DCC 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 deleted in colorectal cancer locus DCC^{5,6} 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 DCC messenger RNA than did patients not treated with trastuzumab, and a single administration of trastuzumab was sufficient to result in differential expression of DCC in primary tumors of the breast, demonstrating that a gene encoding for a netrin receptor, cellular machinery utilized for axon guidance in the central nervous system⁵⁻⁹, is transcriptionally induced in primary tumors of the breast following treatment with trastuzumab.

Keywords: trastuzumab, breast cancer, DCC, deleted in colorectal cancer, 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 DCC is a differentially
expressed gene in the tumors of trastuzumab-treated patients: patients treated with trastuzumab expressed
significantly higher levels of DCC 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 $n=6$ primary breast tumors from
9 patients treated with induction chemotherapy and/or hormone therapy without trastuzumab and $n=35$
10 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. GSE114082 was generated using Illumina HumanHT-12 WG-DASL V4.0 R2
11 expression beadchip technology with $n=17$ primary breast tumors from patients before trastuzumab
treatment and $n=17$ primary tumors from patients after trastuzumab treatment, analysis performed using
platform GPL14951. The Benjamini and Hochberg method of p -value adjustment was used for ranking
12 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
13 generated category of platform annotation was used. A statistical test was performed to evaluate whether
DCC gene expression was significantly between primary breast tumors of patients receiving trastuzumab
14 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).

16 **Results**

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

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

21 We identified the locus deleted in colorectal cancer, DCC, as among the genes whose expression
22 changed 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
23 tumors of breast cancer patients treated with trastuzumab based on significance of change as compared to
that of patients not treated with trastuzumab, DCC ranked 34 out of 1506 total transcripts (Table 1),
24 equating to 97.7% differential expression. DCC differential expression in the tumors of patients treated
with trastuzumab was statistically significant (Table 1; $p=0.03525$).

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 and
27 after a single, brief exposure to trastuzumab, not comparing tumors from different patients treated with a
28

1 course of trastuzumab or not. Thus, while differentially expressed genes when comparing tumors based
2 on trastuzumab treatment can result from a variety of sources, including gene expression changes
3 resulting from tumor evolution over the course of treatment, changes identified in this dataset, occurring
4 after only a single treatment of trastuzumab, are more likely to be a direct transcriptional result of
5 trastuzumab binding events. We found again that DCC was among the genes most differentially
6 expressed in the tumors of patients administered a single treatment of trastuzumab (Table 2). When
7 sorting each of the genes expressed in the tumors of patients administered a single treatment of
8 trastuzumab, DCC ranked 1302 out of 29377 total transcripts (Table 2), equating to 95.6% differential
9 expression. Differential expression of DCC in the primary tumors of breast cancer patients following
10 treatment with trastuzumab was statistically significant (Table 2; $p=1.4E-04$).

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

13 We obtained exact mRNA expression levels for DCC from tumors of patients treated with
14 trastuzumab and from the tumors of patients not treated with trastuzumab to directly evaluate magnitude
15 and change of expression in DCC based on history of trastuzumab treatment. DCC was expressed at
16 higher levels in the tumors of patients treated with trastuzumab as compared to those of patients not
17 treated with trastuzumab, and this difference was statistically significant (Figure 1; $p=0.0431$). We
18 calculated a mean fold change of 2.39 ± 0.89 in DCC expression in the primary tumors of patients treated
19 with trastuzumab relative to that of patients not treated with trastuzumab.

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

26 **Discussion**

27 We provided evidence here that deleted in colorectal cancer, DCC, is a differentially expressed
28 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 DCC in their primary tumors than did
patients not treated with trastuzumab. Thus, transcriptional induction in primary tumors of the breast of a
gene which encodes a netrin receptor, cellular machinery utilized for the guidance of axons as they
migrate and navigate the space of the brain, is a direct consequence of administration of trastuzumab in
patients with breast cancer.

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Rank	ID	p-value	t	B	FC	Gene
34	GI_4885174-S-4	0.03525	2.174127	-4.31	2.39 ± 0.89	DCC

Table 1: DCC 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 DCC 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
1302	ILMN_1665748	1.4E-04	4.2625578	0.94834	DCC

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

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