JARID1A and IGF2R are concomitantly up-regulated in trastuzumab-treated human breast cancers.

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Epigenetic regulation of gene expression is a mechanism by which signals transduced at the plasma membrane be can translated into differences in mRNA quantity and gene product at the protein level^{1,2}. Trastuzumab is widely utilized for the treatment of adjuvant and metastatic breast cancer³. We found, using analysis of public microarray and published multiplexed gene expression data^{4,5}, that the histone lysine H3K4 demethylase JARID1A was among the genes whose expression was most significantly different when comparing the global gene expression profiles of trastuzumab-treated breast cancers and breast cancers not treated with trastuzumab. Expression of JARID1A in cancer cells has been associated with a drug-tolerant state with reduced drug sensitivity, dependent on insulin-like growth factor (IGF) signaling⁶. Interestingly, we found that IGF2R was also among the genes whose expression was most significantly different transcriptome-wide when comparing the primary tumors of breast cancer patients treated with trastuzumab or not. These data clearly demonstrate up-regulation of a receptor for key growth factor signaling and together suggest that trastuzumab could be hard-wiring tumor cells for drug resistance.

Keywords: breast cancer, trastuzumab, JARID1A, IGF2R, systems biology of breast cancer, targeted therapeutics in breast cancer.

Trastuzumab is a monoclonal antibody targeted against the extracellular domain of the human epidermal growth factor receptor 2 (HER2)⁷. While the mechanism of action is thought to involve binding of trastuzumab to HER2⁷ and subsequent proposed decreases in cell proliferation, the transcriptional consequences of these binding events are less well understood.

We compared global transcription in the primary tumors of patients treated with trastuzumab and in patients not treated with trastuzumab using public and published data^{4,5} to understand in an unbiased fashion the genes whose expression was most significantly perturbed by trastuzumab administration.

We identified JARID1A/KDM5A as among the genes whose expression was most significantly different in the primary tumors of breast cancer patients treated with trastuzumab⁴. When sorting each of the genes expressed in the primary tumors of patients treated with trastuzumab based on the significance of change in expression as compared to primary tumors from patients not treated with trastuzumab, JARID1A ranked 101 out of 1506 total transcripts. Differential expression of JARID1A trended towards statistical significance ($p=0.078755$). We validated statistically significant differential expression of JARID1A in the primary tumors of breast cancer patients treated with trastuzumab using a published microarray dataset ($p=1.41E-05$). JARID1A was expressed at significantly higher levels in the primary tumors of patients treated with trastuzumab (Figure 1; $p=0.0213$). We calculated a mean fold change of 1.27 ± 0.30 in JARID1A expression in the primary breast tumors of trastuzumab-treated patients relative to those not treated with trastuzumab.

JARID1A is an H3K4 histone demethylase that has been associated with a “drug-tolerant” transcriptional program in cancer cells, operating through the IGF signaling pathway and resulting in approximately 100-fold decreased sensitivity to chemotherapy and cancer therapeutics⁶. We assessed using the same unbiased methodology whether any components of the IGF signaling pathway were similarly perturbed in human breast cancer in association with trastuzumab treatment.

We identified IGF2R as a differentially expressed gene in human breast cancers treated with trastuzumab. When sorting each of the genes expressed in primary tumors of the breast based on significance of change in expression in the primary tumors of patients treated with trastuzumab as compared to patients not treated with trastuzumab, IGF2R ranked 20 out of 1506 total transcripts. Differential expression of IGF2R in the primary tumors of breast cancer patients treated with trastuzumab was statistically significant ($p=0.02535$). We validated statistically significant differential expression of IGF2R in the primary tumors of breast cancer patients treated with trastuzumab using a published microarray dataset ($p=1.79E-04$). IGF2R was expressed at significantly higher levels in the primary tumors of patients treated with trastuzumab (Figure 2; $p=0.0292$). We calculated a mean fold change of 1.33 ± 0.48 in IGF2R expression in the primary breast tumors of trastuzumab-treated patients relative to those not treated with trastuzumab.

Fig. 1: Trastuzumab treatment is associated with increased expression of JARID1A in human breast cancers.

Fig. 2: Increased expression of IGF2R in the primary tumors of breast cancer patients treated with trastuzumab.

We provided here evidence, obtained through unbiased, systems-level comparative transcriptome analysis of primary tumors that treatment with trastuzumab in humans with breast cancer is associated with significantly increased expression of the H3K4 histone demethylase JARID1A and IGF2R.

Mice over-expressing IGF2 spontaneously develop metastatic mammary tumors; extreme caution should be utilized when administering trastuzumab in human breast cancer⁷. Together with clinical evidence demonstrating an alarming 34% incidence of central nervous system metastases in patients treated with trastuzumab, higher than in patients not treated with trastuzumab, and multiple lines of evidence that demonstrate activation of gene expression programs associated with proliferation, metastasis and growth factor and hormone signaling⁸⁻¹³, these data call for strict re-labeling of trastuzumab with warnings for the real risk of CNS metastasis events, as well as further investigation into the safety of trastuzumab use in humans.

Methods

Datasets GSE38057 and GSE130788 were utilized for this study in conjunction with GEO2R. GSE38057 was generated using DASL Human Cancer Panel by Probe technology with either $n=11$ primary breast tumors from patients treated not treated with trastuzumab and $n=36$ primary breast tumors from patients treated trastuzumab, both of which did not develop brain metastases, or $n=6$ primary breast tumors from patients treated not treated with trastuzumab and $n=35$ primary breast tumors from patients treated trastuzumab, both of which did develop brain metastases; analysis was performed using platform GPL5826. GSE130788 was generated using Whole Human Genome Microarray 4x44K G4112F technology with $n=110$ primary breast tumors from patients before trastuzumab treatment and $n=89$ primary tumors from patients after trastuzumab treatment, 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 JARID1A or IGF2R gene expression was significantly different between primary breast tumors in 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).

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