BMPR1B 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 significant transcriptional changes associated with trastuzumab treatment. We identified BMPR1B 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 BMPR1B messenger RNA than did patients not treated with trastuzumab, and a single administration of trastuzumab was sufficient to result in differential expression of BMPR1B in primary tumors of the breast, demonstrating increased expression of a receptor subunit for a bone morphogenetic protein that is important for development of the ventral retina and optic nerve⁵ as a direct transcriptional consequence of treatment with trastuzumab.

Keywords: trastuzumab, breast cancer, BMPR1B, 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 BMPR1B is a
6 differentially expressed gene in the tumors of trastuzumab-treated patients: patients treated with
7 trastuzumab expressed significantly higher levels of BMPR1B than did those of patients not treated with
8 trastuzumab.

9 **Methods**

10 We used datasets GSE130788³, and GSE114082⁴ for this differential gene expression analysis of
11 the transcriptional response to trastuzumab treatment in patients with breast cancer. GSE130788 was
12 generated using Agilent-014850 Whole Human Genome Microarray 4x44K G4112F technology with
13 $n=11$ primary breast tumors from patients treated with trastuzumab and $n=36$ primary tumors from
14 patients treated with trastuzumab, analysis performed using platform GPL6480. GSE114082 was
15 generated using Illumina HumanHT-12 WG-DASL V4.0 R2 expression beadchip technology with $n=17$
16 primary breast tumors from patients before trastuzumab treatment and $n=17$ primary tumors from patients
17 after trastuzumab treatment, analysis performed using platform GPL14951. The Benjamini and Hochberg
18 method of p -value adjustment was used for ranking of differential expression but raw p -values were used
19 to assess statistical significance of global or multiplexed differential expression. Log-transformation of
20 data was auto-detected, and the NCBI generated category of platform annotation was used. A statistical
21 test was performed to evaluate whether BMPR1B gene expression was significantly between primary
22 breast tumors of patients receiving trastuzumab using a two-tailed, unpaired t-test with Welch's
23 correction. We used PRISM for all statistical analyses of differential gene expression in human breast
24 cancer (Version 8.4.0)(455).

25 **Results**

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

29 **BMPR1B is differentially expressed in the tumors of patients treated with trastuzumab.**

30 We identified neuronal differentiation 4, encoded by BMPR1B as among the genes whose
31 expression changed most significantly in the tumors of breast cancer patients treated with trastuzumab in
32 a multiplexed gene expression dataset³ (Table 1). When sorting each of the genes expressed in the
33 primary tumors of breast cancer patients treated with trastuzumab based on significance of change as
34 compared to that of patients not treated with trastuzumab, BMPR1B ranked 196 out of 41000 total
35 transcripts (Table 1), equating to 99.5% differential expression. BMPR1B differential expression in the
36 tumors of patients treated with trastuzumab trended towards statistical significance (Table 1; $p=1.3E-46$).

37 We turned to a second microarray dataset to validate our findings of BMPR1B differential
38 expression in the patients treated with trastuzumab. The tumors compared in this dataset were sampled
39 from the same patient before and after a single, brief exposure to trastuzumab, not comparing tumors from
40 different patients treated with a course of trastuzumab or not. Thus, while differentially expressed genes
41 when comparing tumors based on trastuzumab treatment can result from a variety of sources, including
42 gene expression changes resulting from tumor evolution over the course of treatment, changes identified

1 in this dataset, occurring after only a single treatment of trastuzumab, are more likely to be a direct
2 transcriptional result of trastuzumab binding events. We found again that BMPR1B was among the genes
3 most differentially expressed in the tumors of patients administered a single treatment of trastuzumab
4 (Table 2). When sorting each of the genes expressed in the tumors of patients administered a single
5 treatment of trastuzumab, BMPR1B ranked 3986 out of 29377 total transcripts (Table 2), equating to
6 86.4% differential expression. Differential expression of BMPR1B in the primary tumors of breast cancer
7 patients following treatment with trastuzumab was statistically significant (Table 2; $p=3.25E-03$).

8 **BMPR1B is expressed at significantly higher levels in the tumors of breast cancer patients treated**
9 **with trastuzumab than in the tumors of those not treated with trastuzumab.**

10 We obtained exact mRNA expression levels for BMPR1B from tumors of patients treated with
11 trastuzumab and from the tumors of patients not treated with trastuzumab to directly evaluate direction
12 and statistical significance of change in expression of BMPR1B based on history of trastuzumab
13 treatment. BMPR1B was expressed at higher levels in the tumors of patients treated with trastuzumab as
14 compared to those of patients not treated with trastuzumab, and this difference was statistically significant
15 (Figure 1; $p<0.0001$).

16 Thus, we found using published multiplexed gene expression and microarray data^{3,4} that
17 BMPR1B was among the genes most differentially expressed in the tumors of patients treated with
18 trastuzumab, that BMPR1B was expressed at higher levels in the tumors of patients treated with
19 trastuzumab as compared to those not treated with trastuzumab, and that this transcriptional change was
20 likely a direct result of trastuzumab treatment as opposed to a gene expression change associated with
21 clonal selection and evolution of the tumor across the course of treatment.

22 **Discussion**

23 We provided evidence here that BMPR1B is a differentially expressed gene in the tumors of
24 patients treated with trastuzumab, and expressed at significantly higher levels in the primary tumors of
25 breast cancer patients treated with trastuzumab as compared to patients not treated with trastuzumab.
26 Deficiency of BMPR1B in mice resulting from *Bmpr1b* mutation results in loss of tissue in or around the
27 eye, known as coloboma, and studies of these mice demonstrated that BMPR1B is important for
28 development of the ventral retina and optic nerve. Thus, trastuzumab, a treatment associated with a 34%
rate of central nervous system metastases⁶, administration in patients with breast cancer results in
increased primary tumor expression of a receptor subunit of a bone morphogenetic protein with functions
in development of the optic system⁵ and in regulation of astrocyte reactivity⁷.

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Table 1: BMPR1B is differentially expressed in the primary tumors of breast cancer patients treated with trastuzumab.

Rank	ID	p-value	t	B	Gene	Gene name
196	A_32_P24939	1.3E-46	19.024859	95.472405	BMPR1B	bone morphogenetic protein receptor type 1B

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.

Table 2: A single exposure to trastuzumab is sufficient to drive differential expression of BMPR1B in the primary tumors of patients with breast cancer.

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Rank	ID	p-value	t	B	Gene
3986	ILMN_1801658	3.25E-03	-3.1542952	-1.95534	BMPR1B

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: BMPR1B is higher levels in the primary tumors of patients treated with trastuzumab.

expressed at significantly tumors of patients treated

The mRNA expression level of BMPR1B 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 BMPR1B expression in the primary tumors of patients treated with trastuzumab or not, a *p*-value, listed above.