BIRC3 is a differentially expressed gene in the tumors of breast cancer patients treated with trastuzumab.

Shahan Mamoor, MS¹

¹shahanmamoor@gmail.com

East Islip, NY USA

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 found that BIRC3 was among the the genes most differentially expressed in the primary tumors of patients treated with trastuzumab. BIRC3, a molecule with the capacity to promote the survival of cancer cells⁵, was expressed at significantly higher levels in the primary tumors of patients treated with trastuzumab as compared to that of patients not treated with trastuzumab.

Keywords: trastuzumab, breast cancer, BIRC3, systems biology of breast cancer, targeted therapeutics in breast cancer.

The mechanism of action by which trastuzumab is thought to exert its therapeutic function in breast cancer is through binding of the Fab domain of trastuzumab to the extracellular region of the human epidermal growth factor receptor 2 (HER2)¹, but the transcriptional changes downstream of trastuzumab binding to HER2 are less well understood. Through unbiased, systems-level comparative transcriptome analysis of published microarray and gene expression data^{3,4}, we found here that the inhibitor of apoptosis protein BIRC3 was differentially expressed in the tumors of trastuzumab-treated breast cancer patients: patients treated with trastuzumab expressed higher levels of BIRC3 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 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. 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 BIRC3 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 using published gene expression and microarray data^{3,4} to identify the most significant transcriptional features of the primary breast tumors of patients treated with trastuzumab (Herceptin).

BIRC3 is differentially expressed in the tumors of patients treated with trastuzumab.

We identified BIRC3 as among the genes whose expression changed most significantly in the tumors of breast cancer patients treated with trastuzumab (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, BIRC3 ranked 55 out of 1506 total transcripts (Table 1), equating to 96.3% differential expression. BIRC3 differential expression in the tumors of patients treated with trastuzumab was statistically significant (Table 1; $p=0.05236$).

The first dataset, although measuring the expression of over 1500 genes, was still limited. We turned to a second microarray dataset, this measuring the expression of genes across the transcriptome -

29377 genes in total - but with another advantage. 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 BIRC3 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, BIRC3 ranked 8773 out of 29377 total transcripts (Table 2), equating to 70.1% differential expression. Differential expression of BIRC3 in the primary tumors of breast cancer patients following treatment with trastuzumab was statistically significant (Table 2; $p=3.53E-02$).

BIRC3 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 BIRC3 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 BIRC3 based on history of trastuzumab treatment. BIRC3 was expressed at higher levels in the tumors of patients treated with trastuzumab as compared to those of patients not treated with trastuzumab, but this difference was not deemed statistically significant (Figure 1: $p=0.1360$). We calculated a mean fold change of 1.72 ± 1.17 in BIRC3 expression in the primary tumors of patients treated with trastuzumab relative to that of patients not treated with trastuzumab.

Thus, we found that BIRC3 was among the genes most differentially expressed in the tumors of patients treated with trastuzumab, that BIRC3 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 tumor evolution.

Discussion

BIRC3, also known as the cellular inhibitor of apoptosis 2 (cIAP2), is an E3 ubiquitin ligase, originally described as an interaction partner of the TNF receptors associated factors 1 and 2 (TRAF1 and TRAF2)⁵, that functions in the ubiquitination of RIP1 kinase to modulate cell survival; ubiquitinated RIP1 associated with the TAK1 kinase to support cell survival, but de-ubiquitinated RIP1 induces cell death by apoptosis through interaction with caspase-8⁶. IAP proteins including BIRC3/cIAP2 are characterized by the presence of the BIR domain⁷. BIRC3/cIAP2 has also been reported as a direct inhibitor of caspase-3 and caspase-7⁸. IAP proteins are characterized by the presence of a BIR domain, which depending on the BIR domain and the IAP protein, serves as an interaction platform to bind caspases or the IAP inhibitory protein SMAC/diablo^{9,10}. In human cancer, BIRC3 has been described as a marker of aggressiveness in glioblastoma, with BIRC3 tumor expression increased in association with disease recurrence, and BIRC3 expression increasing in response to temozolomide and irradiation therapy¹¹.

We provided evidence here that BIRC3, a molecule with the capacity to modulate the survival of cancer cells, is a differentially expressed gene in the tumors of patients treated with trastuzumab, expressed at significantly higher levels in the tumors of patients treated with trastuzumab, and that this is likely a direct transcriptional result of trastuzumab administration patients with breast cancer.

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Rank	ID	p-value	t	B	FC	Gene
55	GI_33946284-A-2	0.05236	1.995463	-4.36	1.72 ± 1.17	BIRC3

Table 1: BIRC3 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 BIRC3 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
8773	ILMN_1776181	3.53E-02	-2.187247	-4.0731	BIRC3

Table 2: A single, brief exposure to trastuzumab is sufficient to drive differential expression of BIRC3 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: BIRC3 is expressed at higher levels in the primary tumors of patients treated with trastuzumab

The mRNA expression level of BIRC3 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 BIRC3 expression in the primary tumors of patients treated with trastuzumab or not, a *p*-value, listed above.