of the F_{ab} domain of trastuzumab to the extracellular region of the human
3 epidermal growth factor receptor 2 (HER2)², possibly resulting in decreased cellular proliferation⁷.
4 Transcriptional modulation resulting from trastuzumab binding events in the primary tumors of treated
5 patients is less well understood. We mined published and public microarray and gene expression data^{3,4}
6 generated using primary tissues from patients treated with trastuzumab to compare tumor global gene
7 expression with that of patients not treated with trastuzumab. Using this unbiased, systems level
8 methodology, we identified differential expression and up-regulation of YES1 in primary tumors of the
9 breast in patients treated with trastuzumab.

10 **Methods**

11 We used datasets GSE38057³ and GSE130788⁴ for this differential gene expression analysis of
12 the transcriptional response to trastuzumab treatment in patients with breast cancer. GSE38057 was
13 generated using DASL Human Cancer Panel by Probe technology with $n=11$ primary breast tumors from
14 patients treated with induction chemotherapy and/or hormone therapy without trastuzumab and $n=36$
15 primary breast tumors from patients treated with induction chemotherapy and/or hormone therapy in
16 addition to trastuzumab; analysis was performed using platform GPL5826. GSE130788 was generated
17 using Agilent-014850 Whole Human Genome Microarray 4x44K G4112F technology with $n=11$ primary
18 breast tumors from patients treated with trastuzumab and $n=36$ primary tumors from patients treated with
19 trastuzumab, analysis performed using platform GPL6480. The Benjamini and Hochberg method of p -
20 value adjustment was used for ranking of differential expression but raw p -values were used to assess
21 statistical significance of global or multiplexed differential expression. Log-transformation of data was
22 auto-detected, and the NCBI generated category of platform annotation was used. A statistical test was
23 performed to evaluate whether YES1 gene expression was significantly between primary breast tumors of
24 patients receiving trastuzumab using a two-tailed, unpaired t-test with Welch's correction. We used
25 PRISM for all statistical analyses of differential gene expression in human breast cancer (Version 8.4.0)
26 (455).

27 **Results**

28 We performed comparative transcriptome analysis of primary tumors from patients treated or not
with trastuzumab (Herceptin), using published multiplexed gene expression data³ and a microarray
dataset⁴, in order to identify the most significant transcriptional changes in the primary tumors of breast
cancer patients following treatment with trastuzumab.

29 **YES1 is differentially expressed in the primary tumors of patients treated with trastuzumab.**

30 We found that the tyrosine kinase YES1 (Yamaguchi sarcoma viral oncogene homolog 1) was
31 among the genes whose expression was most significantly different in the primary tumors of patients with
32 breast cancer treated with trastuzumab³. When sorting each of the genes expressed in the primary tumors
33 of breast cancer patients treated with trastuzumab based on significance of difference in expression as
34 compared to the primary tumors of breast cancer patients not treated with trastuzumab, YES1 ranked 88
35 out of 1506 total transcripts, equating to 94.2% statistical significance (Table 1). Differential expression
36 of YES1 in the primary tumors of breast cancer patients treated with trastuzumab approached the level of
37 statistical significance (Table 1; $p=0.069818$).

38 We queried a second dataset⁶ to validate our findings of YES1 differential expression upon
trastuzumab treatment. In a second microarray dataset analyzing gene expression of patients treated with

trastuzumab, we again found that YES1 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, YES1 ranked 15147 out of 41000 total transcripts, equating to 63.1% differential expression. Differential expression of YES1 in the primary tumors of patients treated with trastuzumab was statistically significant (Table 2; $p=7.86E-05$).

YES1 is expressed at higher levels in the tumors of patients treated with trastuzumab.

We obtained exact mRNA expression levels for YES1 from the tumors of patients treated with trastuzumab and from the tumors of patients not receiving trastuzumab to determine magnitude and direction of YES1 change in the tumors of patients treated with trastuzumab. YES1 expression was 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 YES1 was statistically significant (Figure 1: $p=0.0089$). We calculated a mean fold change of 1.20 ± 0.33 when comparing the expression of YES1 in the tumors of patients treated with trastuzumab as compared to the tumors of patients treated with trastuzumab.

Thus, we found that the tyrosine kinase YES1 was among the genes most differentially expressed in the primary tumors of patients with breast cancer and that YES1 expression was significantly higher in the primary tumors of patients treated with trastuzumab as compared to those not treated.

Discussion

We provided evidence here that the tyrosine kinase YES1 is expressed at 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. Thus, trastuzumab administration in patients with breast cancer results in increased primary tumor expression of a proto-oncogenic tyrosine kinase⁵ with the capacity to fuel the growth and metastasis of lung cancer cells *in vitro* and *in vivo*⁶.

References

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Rank	ID	p-value	t	B	FC	Gene
88	GI_21071041-S-5	0.069818	1.85397	-4.1	1.20 ± 0.33	YES1

Table 1: YES1 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 YES1 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
15147	A_23_P164507	7.86E-05	4.032847	0.225829	YES1	YES proto-oncogene 1, Src family tyrosine kinase

Table 2: YES1 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 YES1 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: YES1 is expressed at higher levels in the primary tumors of patients treated with trastuzumab as compared to those not treated with trastuzumab.

The mRNA expression level of YES1 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 YES1 expression in the primary tumors of patients treated with trastuzumab or not, a *p*-value, listed above.