DVL3 is over-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 and public microarray and gene expression data^{3,4} to discover in an unbiased manner the most striking transcriptional features of trastuzumab treatment. We identified significant differential expression of the gene encoding the Wnt pathway molecule dishevelled 3, DVL3⁵⁻⁷, in the primary tumors of breast cancer patients treated with trastuzumab. DVL3 expression in primary tumors of the breast in patients treated with trastuzumab was significantly higher than in patients not treated with trastuzumab.

Keywords: trastuzumab, breast cancer, DVL3, dishevelled-3, systems biology of breast cancer, targeted therapeutics in breast cancer.

1 The mechanism of action ascribed to trastuzumab in human breast cancer is thought to be binding
2 of the F_{ab} domain of trastuzumab to the extracellular region of the human epidermal growth factor
3 receptor 2 (HER2)¹, but the transcriptional changes that accompany these binding events in the primary
4 tumors of treated patients is less well understood. We used unbiased, systems-level comparative
5 transcriptome analysis of published microarray and gene expression data^{3,4}, we report here that DVL3 is
6 among the genes most differentially expressed in the tumors of trastuzumab-treated patients: patients
7 treated with trastuzumab expressed significantly higher levels of DVL3 than did those of patients not
8 treated with trastuzumab.

9 **Methods**

10 We used datasets GSE38057³ and GSE22226⁴ this differential gene expression analysis of the
11 transcriptional response to trastuzumab treatment in patients with breast cancer. GSE38057 was
12 generated using DASL Human Cancer Panel by Probe technology with either $n=11$ primary breast tumors
13 from patients treated with induction chemotherapy and/or hormone therapy without trastuzumab and
14 $n=36$ primary breast tumors from patients treated with induction chemotherapy and/or hormone therapy in
15 addition to trastuzumab; analysis was performed using platform GPL5826. GSE22226 was generated
16 using Whole Human Genome Oligo Microarray G4112A technology with $n=118$ primary breast tumors
17 from patients treated with chemotherapy and $n=11$ primary tumors from patients treated with
18 chemotherapy and trastuzumab, analysis performed using platform GPL1708. The Benjamini and
19 Hochberg method of p -value adjustment was used for ranking of differential expression but raw p -values
20 were used to assess statistical significance of global or multiplexed differential expression. Log-
21 transformation of data was auto-detected, and the NCBI generated category of platform annotation was
22 used. A statistical test was performed to evaluate whether DVL3 gene expression was significantly
23 between primary breast tumors of patients receiving trastuzumab using a two-tailed, unpaired t-test with
24 Welch's correction. We used PRISM for all statistical analyses of differential gene expression in human
25 breast cancer (Version 8.4.0)(455).

26 **Results**

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

29 **DVL3 is differentially expressed in the tumors of patients treated with trastuzumab.**

30 We identified the gene encoding dishevelled-3 (DVL3) as among the genes whose expression
31 changed most significantly in the tumors of breast cancer patients treated with trastuzumab³ (Table 1).
32 When sorting each of the genes expressed in the primary tumors of breast cancer patients treated with
33 trastuzumab based on significance of change as compared to that of patients not treated with trastuzumab,
34 DVL3 ranked 15 out of 1506 total transcripts (Table 1), equating to 99.0% differential expression. DVL3
35 differential expression in the tumors of patients treated with trastuzumab was statistically significant
36 (Table 1; $p=0.010726$).

37 We mined a second source of published data, this a microarray dataset, to validate differential

expression of DVL3 in the primary tumors of breast cancer patients treated with trastuzumab⁴. We again observed differential expression of DVL3 in the primary tumors of breast cancer patients treated with trastuzumab as compared to that of patients not treated with trastuzumab (Table 2). When sorting each of the genes expressed in the tumors of patients treated with trastuzumab, DVL3 ranked 898 out of 44290 total transcripts (Table 2), equating to 98.0% differential expression. Differential expression of DVL3 in the primary tumors of breast cancer patients treated with trastuzumab was statistically significant (Table 2; $p=7.56E-03$).

DVL3 is expressed at significantly 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 DVL3 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 DVL3 based on history of trastuzumab treatment. DVL3 was expressed at significantly higher levels in the tumors of patients treated with trastuzumab as compared to those of patients not treated with trastuzumab (Figure 1; $p<0.0001$). We calculated a mean fold change of 1.25 ± 0.26 in DVL3 expression in the primary tumors of patients treated with trastuzumab relative to that of patients not treated with trastuzumab.

Thus, we found that DVL3 was among the genes most differentially expressed in the tumors of patients treated with trastuzumab, that DVL3 was expressed at significantly higher levels in the tumors of patients treated with trastuzumab as compared to those not treated with trastuzumab.

Discussion

Dishevelled-3 functions as a scaffolding protein in the Wnt signaling pathway, serving to form supermolecular complexes with the Wnt ligand Wnt3a⁵. Wnt3a, in conjunction with Axin and Dvl3, functionally support transduction of Wnt pathways signals through inhibition of Axin destruction complexes which, when not inhibited, prevent nuclear translocation of β -catenin and its cooperation with TCF/LEF transcriptional proteins by targeting cytoplasmic β -catenin for degradation⁵⁻⁷. In mice, DVL3 is expressed in the brain, heart and ovaries⁸, and in the nervous system of the mouse, dishevelled-3 is important for development of the neural tube and the cochlea⁹. In human cancer, brain metastases from patients with lung cancer express increased levels of DVL3¹⁰ and one report described association of DVL3 with invasion and migration in hepatocellular carcinoma¹¹. While disheveled proteins are otherwise described as scaffolding-type proteins that link Frizzled receptors in the Wnt pathway to intracellular components for the transduction of β -catenin/TCF/LEF signaling, one report described a nuclear function of DVL3, occupying the promoters at the CYP19A1 locus, influencing the expression of aromatase transcripts and of estradiol in the MCF-7 breast cancer cell line¹².

We provided evidence here that the gene encoding the Wnt pathway signaling component dishevelled-3 is differentially expressed in the tumors of patients treated with trastuzumab, and present at significantly higher quantities at the mRNA level in the tumors of breast cancer patients treated with trastuzumab. It appears that in human breast cancer, trastuzumab can influence expression of Wnt signaling pathway molecules.

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Rank	ID	p-value	t	B	FC	Gene
15	GI_6806886-S-6	0.010726	2.65379	-3.31	1.25 ± 0.26	DVL3

Table 1: DVL3 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 DVL3 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
898	9001	7.56E-03	-2.71	-2.49038	DVL3	dishevelled segment polarity protein 3

Table 2: DVL3 is differentially expressed in the primary tumors of patients with breast cancer 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, gene and gene name are listed in this chart.

Figure 1: DVL3 is expressed at significantly higher levels in the primary tumors of patients treated with trastuzumab

The mRNA expression level of DVL3 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 DVL3 expression in the primary tumors of patients treated with trastuzumab or not, a *p*-value, listed above.