Differential expression of Notch4 in the primary tumors of breast cancer patients treated with trastuzumab.

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The mechanism of action underlying trastuzumab (Herceptin) function is ascribed to binding of the F_{ab} region of trastuzumab to the extracellular domain of the human epidermal growth factor receptor (HER2)¹. The transcriptional responses that follow signals transduced after trastuzumab binding of HER2 are less well understood. We mined published microarray and multiplexed gene expression data^{2,3} to understand in an unbiased fashion genes most differentially expressed in the primary tumors of breast cancer patients treated with trastuzumab. We found significant differential and over-expression of Notch4^{4,5}, described as a mammary proto-oncogene⁴, in the primary tumors of breast cancer patients treated with trastuzumab.

Keywords: trastuzumab, breast cancer, Notch4, systems biology of breast cancer, targeted therapeutics in breast cancer.

Trastuzumab (Herceptin), a monoclonal antibody utilized for the treatment of adjuvant and metastatic breast cancer in the United States, targets the human epidermal growth factor receptor 2, or HER2⁶, encoded by the ERBB2 gene. How transcription is most significantly different between the tumors of breast cancer patients treated with trastuzumab and those not treated with trastuzumab is not fully explored. We mined published microarray and multiplexed datasets^{2,3} to understand in an unbiased fashion and at the systems level genes most transcriptionally perturbed by treatment with trastuzumab in the primary tumors of patients with breast cancer. We found significant differential expression of the Notch signaling pathway receptor, Notch4^{4,5}, in the primary tumors of breast cancer patients treated with trastuzumab.

Methods

We used datasets GSE38057² and GSE75685³ for this differential gene expression analysis of the transcriptional response to trastuzumab in human breast cancer. GSE38057 was generated using DASL Human Cancer Panel by Probe technology with either $n=6$ 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. GSE75685 was generated using Whole Human Genome Microarray 4x44K technology with $n=41$ primary breast tumors from patients not treated with trastuzumab and $n=11$ primary tumors from patients treated with trastuzumab, analysis performed using platform GPL4133. 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 Notch4 gene expression was significantly between primary breast tumors of patients receiving trastuzumab or not 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

We performed systems-level transcriptome analysis using published microarray and multiplexed gene expression data^{2,3}, comparing primary tumors of breast cancer patients who had been treated or not with trastuzumab to describe in an unbiased fashion the most significant transcriptional features of trastuzumab treatment in humans with breast cancer.

Notch4 is differentially expressed in the primary tumors of patients treated with trastuzumab.

We found that the Notch pathway signaling component Notch4 was among the genes whose expression was most significantly different in the primary tumors of patients with breast cancer treated with trastuzumab². When sorting each of the genes expressed in trastuzumab-treated breast tumors based on significance of difference in expression as compared to the primary tumors of the breast from patients not treated with trastuzumab, Notch4 ranked 41 out of 1506 total transcripts, equating to 97.3% statistical significance (Table 1). Differential expression of Notch4 in the primary tumors of breast cancer patients treated with trastuzumab was statistically significant (Table 1; $p=0.027522$).

We queried a second dataset³ to validate our findings of Notch4 differential expression upon trastuzumab treatment. In a second microarray dataset analyzing gene expression of patients treated with

trastuzumab, we again found that Notch4 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, Notch4 ranked 3483 out of 45220 total transcripts, equating to 92.3% differential expression. Differential expression of Notch4 was statistically significant (Table 2; $p=8.84E-03$).

Notch4 is expressed at significantly higher levels in the primary tumors of patients treated with trastuzumab as compared to that of patients not treated with trastuzumab.

We obtained exact mRNA expression levels for Notch4 from the tumors of patients treated with trastuzumab and from the tumors of patients not receiving trastuzumab to determine magnitude and direction of Notch4 change in the tumors of patients treated with trastuzumab. Notch4 expression was significantly 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 Notch4 was statistically significant (Figure 1: $p=0.0052$). It is difficult to determine given the information provided whether Notch4 is directly transcriptionally activated by signals transduced following trastuzumab binding to HER2 or whether trastuzumab in some manner selects for the survival of tumor clones with high expression of Notch4. We calculated a mean fold change of 1.95 ± 1.16 when comparing the expression of Notch4 in the tumors of patients treated with trastuzumab as compared to the tumors of patients treated with trastuzumab.

Thus, we found that Notch4 was among the most significant transcriptional features of the primary tumors of breast cancer patients treated with trastuzumab; Notch4 was expressed at significantly increased levels in the primary tumors of patients treated with trastuzumab.

Discussion

Notch receptors interact with ligands including Delta and Jagged at the plasma membrane and are important for diverse biological functions including neural development and specification of T-cell lineage⁶. Notch4, also known as int-3, has been described as a mammary proto-oncogene; interestingly, Notch4 is a target of the mouse mammary tumor virus (MMTV), infection with which results in the development of murine breast cancers⁵. Notch4 differs from other Notch receptors in that it contains fewer EGF-domain repeats and a shorter intracellular tail⁵. The signaling activity of Notch4 is 8-fold higher in stem cell-enriched breast cancer cell populations as compared to differentiated cells, and genetic depletion of Notch4 impaired tumor growth of MCF7 cells in nude mice *in vivo*⁷. Notch4 functions with Nodal in driving an “aggressive” phenotype in melanoma⁹. Expression of Notch4 in TAC-2 mammary epithelial cells impairs contact-inhibition resulting in generation of cell multilayers, and Notch4-expressing TAC-2 cells lose apico-basal polarity¹⁰. In patient-derived xenografts, endocrine therapy results in generation of a breast cancer stem cell population that supports resistance to endocrine therapy through Notch4 and Jag1¹¹.

We found here using published gene expression and microarray data^{2,3} that a gene that functions as a mammary proto-oncogene, whose expression is enriched in breast cancer stem cell populations, and whose expression results in architectural changes consistent with transformation is differentially expressed and likely transcriptionally induced by treatment with trastuzumab in patients with breast cancer.

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Rank	ID	p-value	t	B	FC	Gene
41	GI_27894369-S-3	0.027522	2.27243	-3.71	1.95 ± 1.16	NOTCH4

Table 1: Notch4 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 Notch4 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
3483	42893	8.84E-03	-2.7209643	-2.724998	NOTCH4

Table 2: Notch4 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, gene and gene name are listed in this chart.

Figure 1: Notch4 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 Notch4 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 Notch4 expression in the primary tumors of patients treated with trastuzumab or not, a *p*-value, listed above.