Differential expression of CDC28 protein kinase regulatory subunit 1B in triple negative breast cancer.

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Women diagnosed with triple negative breast cancer can benefit neither from endocrine therapy nor from HER2-targeted therapies¹. We mined published microarray datasets^{2,3} to determine in an unbiased fashion and at the systems level genes most differentially expressed in the primary tumors of patients with breast cancer. We report here significant differential expression of the gene encoding CDC28 protein kinase regulatory subunit 1B, CKS1B, when comparing the tumor cells of patients with triple negative breast cancer to normal mammary ductal cells². CKS1B was also differentially expressed in the brain metastases of patients with metastatic breast cancer³. CKS1B mRNA was present at significantly increased quantities in TNBC tumor cells relative to normal mammary ductal cells. Analysis of human survival data revealed that expression of CKS1B in primary tumors of the breast was correlated with distant metastasis-free survival in patients with luminal A type cancer, while within triple negative breast cancer, primary tumor expression of CKS1B was correlated with overall survival in patients with basal-like 2 and mesenchymal stem-like subtype disease. CKS1B may be of relevance to initiation, maintenance or progression of triple negative breast cancers.

Keywords: breast cancer, CKS1B, CDC28 protein kinase regulatory subunit 1B, systems biology of breast cancer, targeted therapeutics in breast cancer.

Patients with hormone receptor positive disease can benefit from endocrine therapy⁴: aromatase inhibition such as that provided by exemestane or letrozole, or estrogen receptor antagonism such as that provided by tamoxifen or fulvestrant. Patients whose tumors overexpress the human epidermal growth factor 2 (HER2) can be treated with HER2-targeted therapies: HER2 inhibitors, immunoglobulin⁵- or small molecule-based⁶. As triple negative breast cancer (TNBC) is defined by its lack of HER2 expression as well as by its lack of expression of the hormone receptor estrogen, patients diagnosed with triple negative breast cancer will not benefit from either of these treatment strategies.

Understanding the transcriptional composition of triple negative breast cancers in humans can facilitate development of novel therapeutics and contribute to efforts to prevent progression to metastatic stages. We mined published microarray data^{2,3} to understand at the transcriptome level and in an unbiased fashion genes most differentially expressed in the tumor cells of patients with triple negative breast cancer.

Methods

We utilized datasets GSE38959² and GSE52604³ for this differential gene expression analysis of female breast cancer in conjunction with GEO2R. GSE38959 was generated using Agilent-014850 Whole Human Genome Microarray 4x44K G4112F technology with $n=13$ samples of normal mammary gland ductal cells and $n=30$ samples of tumor cells from patients with triple negative breast cancer; analysis was performed using platform GPL4133. GSE52604 was generated using Agilent-014850 Whole Human Genome Microarray 4x44K G4112F with $n=10$ normal breast tissues and $n=35$ brain metastases from patients with breast cancer; analysis was 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 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 CKS1B expression was significantly different between primary breast tumors and breast tissue using a two-tailed t-test.

For Kaplan-Meier survival analysis, we used the Kaplan-Meier plotter tool⁷ for correlation of CKS1B mRNA expression levels with distant metastasis-free survival (DMFS) in $n=630$ patients with basal subtype cancer, $n=998$ patients with luminal A subtype cancer, $n=673$ patients with luminal B subtype cancer, $n=401$ patients with HER2+ cancer, and $n=63$ patients with normal-like subtype cancer. In patients with triple negative breast cancer⁷, we analyzed overall survival (OS) for $n=103$ patients with basal-like 1 subtype TNBC, $n=58$ patients with basal-like 2 subtype TNBC, $n=149$ patients with immunomodulatory subtype TNBC, $n=116$ patients with luminal androgen receptor subtype TNBC, $n=114$ patients with mesenchymal subtype TNBC, and $n=39$ patients with mesenchymal stem-like subtype TNBC.

Results

We performed discovery of genes associated with triple negative breast cancer in humans by mining two independently published microarray datasets^{2,3}.

CKS1B is differentially expressed in primary and metastatic states in human breast cancer.

Comparison of the tumor cells of patients with triple negative breast cancer to normal mammary ductal cells² revealed CDC28 protein kinase regulatory subunit 1B, encoded by CKS1B, as among the genes whose expression was most significantly different in human TNBC as compared to the normal breast (Chart 1). When sorting each of the genes expressed in the tumor cells of patients with triple negative breast cancer based on significance of difference as compared to normal mammary ductal cells, CKS1B ranked 245 out of 45015 total transcripts, equating to 99.5% differential expression (Chart 1). Differential expression of CKS1B in the tumor cells of patients with triple negative breast cancer was

statistically significant (Chart 1; $p=7.84\text{E-}11$).

