Significant up-regulation of the cyclin-dependent kinase CDK10 in primary tumors of patients treated with trastuzumab.

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Cancer is defined by uncontrolled cell division^{1,2}. Cell division is governed by the cell cycle, consisting in part of a system of molecules called cyclins, which are activated by cyclin-dependent kinases, operating together to orchestrate controlled progression through G1-, S-, G2-, and M-phases³. We report here that in human breast cancer, trastuzumab treatment is associated with significantly increased expression of the cyclin-dependent kinase CDK10. This piece of evidence, obtained through unbiased comparative transcriptome analysis of published data^{4,5}, continues to illustrate a portrait of a cancer therapeutic with molecular properties associated with proliferation and metastasis, reconciling a 34% incidence of central nervous system metastases⁶ in patients treated with what is considered a front-line treatment for adjuvant and metastatic breast cancer.

Keywords: breast cancer, cell cycle, CDK10, systems biology of breast cancer, targeted therapeutics in breast cancer.

Expression of cyclin-dependent kinases are critical for progression of the cell cycle. CDK inhibitors have recently been implemented into clinical use for the treatment of breast cancer⁷, including CDK4/6 inhibitors palbociclib⁸, ribociclib⁹, and abemaciclib¹⁰. The use of these inhibitors is predicated upon the concept that cancer cells express or over-express cyclin-dependent kinases to support their growth. We used systems-level, unbiased transcriptome analysis of published data^{4,5} to discover genes transcriptionally perturbed in primary tumors of patients treated with trastuzumab, a monoclonal antibody targeting HER2, finding not only that the cyclin-dependent kinase CDK10 was among the genes most differentially expressed in the tumors of patients treated with trastuzumab, but that it was expressed at significantly higher levels than in patients not treated with trastuzumab.

Methods

We used datasets GSE38057⁴ and GSE75685⁵ for this differential gene expression analysis of the transcriptional landscape of trastuzumab. GSE38057 was generated using DASL Human Cancer Panel by Probe technology with either $n=11$ 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. GSE75685 was generated using Whole Human Genome Microarray 4x44K technology with $n=41$ primary breast tumors from patients not treated with trastuzumab and $n=11$ primary tumors from patients treated with trastuzumab, analysis performed using platform GPL4133. 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 CDK10 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 systems-level comparative transcriptome analysis to describe in an unbiased fashion the most significant transcriptional features of trastuzumab treatment in patients with breast cancer, using published microarray and multiplexed gene expression data^{4,5}.

CDK10 is differentially expressed in the primary tumors of patients treated with trastuzumab.

We found that the gene encoding the cyclin dependent kinase 10, CDK10, was among the genes whose expression was most significantly different in the primary tumors of patients with breast cancer treated with trastuzumab⁴. 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, CDK10 ranked 53 out of 1506 total transcripts, ranking at 96.5% statistical significance (Table 1). Differential expression of CDK10 in the primary tumors of breast cancer patients treated with trastuzumab trended towards statistical significance (Table 1; $p=0.043892$).

We sought to validate our findings of CDK10 differential expression upon trastuzumab treatment in an independent dataset. In a second microarray dataset analyzing gene expression of patients treated with trastuzumab⁵, we again found that CDK10 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, CDK10 ranked 5479 out of 45220 total transcripts, equating to 87.9% differential expression. Differential expression of CDK10 was statistically significant (Table 2; $p=2.05E-02$).

CDK10 is expressed at significantly higher levels in the primary tumors of patients treated with trastuzumab as compared to that of patients not treated with trastuzumab.

We obtained exact mRNA expression levels for CDK10 from the tumors of patients treated with trastuzumab and from the tumors of patients not receiving trastuzumab to determine magnitude and direction of CDK10 change in the tumors of patients treated with trastuzumab. We were alarmed to find that CDK10 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 CDK10 was statistically significant (Figure 1: $p=0.0187$). It is difficult to determine at this point whether CDK10 is being transcriptionally induced by trastuzumab treatment or whether trastuzumab in some manner selects and enriches for the survival of tumor clones with high expression of CDK10. We calculated a mean fold change of 1.64 ± 1.14 when comparing the expression of CDK10 in the tumors of patients treated with trastuzumab as compared to the tumors of patients treated with trastuzumab.

Thus, we found that CDK10 was among the most significant transcriptional features of the primary tumors of breast cancer patients treated with trastuzumab, and that CDK10 was expressed at significantly increased levels in the primary tumors of patients treated with trastuzumab.

Discussion

We provided evidence here that messenger RNA for the cyclin-dependent kinase CDK10 is present in significantly higher quantities in the tumors of patients treated with trastuzumab than in the tumors of patients never treated with trastuzumab. CDK4/6 inhibition is currently utilized as a clinical tool for the treatment of human breast cancer. We showed here that CDK10 is over-expressed in the tumors of patients treated with trastuzumab; this is either a direct result of trastuzumab treatment (i.e., trastuzumab results in transcriptional induction of CDK10), or an evolutionary byproduct of trastuzumab treatment (i.e., trastuzumab treatment results in selection of tumor clones with higher expression of CDK10). In any case, CDK10 expression is strongly associated with resistance to endocrine (tamoxifen) therapy in human breast cancer¹¹. We argue that increased CDK10 expression associated with trastuzumab treatment is a dangerous consequence of trastuzumab administration in patients with breast cancer. It should now be considered, regardless of efficacy, whether the safety profile of trastuzumab⁶ is acceptable.

References

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Rank	ID	p-value	t	B	FC	Gene
53	GI_32528264-A-1	0.043892	2.06894	-3.91	1.64 ± 1.14	CDK10

Table 1: Differential expression of CDK10 in primary tumors of trastuzumab-treated 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 CDK10 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
5479	30348	2.05E-02	-2.3902025	-3.442759	CDK10	cyclin dependent kinase 10

Table 2: Differential expression of CDK10 in primary tumors of trastuzumab-treated 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.

Figure 1: CDK10 is expressed at significantly higher levels in the tumors of breast cancer patients treated with trastuzumab.

The mRNA expression level of CDK10 is graphically depicted in this chart in the primary tumors of breast cancer patients not treated with trastuzumab and in the primary tumors of breast cancer patients treated with trastuzumab, in standard scale (left) and scaled on natural logarithm (right) with mean mRNA expression level marked and the result of a statistical test evaluating significance of difference between CDK10 expression in the primary tumors of patients treated with trastuzumab or not, a *p*-value, listed above.