ITGβ1 (CD29) 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 cell adhesion molecule integrin β1⁵⁻⁹ 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 significantly higher levels of ITGβ1 messenger RNA than did patients not treated with trastuzumab, and a single administration of trastuzumab was sufficient to result in differential expression of ITGβ1 in primary tumors of the breast, suggesting that increased primary tumor expression of ITGβ1 in the primary tumors of patients with breast cancer, a molecule whose increased expression in human breast cancer is correlated with overall survival, metastasis and significantly decreased time from diagnosis to death^{10,11}, is a direct transcriptional result of treatment with trastuzumab.

Keywords: trastuzumab, breast cancer, ITGβ1, systems biology of breast cancer, targeted therapeutics in breast cancer.

The mechanism of action ascribed to trastuzumab function is thought to be binding of the F_{ab} domain of trastuzumab to the extracellular region of the human epidermal growth factor receptor 2 (HER2)¹, but the transcriptional consequences resulting from signaling downstream of trastuzumab 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 ITGβ1, also known as CD29, is a differentially expressed gene in the tumors of trastuzumab-treated patients: patients treated with trastuzumab expressed significantly higher levels of ITGβ1 than did those of patients not treated with trastuzumab.

Methods

We used datasets GSE38057³ and GSE114082⁴ 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*=11 primary breast tumors from patients treated with induction chemotherapy and/or hormone therapy without trastuzumab and *n*=36 primary breast tumors from patients treated with induction chemotherapy and/or hormone therapy in addition to trastuzumab; patients in both groups compared had not developed brain metastases at time of data collection by investigators; analysis was performed using platform GPL5826. GSE114082 was generated using Illumina HumanHT-12 WG-DASL V4.0 R2 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 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 ITGβ1 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

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

ITGβ1 is differentially expressed in the tumors of patients treated with trastuzumab.

We identified the cell adhesion molecule ITGβ1 as among the genes whose expression 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 tumors of breast cancer patients treated with trastuzumab based on significance of change as compared to that of patients not treated with trastuzumab, ITGβ1 ranked 167 out of 1506 total transcripts (Table 1), equating to 88.9% differential expression. ITGβ1 differential expression in the tumors of patients treated with trastuzumab was statistically significant (Table 1; *p*=0.134325).

The first dataset, although measuring the expression of over 1500 genes, was still limited. We 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 after a single, brief exposure to trastuzumab, not comparing tumors from different patients treated with a course of trastuzumab or not. Thus, while differentially expressed genes when comparing tumors based on trastuzumab treatment can result from a variety of sources, including gene expression changes resulting from tumor evolution over the course of treatment, changes identified in this dataset, occurring after only a single treatment of trastuzumab, are more likely to be a direct transcriptional result of trastuzumab binding events. We found again that ITGβ1 was among the genes most differentially

expressed in the tumors of patients administered a single treatment of trastuzumab (Table 2). When sorting each of the genes expressed in the tumors of patients administered a single treatment of trastuzumab, ITGβ1 ranked 6945 out of 29377 total transcripts (Table 2), equating to 76.4% differential expression. Differential expression of ITGβ1 in the primary tumors of breast cancer patients following treatment with trastuzumab was statistically significant (Table 2; $p=1.72E-02$).

ITGβ1 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 ITGβ1 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 ITGβ1 based on history of trastuzumab treatment. ITGβ1 was expressed at higher levels in the tumors of patients treated with trastuzumab as compared to those of patients not treated with trastuzumab, and this difference was deemed statistically significant (Figure 1; $p=0.0191$). We calculated a mean fold change of 1.13 ± 0.24 in ITGβ1 expression in the primary tumors of patients treated with trastuzumab relative to that of patients not treated with trastuzumab.

Thus, we found using published multiplexed gene expression and microarray data^{3,4} that ITGβ1 was among the genes most differentially expressed in the tumors of patients treated with trastuzumab, that ITGβ1 was expressed at higher levels in the tumors of patients treated with trastuzumab as compared to those not treated with trastuzumab, and that this transcriptional change was likely a direct result of trastuzumab treatment as opposed to a gene expression change associated with clonal selection and evolution of the tumor across the course of treatment.

Discussion

Integrins are transmembrane cell adhesion molecules that form heterodimers consisting of an α and β subunit; broadly, integrin β 1 functions in recognition of ligands containing an RGD tripeptide active site⁵. β integrin ectodomains contain four epidermal growth factor modules that are cysteine-rich and a β -tail domain, both of which are preceded by a β -I domain contained within a hybrid domain, which is further contained with a plexin-semaphorin-integrin (PSI) domain⁵. Integrin heterodimers α 1 β 1 and α 6 β 1 can function in binding to laminin⁶. Evidence that integrin β 1 is involved in productive signaling events in the cytosol is demonstration of clustering of ITGβ1 and increased tyrosine phosphorylation of pp130 in KB human epidermal carcinoma cells following incubation with an antibody binding integrin β 1⁷. Integrin α 2 β 1 can function in migration of human umbilical vein endothelial cells on collagen⁸. In human lung cancer, one study demonstrated that integrin β 1; could function in a signaling pathway in conjunction with focal adhesion complexes wherein pyruvate kinase 2 supported metastasis⁹.

At least three studies have demonstrated that integrin β 1 expression is associated with poor outcomes in human breast cancer and in a mouse model of breast cancer¹⁰⁻¹². One showed a very significant correlation between expression levels of ITGβ1 and the number of months between diagnosis and death, as well as independent statistically significant correlations with death and metastasis¹⁰. Another demonstrated that highest expression of ITGβ1 was correlated with significantly decreased overall survival on univariate analysis, as was high fibronectin expression¹¹. ITGβ1 expression was also associated with expression of fibronectin, which we recently demonstrated was over-expressed in human breast cancer¹². ITGβ1 was also correlated with disease-free survival and overall survival on multivariate analysis as well. An important mechanistic insight can be gained by understanding that MDA-MD-435 breast cancer cells expressing an β 4 mutant lacking the cytoplasmic domain that prevents formation of the α 6 β 1 heterodimer (β 4 Δ CYT), when grown in athymic mice underwent significant apoptotic cell death, their growth at the level of the tumor was impaired, and were unable to form metastases in the lungs¹³.

We provided evidence here that the cell adhesion molecule ITGβ1 is a differentially expressed 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 ITGβ1 than did patients not treated with trastuzumab. These data, together with the knowledge that increased expression of ITGβ1 correlates with with death, metastasis and decreased survival in humans, reveal a potentially dangerous transcriptional consequence of trastuzumab administration in patients with breast cancer.

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Rank	ID	p-value	t	B	FC	Gene
167	GI_19743818-A-6	0.134325	1.52266	-4.37	1.13 ± 0.24	ITGB1

Table 1: ITGβ1 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 ITGβ1 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
6945	ILMN_1784454	1.72E-02	-2.4979953	-3.4491	ITGB1

Table 2: A single exposure to trastuzumab is sufficient to drive differential expression of ITGβ1 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: ITGβ1 is expressed at significantly higher levels in the primary tumors of patients treated with trastuzumab.

The mRNA expression level of ITGβ1 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 ITGβ1 expression in the primary tumors of patients treated with trastuzumab or not, a *p*-value, listed above.