Analysis of a second microarray dataset³, here studying global gene expression patterns in the brain metastases of patients with metastatic breast cancer, again revealed significant differential expression of CKS1B in tumor tissue of patients with human breast cancer (Chart 2). When sorting each of the genes expressed in the brain metastases of patients with breast cancer based on significance of difference as compared to normal breast tissue, CKS1B ranked 10137 out of 41093 total transcripts, equating to 75.3% differential expression (Chart 2). Differential expression of CKS1B in the brain metastases of patients with breast cancer was statistically significant (Chart 2; $p=5.28\text{E-}03$). These data suggested that differential expression of CKS1B was not an artifact of a single microarray dataset, nor was it strictly associated with primary tumors of the breast, rather a reflection of the spectrum of transformation in human cancer.

CKS1B is expressed at significantly higher levels in triple negative tumor cells as compared to normal cells of the breast.

We obtained exact mRNA expression levels for CKS1B - from normal mammary ductal cells of the breast and from tumor cells of patients with triple negative breast cancer - to understand the magnitude and direction of CKS1B expression change in TNBC. CKS1B mRNA transcript was present at higher levels in triple negative tumor cells of the breast as compared to normal mammary ductal cells (Figure 1). Increased expression of CKS1B in primary tumor-isolated triple negative breast cancer cells was statistically significant (Figure 1: $p<0.0001$). CKS1B was expressed at 1258.5 ± 1078.1 arbitrary units (A.U.) in normal mammary ductal cells, while it was expressed at 3967 ± 1501.7 A.U. in tumor cells of patients with triple negative breast cancer. We calculated a mean fold change of 3.2 when comparing triple negative breast cancer cells to normal mammary ductal cells.

CKS1B expression correlates with survival outcomes in luminal A human breast cancer.

We performed Kaplan-Meier survival analysis to evaluate potential relationships between primary tumor CKS1B mRNA expression levels and survival outcomes in patients with breast cancer, when analyzing outcomes based on the molecular subtype of the patient (PAM50 classification): luminal A, luminal B, HER2+, normal-like, and basal-like. We observed a correlation between CKS1B expression and distant metastasis-free survival (DMFS) in patients with luminal A subtype breast cancer which trended towards statistical significance (Figure 2; log rank p -value: 0.09 for distant metastasis-free survival, hazard ratio: 1.33 (0.96-1.85) (Fig. 2)).

CKS1B mRNA levels were a negative prognostic indicator in luminal A breast cancer patients. Median DMFS was 152.61 months for luminal A patients with low tumor expression of CKS1B while median DMFS was 140 months for luminal A patients with high tumor expression of CKS1B (Chart 3).

CKS1B primary tumor expression was not correlated with distant metastasis-free survival in basal subtype (Figure 2; log rank p -value: 0.46 for DMFS, hazard ratio: 0.89 (0.67-1.2) (Fig. 2)), luminal B subtype (Figure 2; log rank p -value: 0.17 for DMFS, hazard ratio: 1.23 (0.92-1.65) (Fig. 2)), in HER2+ breast cancer (Figure 2; log rank p -value: 0.72 for DMFS, hazard ratio: 0.94 (0.66-1.33) (Fig. 2)), or in the normal-like subtype (Figure 2; log rank p -value: 0.58 for DMFS, hazard ratio: 1.31 (0.5-3.4) (Fig. 2)).

Influence of primary tumor CKS1B expression on survival outcomes based on TNBC molecular subtype.

We also performed Kaplan-Meier survival analysis to examine relationships between CKS1B mRNA expression levels with survival strictly in patients with triple negative breast cancer, analyzing outcomes based on the TNBC molecular subtype of the patient: basal-like 1, basal-like 2,

immunomodulatory, mesenchymal, mesenchymal stem-like, and luminal androgen receptor. We observed a correlation between CKS1B expression and overall survival (OS) in TNBC patients with basal-like 2 subtype disease which trended towards statistical significance (Figure 3; log rank p -value: 0.093 for overall survival, hazard ratio: 0.42 (0.14-1.2) (Fig. 3)). CKS1B mRNA levels were a positive prognostic indicator in patients with basal-like 2 TNBC. OS was, on average, markedly shorter for basal-like 2 TNBC patients with low tumor expression of CKS1B as compared to basal-like 2 TNBC patients with high tumor expression of CKS1B (Chart 4).

We also observed a statistically significant correlation between CKS1B expression and overall survival (OS) in TNBC patients with mesenchymal stem-like subtype disease (Figure 3; log rank p -value: 0.014 for overall survival, hazard ratio: 3.86 (1.22-12.24) (Fig. 3)). CKS1B mRNA levels were a negative prognostic indicator in patients with mesenchymal stem-like TNBC. OS was, on average, markedly greater for mesenchymal stem-like TNBC patients with low tumor expression of CKS1B as compared to mesenchymal stem-like TNBC patients with high tumor expression of CKS1B (Fig. 3).

