Casitas B-lineage Lymphoma (CBL) is differentially expressed in the tumors of breast cancer patients treated with trastuzumab.

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Trastuzumab, a monoclonal antibody that targets HER2, is utilized for the treatment of breast cancer¹. While the mechanism of action of trastuzumab function in breast cancer is ascribed to trastuzumab binding to the extracellular region of HER2², the transcriptional consequences that follow this binding event are less well understood. We report here, using analysis of published microarray and gene expression data^{3,4}, that the gene encoding the E3 ubiquitin ligase Casitas B-lineage Lymphoma, CBL⁵⁻⁸, is differentially expressed in the tumors of patients with breast cancer treated with trastuzumab. These data demonstrate that a *bona fide* proto-oncogene is expressed at higher levels in the primary tumors of trastuzumab-treated patients with breast cancer.

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

Central nervous system metastases can affect women with breast cancer, but the rate of CNS metastases in breast cancer patients treated with trastuzumab was described at 34% in one report, and said to be higher than one might anticipate in the absence of trastuzumab administration⁹. The mechanism of action ascribed to trastuzumab is thought to be binding of trastuzumab to HER2² but the transcriptional consequences resulting from HER2 binding are less well understood. We found that the E3 ubiquitin ligase CBL, described to possess proto-oncogenic function⁶, was among the genes most differentially expressed in the primary tumors of patients with breast cancer treated with trastuzumab.

Methods

We used datasets GSE38057³ and GSE130788⁴ 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 either $n=11$ 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; analysis was performed using platform GPL5826. 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 CBL 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 harnessed the power of blind, comparative transcriptome analysis of 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).

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

We identified the proto-oncogene and E3 ubiquitin ligase casitas B-lineage lymphoma, CBL, as among the genes whose expression changed most significantly in the tumors of breast cancer patients treated with trastuzumab³ (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, CBL ranked 115 out of 1506 total transcripts (Table 1), equating to 92.4% differential expression. CBL differential expression in the tumors of patients treated with trastuzumab trended towards statistical significance (Table 1; $p=0.088081$).

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 - 41000 genes in total. We found again that CBL 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, CBL ranked 15344 out of 41000 total transcripts (Table 2), equating to 62.6% differential expression. Differential expression of CBL was statistically significant (Table 2; $p=9.26E-05$).

CBL is expressed at 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 CBL 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 CBL based on history of trastuzumab treatment. CBL was expressed at higher levels in the tumors of patients treated with trastuzumab as compared to those of patients not treated with trastuzumab; this difference approached the level of statistical significance (Figure 1; $p=0.0744$). We calculated a mean fold change of 1.26 ± 0.53 in CBL expression in the primary tumors of patients treated with trastuzumab relative to that of patients not treated with trastuzumab.

Thus, we found that the proto-oncogene and E3 ubiquitin ligase CBL was among the genes most differentially expressed in the tumors of patients treated with trastuzumab, and that CBL was expressed at higher levels in the tumors of patients treated with trastuzumab as compared to those not treated with trastuzumab.

Discussion

Cbl, or c-Cbl is a cellular homolog of a viral oncogene, v-Cbl; v-Cbl expression is sufficient to drive transformation, and v-Cbl is a truncated form of c-Cbl^{5,6}. While over-expression of c-Cbl is not sufficient to drive transformation, mutation at a splice acceptor site deleted from Cbl in 70Z/3 pre-B lymphoma can activate can convert Cbl from a proto-oncogene to oncogene^{5,6}. Cbl functions in signal transduction by catalyzing the covalent modification of multiple substrates with ubiquitin chains, including platelet-derived growth factor (PDGF), the epidermal growth factor (EGF), and the colony-stimulating factor-1 receptors⁷. Tyrosine kinase-binding by CBL allows it to utilize its RING domain to conjugate ubiquitin chains to receptor tyrosine kinases and down-regulate tyrosine kinase function⁷. Deletion of Cbl-b functions to permit natural killer cells to reject metastatic tumors, functioning through ubiquitylation of TAM family kinase receptors Axl, Mertk and Tyro3⁸. One study described increased expression of CBL in breast cancers, and depletion of CBL in MDA-MB231 breast cancer cells resulted in decreased tumor volume in athymic nude mice *in vivo*⁹.

We provided evidence here that an E3 ubiquitin ligase with proto-oncogenic function described to possess the capacity to regulate metastasis in certain cellular contexts is among the genes whose expression changes most significantly in the tumors of patients treated with trastuzumab, a treatment associated with a 34% incidence of central nervous system metastases in patients with breast cancer¹⁰.

References

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Rank	ID	p-value	t	B	FC	Gene
115	GI_4885116-S-1	0.088081	1.74061	-4.2	1.26 ± 0.53	CBL

Table 1: CBL is differentially expressed in the 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 CBL 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
15344	A_23_P339191	9.26E-05	-3.990398	0.066187	CBL	Cbl proto-oncogene

Table 2: CBL is differentially expressed in the 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: CBL is expressed at significantly higher levels in the primary tumors of breast cancer patients treated with trastuzumab.

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