Trastuzumab administration in patients with breast cancer is associated with increased primary tumor expression of Wnt2 and Wnt10b.

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The Wnt pathway transduces signals through a series of intracellular mediators including Axin, dishevelled proteins, and through regulation of an Axin destruction complex by casein kinases to effect nuclear translocation of β -catenin and β -catenin cooperation with TCF/LEF proteins at nuclear transactivation targets following binding of Wnt ligands to Frizzled receptors which act in concert with LRP co-receptors at the plasma membrane¹⁻³. Trastuzumab, a monoclonal antibody targeted against the human epidermal growth factor receptor 2 (HER2) is utilized for the treatment of human breast cancer⁴, 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 data⁵⁻⁷ from the primary tumors of patients treated with trastuzumab, we found that the Wnt ligands Wnt2 and Wnt10b, molecules with the capacity to support proliferation of ventral midbrain progenitors⁸ and to induce transformation of the mammary gland⁹, were among the genes most differentially expressed in the primary tumors of patients treated with trastuzumab. Increased expression of Wnt2 and Wnt10b implies that the Wnt pathway may be activated in primary tumors of patients treated with trastuzumab.

Keywords: breast cancer, trastuzumab, Wnt signaling pathway, Wnt2, Wnt10b, systems biology of breast cancer, targeted therapeutics in breast cancer.

The mechanism of action by which trastuzumab exerts its therapeutic function in human breast cancer is said to be binding of the F_{ab} domain of trastuzumab to the extracellular region of the human epidermal growth factor receptor 2 (HER2)¹⁰, possibly resulting in decreased cellular proliferation¹¹. Transcriptional modulation resulting from trastuzumab binding events in the primary tumors of treated patients is less well understood. We mined published and public microarray and gene expression data⁵⁻⁷ generated using primary tissues from patients treated with trastuzumab to compare tumor global gene expression with that of patients not treated with trastuzumab. Using this unbiased, systems level methodology, we identified differential expression and up-regulation of the Wnt pathway ligands Wnt2 and Wnt10b in the primary tumors of patients treated with trastuzumab.

Methods

We used datasets GSE38057⁵, GSE58644⁶ and GSE130788⁷ for this differential gene expression analysis of the transcriptional response to trastuzumab treatment in patients with breast cancer. GSE38057 was generated using DASL Human Cancer Panel by Probe technology with *n*=6 primary breast tumors from patients treated with induction chemotherapy and/or hormone therapy without trastuzumab and *n*=35 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. GSE58644 was generated using Affymetrix Human Gene 1.0 ST Array technology with *n*=191 primary breast tumors from patients not treated with trastuzumab and *n*=12 primary tumors from patients treated with trastuzumab, analysis performed using platform GPL6244. GSE130788 was generated using Agilent-014850 Whole Human Genome Microarray 4x44K G4112F technology with *n*=11 primary breast tumors from patients treated with trastuzumab and *n*=36 primary tumors from patients treated with trastuzumab, analysis performed using platform GPL6480. The Benjamini and Hochberg method of *p*-value adjustment was used for ranking 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 generated category of platform annotation was used. A statistical test was performed to evaluate whether Wnt2 or Wnt10b gene expression was significantly between primary breast tumors of patients receiving trastuzumab 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).

Results

Wnt2 is differentially expressed in the primary tumors of patients treated with trastuzumab.

We found that Wnt2 was among the genes whose expression was most significantly different in the primary tumors of patients with breast cancer treated with trastuzumab⁵. When sorting each of the genes expressed in trastuzumab-treated breast tumors based on significance of difference in expression as compared to the primary breast tumors of patients not treated with trastuzumab, Wnt2 ranked 5 out of 1506 total transcripts, equating to 99.7% statistical significance (Table 1). Differential expression of Wnt2 in the primary tumors of breast cancer patients treated with trastuzumab was statistically significant (Table 1; *p*=0.0079).

We queried a second dataset⁶ to validate our findings of Wnt2 differential expression upon trastuzumab treatment. In a second microarray dataset analyzing gene expression of patients treated with trastuzumab, we again found that Wnt2 was among the genes whose expression was most significantly different transcriptome-wide when comparing the global gene expression profiles of tumors from patients

1 treated with trastuzumab as compared to those not treated with trastuzumab. When sorting each of the
2 genes expressed in the tumors of patients treated with trastuzumab based on significance of change as
3 compared to the tumors of patients not treated with trastuzumab, Wnt2 ranked 1965 out of 32321 total
4 transcripts, equating to 93.9% differential expression. Differential expression of Wnt2 in the primary
5 tumors of patients treated with trastuzumab was statistically significant (Table 2; $p=4.6E-02$).

6 **Wnt2 is expressed at higher levels in the tumors of patients treated with trastuzumab.**

7 We obtained exact mRNA expression levels for Wnt2 from the tumors of patients treated with
8 trastuzumab and from the tumors of patients not receiving trastuzumab to determine magnitude and
9 direction of Wnt2 change in the tumors of patients treated with trastuzumab. Wnt2 expression was
10 significantly higher in the tumors of patients treated with trastuzumab as compared to the tumors of
11 patients not treated with trastuzumab (Figure 1). Increased expression of Wnt2 was statistically
12 significant (Figure 1: $p=0.0004$). We calculated a mean fold change of 1.51 ± 0.48 when comparing the
13 expression of Wnt2 in the tumors of patients treated with trastuzumab as compared to the tumors of
14 patients treated with trastuzumab.

