Frizzled class receptor 7 is differentially expressed in the primary tumors of breast cancer patients treated with trastuzumab.

Shahan Mamoor, MS¹

¹shahanmamoor@gmail.com

East Islip, NY USA

Trastuzumab, a monoclonal antibody targeted against the human epidermal growth factor receptor 2 (HER2) is utilized for the treatment of human breast cancer^{1,2}, but a complete understanding of how tumor signal transduction is modulated by trastuzumab treatment is lacking. By mining published and public microarray and gene expression data^{3,4} from the primary tumors of patients treated with trastuzumab, we found that the Wnt pathway receptor frizzled-7, FZD7, was among the genes most differentially expressed in the primary tumors of patients treated with trastuzumab, and expressed at significantly higher levels in the tumors of patients treated with trastuzumab. Thus, the use of trastuzumab in patients with breast cancer is associated with increased expression of a signal transduction receptor that can support proliferation of triple negative breast cancer cells⁵ and is required for the maintenance of pluripotency of human embryonic stem cells⁶.

Keywords: breast cancer, trastuzumab, FZD7, frizzled-7, frizzled class receptor 7, systems biology of breast cancer, targeted therapeutics in breast cancer.

The mechanism of action by which trastuzumab exerts its therapeutic function in human breast cancer is said to be binding of the F_{ab} domain of trastuzumab to the extracellular region of the human epidermal growth factor receptor 2 (HER2)², possibly resulting in decreased cellular proliferation⁷. Transcriptional modulation resulting from trastuzumab binding events in the primary tumors of treated patients is less well understood. We mined published and public microarray and gene expression data^{3,4} generated using primary tissues from patients treated with trastuzumab to compare tumor global gene expression with that of patients not treated with trastuzumab. Using this unbiased, systems level methodology, we identified differential expression and up-regulation of FZD7 in primary tumors of the breast in patients treated with trastuzumab.

Methods

We used datasets GSE38057³ and GSE130788⁴ for 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 $n=11$ 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 FZD7 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 performed comparative transcriptome analysis of primary tumors from patients treated or not with trastuzumab (Herceptin), using published multiplexed gene expression data³ and a microarray dataset⁴, in order to identify the most significant transcriptional changes in the primary tumors of breast cancer patients following treatment with trastuzumab.

FZD7 is differentially expressed in the primary tumors of patients treated with trastuzumab.

We found that the frizzled class receptor 7, encoded by FZD7, was among the genes whose expression was most significantly different in the primary tumors of patients with breast cancer treated with trastuzumab³. When sorting each of the genes expressed in the primary tumors of breast cancer patients treated with trastuzumab based on significance of difference in expression as compared to the primary tumors of breast cancer patients not treated with trastuzumab, FZD7 ranked 66 out of 1506 total transcripts, equating to 95.6% statistical significance (Table 1). Differential expression of FZD7 in the primary tumors of breast cancer patients treated with trastuzumab was statistically significant (Table 1; $p=0.054427$).

We queried a second dataset⁶ to validate our findings of FZD7 differential expression upon trastuzumab treatment. In a second microarray dataset analyzing gene expression of patients treated with

trastuzumab, we again found that FZD7 was among the genes whose expression was most significantly different transcriptome-wide when comparing the global gene expression profiles of tumors from patients treated with trastuzumab as compared to those not treated with trastuzumab. When sorting each of the genes expressed in the tumors of patients treated with trastuzumab based on significance of change as compared to the tumors of patients not treated with trastuzumab, FZD7 ranked 7818 out of 41000 total transcripts, equating to 80.9% differential expression. Differential expression of FZD7 in the primary tumors of patients treated with trastuzumab was statistically significant (Table 2; $p=4.31E-09$).

FZD7 is expressed at higher levels in the tumors of patients treated with trastuzumab.

We obtained exact mRNA expression levels for FZD7 from the tumors of patients treated with trastuzumab and from the tumors of patients not receiving trastuzumab to determine magnitude and direction of FZD7 change in the tumors of patients treated with trastuzumab. FZD7 expression was higher in the tumors of patients treated with trastuzumab as compared to the tumors of patients not treated with trastuzumab (Figure 1). Increased expression of FZD7 was statistically significant (Figure 1: $p=0.0058$). We calculated a mean fold change of 1.46 ± 0.69 when comparing the expression of FZD7 in the tumors of patients treated with trastuzumab as compared to the tumors of patients treated with trastuzumab.

Thus, we found that the frizzled class receptor 7, encoded by FZD7, was among the genes most differentially expressed in the primary tumors of patients with breast cancer and that FZD7 expression was significantly higher in the primary tumors of patients treated with trastuzumab as compared to those not treated.

Discussion

We provided evidence here that the frizzled class receptor 7, encoded by FZD7 is expressed at higher levels in the tumors of patients treated with trastuzumab and among the genes most differentially expressed in the primary tumors of patients treated with trastuzumab. Thus, administration of trastuzumab in patients with breast cancer results in increased expression of a cell surface signal transduction receptor that is required for the pluripotency of human embryonic stem cells⁶.

References

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Rank	ID	p-value	t	B	FC	Gene
66	GI_4503832-S-1	0.054427	1.97105	-4	1.46 ± 0.69	FZD7

Table 1: FZD7 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 FZD7 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
7818	A_23_P209449	4.31E-09	6.143313	9.710516	FZD7	frizzled class receptor 7

Table 2: FZD7 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 FZD7 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: FZD7 is expressed at higher levels in the primary tumors of patients treated with trastuzumab as compared to those not treated with trastuzumab.

The mRNA expression level of FZD7 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 FZD7 expression in the primary tumors of patients treated with trastuzumab or not, a *p*-value, listed above.