The recombination activating gene 2, RAG2, 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 data^{3,4} to discover in an unbiased manner the most significant transcriptional changes associated with trastuzumab treatment. We identified the recombination activating gene, RAG2, as among those most differentially expressed in the primary tumors of patients with breast cancer treated with trastuzumab. A single administration of trastuzumab was sufficient to transcriptionally activate RAG2 in primary tumors of the breast, demonstrating increased expression of a molecule thought to possess cell type-specific expression in B-lymphocytes as a direct transcriptional consequence of treatment with trastuzumab.

Keywords: trastuzumab, breast cancer, RAG2, recombination activating gene 2, 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 data^{3,4}, we report here that RAG2 is a differentially expressed gene in the tumors of trastuzumab-treated patients.

Methods

We used datasets GSE114082³ and GSE20194⁴ for this differential gene expression analysis of the transcriptional response to trastuzumab treatment in patients with breast cancer. 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. GSE20194 was generated using Affymetrix Human Genome U133A Array with $n=256$ primary breast tumors from patients treated with chemotherapy and $n=12$ primary tumors from patients treated with chemotherapy and trastuzumab, analysis performed using platform GPL96. 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 RAG2 gene expression was significantly between primary breast tumors of patients before and after administration of a single dose of 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).

RAG2 is differentially expressed in the tumors of patients treated with trastuzumab.

We identified RAG2 as a differentially expressed gene in the primary tumors of patients treated with trastuzumab using a published microarray dataset. The tumors compared in this dataset were sampled from the same patient before and after administration of a single dose of trastuzumab, not comparing tumors from different patients treated with a course of trastuzumab or not. Thus, while differential expression of genes identified through comparison of tumors based on trastuzumab treatment can arise due to a variety of reasons, 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 likely to be a direct transcriptional result of trastuzumab binding events. We found that RAG2 was among the genes most differentially expressed in the tumors of patients administered a single treatment of trastuzumab (Table 1). When sorting each of the genes expressed in the tumors of patients administered a single treatment of trastuzumab, RAG2 ranked 59 out of 29377 total transcripts (Table 1), equating to 99.8% differential expression. Differential expression of RAG2 in the primary tumors of breast cancer patients following treatment with trastuzumab was statistically significant (Table 1; $p=4.88E-07$).

We turned to a second microarray dataset⁴ to validate our findings of RAG2 differential expression in the patients treated with trastuzumab. We found again that RAG2 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, RAG2 ranked 8552 out of 22283 total transcripts (Table 2), equating to 61.6% differential expression. Differential expression of RAG2 in the primary tumors of breast cancer patients following treatment with trastuzumab was not deemed statistically significant (Table 2; $p=3.56E-01$).

RAG2 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 RAG2 from tumors of patients before and after a single administration of trastuzumab to directly evaluate direction and statistical significance of change in expression of RAG2 based on history of trastuzumab treatment. RAG2 was expressed at higher levels in the tumors of patients following a single dose of trastuzumab, and this difference was statistically significant (Figure 2; $p=0.0019$).

Thus, we found using published microarray data^{3,4} that RAG2 was among the genes most differentially expressed in the tumors of patients treated with trastuzumab, that RAG2 was expressed at significantly higher levels in the tumors of patients following a single dose of 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

We provided evidence here that recombination activating gene RAG2 is a differentially expressed genes in the tumors of patients treated with trastuzumab, expressed at significantly higher levels in the following administration of a single dose of trastuzumab. These data demonstrate increased primary tumor expression of an enzyme with functions in rearrangement of immunoglobulin genes as a transcriptional consequence of treatment with trastuzumab in patients with breast cancer. We have recently demonstrated that the light chain gene segment IGKC, the heavy chain gene segment IGHV 4-31, the terminal deoxynucleotidyl transferase TdT, and the joining chain of multimeric IgA and IgM, JCHAIN are all among the genes whose expression is most significantly different, transcriptome-wide, in the brain metastases of patients with metastatic breast cancer as compared to benign or transformed tissues of the breast⁵⁻⁸. RAG2 is generally thought to function in concert with RAG1 during V(D)J rearrangement events⁹⁻¹¹. Only experimental evaluation can determine whether induction of RAG2 expression resulting from trastuzumab administration can ectopically trigger immature immunoglobulin rearrangement events in primary tumors of the breast.

References

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Rank	ID	p-value	t	B	FC	Gene
59	ILMN_1702072	4.88E-07	6.1202123	6.25465	8.27 ± 8.05	RAG2

Table 1: A single exposure to trastuzumab is sufficient to drive differential expression of RAG2 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.

Rank	ID	p-value	t	B	Gene	Gene name
8552	215117_at	3.56E-01	0.9252645	-5.5714	RAG2	recombination activating gene 2

Table 2: RAG2 is differentially expressed based on percentile-rank but not statistical significance in the primary tumors of patients with breast cancer 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: RAG2 is transcriptionally induced to a statistically significant extent in the primary tumors of patients treated with trastuzumab.

The mRNA expression level of RAG2 is graphically depicted in this chart in the primary tumors of breast cancer patients before treatment with trastuzumab (left) and in the primary tumors of breast cancer patients following administration of a single dose with trastuzumab (right), with mean mRNA expression level marked and the result of a statistical test evaluating significance of difference between RAG2 expression in the primary tumors of patients before and after administration of a single dose of trastuzumab, a *p*-value, listed above.