Cyclin H is differentially expressed in the primary tumors of breast cancer patients treated with trastuzumab.

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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 regulatory subunit of the CDK-activating kinase (CAK)^{5,6}, cyclin H, encoded by CCNH, was among the genes most differentially expressed in the primary tumors of patients treated with trastuzumab. Thus, trastuzumab may likely be associated with transcriptional activation of a central cell cycle component⁵⁻⁸ that functions in phosphorylation of the carboxy-terminal domain of the RNA polymerase II⁹⁻¹¹ in primary tumors of patients with breast cancer.

Keywords: breast cancer, trastuzumab, CCNH, cyclin H, CAK, CDK-activating kinase, 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 proliferation⁸. 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 cyclin H (CCNH) 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 $n=6$ 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. 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 CCNH 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), published and public microarray and multiplexed gene expression data^{3,4}, in order to identify the most significant transcriptional changes in the primary tumors of breast cancer patients following treatment with trastuzumab.

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

We found that CCNH, 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 trastuzumab-treated breast tumors based on significance of difference in expression as compared to the primary breast tumors of patients not treated with trastuzumab, CCNH ranked 4 out of 1506 total transcripts, equating to 99.7% statistical significance (Table 1). Differential expression of CCNH in the primary tumors of breast cancer patients treated with trastuzumab was trended towards statistical significance (Table 1; $p=0.00624$).

We queried a second dataset⁴ to validate our findings of CCNH differential expression upon trastuzumab treatment. In a second microarray dataset analyzing gene expression of patients treated with trastuzumab, we again found that CCNH 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, CCNH ranked 6042 out of 32321 total transcripts, equating to 81.3% differential expression. Differential expression of CCNH in the primary tumors of patients treated with trastuzumab was statistically significant (Table 2; $p=5.98E-11$).

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

We obtained exact mRNA expression levels for CCNH from the tumors of patients treated with trastuzumab and from the tumors of patients not receiving trastuzumab to determine magnitude and direction of CCNH change in the tumors of patients treated with trastuzumab. CCNH expression was significantly 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 CCNH was statistically significant (Figure 1: $p=0.0358$). We calculated a mean fold change of 1.55 ± 0.59 when comparing the expression of CCNH in the tumors of patients treated with trastuzumab as compared to the tumors of patients treated with trastuzumab.

Thus, by mining published and public microarray and multiplexed gene expression data, found that cyclin H, encoded by CCNH, was among the genes most differentially expressed in the primary tumors of patients with breast cancer and that CCNH 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 cyclin H is expressed at significantly 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 primary tumor expression of a component of the CAK, CDK-activating kinase⁵⁻⁸, that has the ability to phosphorylate the carboxy-terminal domain of RNA polymerase II, a critical event for general transcriptional activation⁹⁻¹¹.

References

1. Slamon, D., Eiermann, W., Robert, N., Pienkowski, T., Martin, M., Press, M., Mackey, J., Glaspy, J., Chan, A., Pawlicki, M. and Pinter, T., 2011. Adjuvant trastuzumab in HER2-positive breast cancer. *New England Journal of Medicine*, 365(14), pp.1273-1283.
2. Hudis, C.A., 2007. Trastuzumab—mechanism of action and use in clinical practice. *New England Journal of Medicine*, 357(1), pp.39-51.
3. Duchnowska, R., Jassem, J., Goswami, C.P., Dundar, M., Gökmen-Polar, Y., Li, L., Woditschka, S., Biernat, W., Sosińska-Mielcarek, K., Czartoryska-Arlukowicz, B. and Radecka, B., 2015. Predicting early brain metastases based on clinicopathological factors and gene expression analysis in advanced HER2-positive breast cancer patients. *Journal of Neuro-oncology*, 122(1), pp.205-216.
4. GSE130788. A Phase II Randomized Trial of Neoadjuvant Trastuzumab or Lapatinib or the Combination of Trastuzumab and Lapatinib, Followed by Docetaxel and Carboplatin with Trastuzumab and/or Lapatinib in Patients with HER2+ Breast Cancer. [https:// www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE130788](https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE130788). Hsiaowang Chen. UCLA, Department of Medicine. Santa Monica, CA.
5. Kim, K.K., Chamberlin, H.M., Morgan, D.O. and Kim, S.H., 1996. Three-dimensional structure of human cyclin H, a positive regulator of the CDK-activating kinase. *Nature structural biology*, 3(10), pp.849-855.
6. Drapkin, R., Le Roy, G., Cho, H., Akoulitchiev, S. and Reinberg, D., 1996. Human cyclin-dependent kinase-activating kinase exists in three distinct complexes. *Proceedings of the National Academy of Sciences*, 93(13), pp.6488-6493.
7. Rickert, P., Corden, J.L. and Lees, E., 1999. Cyclin C/CDK8 and cyclin H/CDK7/p36 are biochemically distinct CTD kinases. *Oncogene*, 18(4), pp.1093-1102.
8. Patel, H., Abduljabbar, R., Lai, C.F., Periyasamy, M., Harrod, A., Gemma, C., Steel, J.H., Patel, N., Busonero, C., Jerjees, D. and Remenyi, J., 2016. Expression of CDK7, cyclin H, and MAT1 is elevated in breast cancer and is prognostic in estrogen receptor-positive breast cancer. *Clinical Cancer Research*, 22(23), pp.5929-5938.
9. Chapman, R.D., Heidemann, M., Albert, T.K., Mailhammer, R., Flatley, A., Meisterernst, M., Kremmer, E. and Eick, D., 2007. Transcribing RNA polymerase II is phosphorylated at CTD residue serine-7. *Science*, 318(5857), pp.1780-1782.
10. Mayer, A., Heidemann, M., Lidschreiber, M., Schreieck, A., Sun, M., Hintermair, C., Kremmer, E., Eick, D. and Cramer, P., 2012. CTD tyrosine phosphorylation impairs termination factor recruitment to RNA polymerase II. *Science*, 336(6089), pp.1723-1725.
11. Kim, H., Erickson, B., Luo, W., Seward, D., Graber, J.H., Pollock, D.D., Megee, P.C. and Bentley, D.L., 2010. Gene-specific RNA polymerase II phosphorylation and the CTD code. *Nature structural & molecular biology*, 17(10), p.1279.

Rank	ID	p-value	t	B	FC	Gene
4	GI_17738313-S-2	0.00624	2.876219	-4.07	1.55 ± 0.59	CCNH

Table 1: CCNH 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 CCNH 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
6042	A_23_P30338	5.98E-11	6.921175	13.902865	CCNH	cyclin H

Table 2: CCNH 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 CCNH 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: Cyclin H (CCNH) is expressed at significantly higher levels in the primary tumors of patients treated with trastuzumab as compared to those not treated with trastuzumab.

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