15 **Wnt10b is differentially expressed in the primary tumors of patients treated with trastuzumab.**

16 We found that Wnt10b was among the genes whose expression was most significantly different in
17 the primary tumors of patients with breast cancer treated with trastuzumab⁵. When sorting each of the
18 genes expressed in trastuzumab-treated breast tumors based on significance of difference in expression as
19 compared to the primary breast tumors of patients not treated with trastuzumab, Wnt10b ranked 42 out of
20 1506 total transcripts, equating to 97.2% statistical significance (Table 3). Differential expression of
21 Wnt10b in the primary tumors of breast cancer patients treated with trastuzumab was statistically
22 significant (Table 3; $p=0.04012$).

23 We queried a second dataset⁷ to validate our findings of Wnt10b differential expression upon
24 trastuzumab treatment. In a second microarray dataset analyzing gene expression of patients treated with
25 trastuzumab, we again found that Wnt10b was among the genes whose expression was most significantly
26 different transcriptome-wide when comparing the global gene expression profiles of tumors from patients
27 treated with trastuzumab as compared to those not treated with trastuzumab. When sorting each of the
28 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, Wnt10b ranked 19158 out of 41000 total
transcripts, equating to 53.3% differential expression. Differential expression of Wnt10b in the primary
tumors of patients treated with trastuzumab was statistically significant (Table 4; $p=1.57E-03$).

29 **Wnt10b is expressed at higher levels in the tumors of patients treated with trastuzumab.**

30 We obtained exact mRNA expression levels for Wnt10b from the tumors of patients treated with
31 trastuzumab and from the tumors of patients not receiving trastuzumab to determine magnitude and
32 direction of Wnt10b change in the tumors of patients treated with trastuzumab. Wnt10b expression was
33 significantly higher in the tumors of patients treated with trastuzumab as compared to the tumors of
34 patients not treated with trastuzumab (Figure 1). Increased expression of Wnt10b was not deemed
35 statistically significant (Figure 1: $p=0.1544$). We calculated a mean fold change of 1.55 ± 0.75 when
36 comparing the expression of Wnt10b in the tumors of patients treated with trastuzumab as compared to
37 the tumors of patients treated with trastuzumab.

Discussion

Wnt2 has important functions in development, as evidenced by the fact that Wnt2-null mice manifest defects in the structure and size of the placenta in the labyrinthine zone resulting in the accumulation of large pools of maternal blood¹². Wnt2 also has developmental functions in the brain, as it has the capacity to influence the proliferation of ventral midbrain progenitors⁸. One report described the existence of an autocrine Wnt2 signaling loop originating from fibroblasts that supported the progression of colorectal cancers¹³, and another described the activation of Wnt pathway signaling in pancreatic cancer through Wnt2 which supported metastasis¹⁴.

Wnt10b also has important functions in the development, in the skeletal structure as it can regulate bone mass and osteoblastogenesis¹⁵. Expression of Wnt10b has been documented in human breast cancer¹⁶. Importantly, Wnt10b expression marks the mammary line in the mouse, a developmental structure that defines the space at which mammaryogenesis occurs¹⁷, and in both male and female mice Wnt10b transgenic expression can result in transformation of the mammary gland, as expression of Wnt10b under the control of the mouse mammary tumor virus (MMTV) promoter results in increased numbers of both mammary ducts and alveoli and early-onset mammary adenocarcinoma⁹.

We provided evidence here that two separate Wnt pathway ligands, Wnt2 and Wnt10b, signaling molecules with the potential to influence proliferation of progenitor cells of the central nervous system, and to support transformation of the mammary glands in the mouse, are expressed at significantly higher levels in the tumors of patients treated with trastuzumab and among the genes most differentially expressed when comparing the tumors of patients treated with trastuzumab as opposed to those not treated with trastuzumab.

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Rank	ID	p-value	t	B	FC	Gene
5	GI_4507926-S-4	0.0079	2.786718	-4.1	1.51 ± 0.48	WNT2

Table 1: Wnt2 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 Wnt2 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
1965	8142471	4.6E-02	2.0076899	-3.9364	WNT2	Wnt family member 2

Table 2: Wnt2 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 Wnt2 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	FC	Gene
42	GI_16936521-S-1	0.04012	2.116731	-4.33	1.55 ± 0.75	WNT10B

Table 3: Wnt10b 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 Wnt10b 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
19158	A_23_P162322	1.57E-03	-3.206078	-2.588656	WNT10B	Wnt family member 10B

Table 4: Wnt10b 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 Wnt10b 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: Wnt2 is expressed at significantly higher levels in the primary tumors of patients treated with trastuzumab as compared to those not treated with trastuzumab.

The mRNA expression level of Wnt2 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 Wnt2 expression in the primary tumors of patients treated with trastuzumab or not, a *p*-value, listed above.

Figure 2: Wnt10b 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 Wnt10b 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 Wnt10b expression in the primary tumors of patients treated with trastuzumab or not, a *p*-value, listed above.