SLITRK3 is differentially expressed 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 data^{3,4} to discover in an unbiased manner the most significant transcriptional changes associated with trastuzumab treatment. We identified SLIT and NTRK-like family member 3 (encoded by *SLITRK3*) as among the genes most differentially expressed in the primary tumors of patients with breast cancer treated with trastuzumab. A single dose of trastuzumab was sufficient to result in differential expression of *SLITRK3* in the primary tumors of patients with breast cancer, suggesting that transcriptional induction of *SLITRK3* is a direct result of trastuzumab administration.

Keywords: trastuzumab, breast cancer, *SLITRK3*, SLIT and NTRK-like family member 3, 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 are less well understood. Through unbiased, systems-level comparative transcriptome analysis of published microarray data^{3,4}, we report here that SLITRK3 is a differentially expressed gene in the tumors of trastuzumab-treated patients: patients treated with a single dose of trastuzumab expressed significantly higher levels of SLITRK3 than before treatment with trastuzumab.

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 technology with n=256 samples of fine needle aspiration-isolated breast cancer cells of patients not treated with trastuzumab and n=12 samples of fine needle aspiration isolated breast cancer cells of patients treated with 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 SLITRK3 gene expression was significantly between primary breast tumors of patients receiving trastuzumab using a two-tailed t-test. 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 data^{3,4} to identify the most significant transcriptional features of the primary breast tumors of patients treated with trastuzumab (Herceptin).

SLITRK3 is differentially expressed in the tumors of patients treated with trastuzumab.

We identified SLIT and NTRK-like family member 3, encoded by SLITRK3 as among the genes whose expression changed most significantly in the tumors of breast cancer patients treated with trastuzumab in a microarray dataset³ (Table 1). The tumors compared in this dataset were sampled from the same patient before and after administration of a single dose of trastuzumab. 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 the same patients before a single administration of trastuzumab, SLITRK3 ranked 15 out of 29377 total transcripts (Table 1), equating to 99.9% differential expression. SLITRK3 differential expression in the tumors of patients following a single administration of trastuzumab was statistically significant (Chart 1; *p*=1.25E-07).

We queried a second microarray dataset to attempt to validate transcriptome-wide differential expression of SLITRK3 in the primary tumors of patients treated with trastuzumab. We again found that SLITRK3 was among the genes most differentially expressed in the tumors of patients treated with trastuzumab, but here, in fine needle-aspirated tumor specimens (Chart 2). When sorting each of the genes expressed in the tumor cells of patients treated with trastuzumab, SLITRK3 ranked 8555 out of 22283 total transcripts (Chart 2), equating to 61.6% differential expression, when compared to patients not

1 treated with trastuzumab. Differential expression of SLITRK3 in the tumor cells of breast cancer patients
2 treated with trastuzumab, in this dataset, was not deemed statistically significant (Chart 2; $p=3.14E-01$).

3 **SLITRK3 is expressed at significantly higher levels in the tumors of breast cancer patients following** 4 **a single administration of trastuzumab.**

5 We obtained exact mRNA expression levels for SLITRK3 from tumors of the same patients, before
6 and after a single administration of trastuzumab to directly evaluate direction and statistical significance of
7 change in expression of SLITRK3 following exposure to trastuzumab. SLITRK3 was expressed at higher
8 levels in the tumors of patients after treatment with trastuzumab as compared to before, and this difference
9 was statistically significant (Figure 1; $p=0.0002$). We calculated a mean fold change of 4.29 in SLITRK3
10 mRNA expression, as SLITRK3 was expressed at 58.87 ± 26.47 arbitrary units (AU) before
11 administration of trastuzumab, and at 252.29 ± 141.74 AU following administration of a single dose of
12 trastuzumab.

13 Thus, we found using published microarray data^{3,4} that SLITRK3 was among the genes most
14 differentially expressed in the tumors of patients following a single exposure to trastuzumab, with
15 significantly higher mRNA expression of SLITRK3 in the tumors of patients following administration of a
16 single dose of trastuzumab.

17 **Discussion**

18 We provided evidence here that SLITRK3 is a differentially expressed gene in the tumors of
19 patients treated with trastuzumab, and expressed at significantly higher levels in the primary tumors of
20 breast cancer patients treated with a single dose of trastuzumab as compared to the same patient tumors
21 before trastuzumab dosing. These data demonstrate that trastuzumab administration in patients with breast
22 cancer, a treatment according to one report associated with a 34% rate of brain metastasis⁵, results in
23 increased expression of a molecule that functions in axon guidance - exerting inhibitory influences on
24 synapse formation through interactions with axonally-localized protein tyrosine phosphatase PTP δ ⁶ - and
25 thus the spatially (anatomically) appropriate development of a neural network⁷. One group, demonstrating
26 the differential expression of the SLITRK gene family (including SLITRK3) in a variety of human brain
27 cancers, has suggested that expression of the SLITRKs be considered indicators of molecular properties
28 associated with the brain tumor⁸.

References

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Rank: 131
Probe ID: ILMN_1736969
p-value: 1.43E-06
t: -5.7691049
B: 5.24297
Gene: SLITRK3
Gene name: SLIT and NTRK-like family member 3

Chart 1: SLITRK3 is differentially expressed in the primary tumors of breast cancer patients treated with trastuzumab, and a single dose of trastuzumab is sufficient to result in differential expression of SLITRK3 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, fold change of expression in SLITRK3 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: 8555
Probe ID: 206732_at
p-value: 3.14E-01
t: -1.011538
B: -5.53913
Gene: SLITRK3

Chart 2: SLITRK3 is differentially expressed in the fine needle-aspirated tumor cells 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 SLITRK3 between the tumors of patients treated with trastuzumab and the tumors of patients not treated with trastuzumab, and gene are listed in this chart.

Figure 1: SLITRK3 is expressed at significantly higher levels with trastuzumab in the primary tumors of patients following a single administration of trastuzumab.

The mRNA expression level of SLITRK3 in the tumors of patients before treatment with trastuzumab (left) and in the primary tumors of patients after treatment with trastuzumab (right) is graphically depicted with mean mRNA level marked and the result of a statistical test evaluating significance of difference in mRNA expression between the groups, a *p*-value, is listed above.