CKS1B primary tumor expression was not correlated with overall survival in basal-like 1 subtype (Figure 3; log rank p -value: 0.63 for OS, hazard ratio: 1.21 (0.56-2.62) (Fig. 3), immunomodulatory subtype (Figure 3; log rank p -value: 0.71 for OS, hazard ratio: 0.86 (0.39-1.89) (Fig. 3)), in the mesenchymal subtype (Figure 3; log rank p -value: 0.75 for OS, hazard ratio: 0.9 (0.46-1.73) (Fig. 3)), or in the luminal androgen receptor subtype (Figure 3; log rank p -value: 0.65 for OS, hazard ratio: 0.87 (0.47-1.59) (Fig. 3)).

Discussion

We performed comparative transcriptome analysis using two independently published microarray datasets^{2,3} to define and catalog transcriptional differences in human triple negative breast cancer. We provided evidence here that differential expression of CDC28 protein kinase regulatory subunit 1B, encoded by the CKS1B gene, is a defining transcriptional feature of triple negative breast cancer. CKS1B was also differentially expressed in brain metastasis in human breast cancer. Expression of CKS1B was correlated with distant metastasis-free survival in patients with luminal A subtype breast cancer, and within triple negative breast cancer patients, primary tumor expression of CKS1B was correlated with overall survival in patients with basal-like 2 and mesenchymal stem-like subtype disease. CDC28 protein kinase regulatory subunit 1B and the molecular processes to which it pertains to may be relevant to the initiation or progression of triple negative breast cancer in humans.

References

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Rank: 245
Probe ID: 12505
p-value: 7.84E-11
t: -8.4361285
B: 14.60419
Gene: CKS1B
Gene name: CDC28 protein kinase regulatory subunit 1B

Chart 1: CKS1B is differentially expressed in triple negative breast cancer.

Rank of differential expression, probe ID, p-value of global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the groups compared, gene and gene name are listed in this chart.

Rank: 10137
Probe ID: A_23_P45917
p-value: 5.28E-03
t: -2.925458
B: -2.772692
Gene: CKS1B
Gene name: CDC28 protein kinase regulatory subunit 1B

Chart 2: CKS1B is differentially expressed in brain metastasis in human metastatic breast cancer.

Rank of differential expression, probe ID, p-value of global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the groups compared, gene and gene name are listed in this chart.

Figure 1: CKS1B is expressed at significantly higher levels in primary TNBC tumor cells as compared to normal cells of the breast.

The mRNA expression level of CKS1B in normal mammary ductal cells (left) and in the isolated primary tumor cells of patients with triple negative breast cancer (right) is graphically depicted with the result of a statistical test evaluating significance of difference in CKS1B expression between normal cells of the breast and TNBC tumor cells, a *p*-value, listed above.

Figure 2: CKS1B expression correlates with distant metastasis-free survival in patients with luminal A breast cancer, but not in patients with basal, luminal B, HER2+, or normal-like subtype cancer.

Depicted in this Kaplan-Meier plot is the probability of distant metastasis-free survival (DMFS) for $n=630$ patients with basal subtype breast cancer (upper left), $n=401$ patients with HER2+ breast cancer (upper right), $n=998$ patients with luminal A breast cancer (middle left), $n=673$ patients with luminal B breast cancer (middle right), and $n=63$ patients with normal-like breast cancer (below) stratified into two groups, based on low or high expression of CKS1B in patient primary tumors. The log rank p -value denoting statistical significance of difference in distant metastasis-free survival when comparing the two groups, as well as hazard ratio for this comparison is listed above. Listed below is the number of patients at risk (number of patients alive) per interval, after stratification based on CKS1B expression; in the first interval, number at risk is number of patients alive; in each subsequent interval, number at risk is the number at risk less those who have expired or are censored.

1 Low CKS1B expression: 152.61 months

2 High CKS1B expression: 140 months

3 **Chart 3: Median distant metastasis-free survival is superior in luminal A subtype breast cancer**
4 **patients with low primary tumor expression of CKS1B.**

5 The distant metastasis-free survival of $n=998$ luminal A subtype breast cancer patients based on
6 stratification into low or high tumor expression of CKS1B is listed in this chart.

Figure 3: CKS1B expression correlates with overall survival in triple negative breast cancer patients whose tumors are of the basal-like 2 and mesenchymal stem-like molecular subtype.

Depicted in this Kaplan-Meier plot is the probability of overall survival (OS) for $n=103$ patients with basal-like 1 subtype TNBC (upper left), $n=58$ patients with basal-like 2 subtype TNBC (upper middle), $n=149$ patients with immunomodulatory subtype TNBC (upper right), $n=116$ patients with luminal androgen receptor subtype TNBC (lower left), $n=114$ patients with mesenchymal subtype TNBC (lower middle), and $n=39$ patients with mesenchymal stem-like subtype TNBC (lower right), stratified into two groups, based on low or high expression of CKS1B in the primary tumors of patients with TNBC. The log rank p-value denoting statistical significance of difference in overall survival when comparing the two groups, as well as hazard ratio for this comparison is listed above. Listed below is the number of patients at risk (number of patients alive) per interval, after stratification based on CKS1B expression; in the first interval, number at risk is number of patients alive; in each subsequent interval, number at risk is the number at risk less those who have expired or are censored.