Over-expression of cyclin B2 in human endometrial cancer.

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Gynecologic cancers including cancers of the endometrium are a clinical problem^{1,24}. We mined published microarray data^{5,6} to discover genes associated with endometrial cancers by comparing transcriptomes of the normal endometrium and endometrial tumors from humans. We identified the cell cycle component cyclin B2, encoded by CCNB2, as among the most differentially expressed genes, transcriptome-wide, in cancers of the endometrium. CCNB2 was expressed at significantly higher levels in endometrial tumor tissues as compared to the endometrium. Importantly, high primary tumor expression of CCNB2 was correlated with worse overall survival in patients with endometrial cancer. Together, the data reveal over-expression of CCNB2 in human endometrial cancer and describe specific activation of the cell cycle in cancers of the endometrium.

Keywords: endometrial cancer, gynecologic cancers, endometrium, CCNB2, cyclin B2, systems biology of endometrial cancer, targeted therapeutics in endometrial cancer.

Endometrial cancer is the most common gynecologic cancer in the developed world¹. Over the last three decades, the incidence of endometrial cancer has increased 21%⁴ and the death rate has increased 100%³. We harnessed the power of independently published microarray datasets^{5,6} to determine in an unbiased fashion and at the systems-level genes most differentially expressed in endometrial tumors. We report here the differential and increased expression of the cyclin B2 (CCNB2) in human endometrial cancer.

Methods

We utilized datasets GSE63678⁵ and GSE32507⁶ for this global differential gene expression analysis of human endometrial cancer in conjunction with GEO2R. GSE63678 was generated using Affymetrix Human Genome U133A 2.0 Array technology with $n=5$ control endometrial tissues (including $n=4$ uterine myomas and $n=1$ benign cyst) and $n=7$ endometrial cancers (including $n=2$ endometrial adenocarcinomas, $n=3$ mixed endometrioid adenocarcinomas, and $n=2$ adenocarcinomas with squamous differentiation); analysis was performed using platform GPL571. GSE32507 was generated using Agilent-014850 Whole Human Genome Microarray 4x44K G4112F technology with $n=24$ endometrioid adenocarcinoma and $n=8$ sarcoma of uterine corpus; 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 CCNB2 gene expression was significantly different between primary tumors of the breast and brain metastases in humans with breast cancer using a two-tailed t-test. For Kaplan-Meier survival analysis, we used the Kaplan-Meier plotter tool⁷ for correlation of CCNB2 mRNA expression levels with overall survival in $n=543$ endometrial cancer patients.

Results

We harnessed the power of blind comparative transcriptome analysis using published microarray data^{5,6} to discover in an unbiased fashion genes associated with endometrial cancer in humans.

CCNB2 is differentially expressed in endometrial cancer.

We identified cyclin B2, encoded by CCNB2, as among the genes most differentially expressed in cancers of the endometrium when compared to benign endometrial tissues (Chart 1). When sorting each of the genes expressed in endometrial tumor tissue based on significance of change in expression as compared to benign endometrial tissue, CCNB2 ranked 4 out of 22273 transcripts, equating to 99.98% differential expression (Chart 1). Differential expression of CCNB2 in human endometrial cancers was statistically significant (Chart 1; $p=4.95\text{e-}06$).

We queried a second microarray data to validate differential expression of CCNB2 in endometrial cancer. Again, we observed differential expression of CCNB2 when comparing endometrial tumor tissue to benign endometrial tissue (Chart 2). When sorting each of the genes expressed in endometrial tumor tissue based on significance of change in expression as compared to benign endometrial tissue, CCNB2 ranked 291 out of 54675 transcripts, equating to 99.5% differential expression (Chart 2). Differential expression of CCNB2 in human endometrial cancers trended towards statistical significance (Chart 2; $p=2.82e-06$).

CCNB2 is expressed at significantly higher levels in endometrial cancers as compared to benign endometrial tissue.

We obtained exact mRNA expression levels for CCNB2 in endometrial tumor tissues and from benign endometrial tissue to evaluate direction and statistical significance of change in expression of CCNB2 in human endometrial cancer. CCNB2 was expressed at higher levels in endometrial tissue as compared to normal endometrial tissue, and this difference was statistically significant (Figure 1; $p=0.000039$).

CCNB2 expression is correlated with patient survival outcomes in endometrial cancer.

We performed Kaplan-Meier survival analysis to evaluate correlation between CCNB2 primary tumor expression and survival outcomes in 543 endometrial cancer patients. We observed a correlation between primary tumor expression of CCNB2 and overall survival in patients with endometrial cancer (Figure 2). Patients whose primary tumors expressed low levels of CCNB2 possessed median OS of 78.4 months, while patients whose tumors expressed high levels of CCNB2 possessed median OS of 47.43 months. This difference in OS based on CCNB2 tumor expression in patients with endometrial cancer trended towards statistical significance (Figure 2, Chart 3; logrank p -value: 0.093; hazard ratio: 1.43 (0.94-2.17)).

Thus, by mining published microarray data^{5,6} in an unbiased and systematic fashion, we identified cyclin B2, encoded by CCNB2, as among the genes whose expression was most different, transcriptome-wide, in the endometrial tumor tissue of patients with endometrial cancer when compared to benign endometrial tissue; we observed significantly increased expression of CCNB2 in endometrial tumor tissue as compared to benign endometrial tissue. Further, we found a correlation between CCNB2 expression and patient survival outcomes in human endometrial cancer, as overall survival was greater in patients whose tumors expressed higher levels of CCNB2 as compared to patients whose tumors expressed lower levels of CCNB2.

Discussion

We provided evidence here that the cell cycle component cyclin B2 is among the genes most differentially expressed in human endometrial cancer, that mRNA for CCNB2 is present at significantly increased quantity in endometrial tumor tissue as compared to benign endometrium, and that CCNB2 primary tumor expression is correlated with overall survival in patients with endometrial cancer. These data, by demonstrating differential over-expression of cell cycle machinery, describe a specific mechanism by which the endometrial cell cycle is dysregulated, potentially to support transformation and/or tumor maintenance.

References

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Rank: 4
Probe ID: 202705_at
p-value: 4.95e-06
t: -7.7226405
B: 4.37164
Gene: CCNB2
Gene name: cyclin B2

Chart 1: CCNB2 is differentially expressed in endometrial cancer when comparing primary endometrial tumors to benign endometrial tissue.

The rank of global differential expression, probe/transcript ID, the *p*-value with respect to differential expression transcriptome-wide, *t*, a moderated t-statistic, *B*, the log-odds of differential expression between the groups compared, the gene and gene name are listed in this chart.

Rank: 291
probe ID: 202705_at
p-value: 2.82e-06
t: -4.9707725
B: 4.43263
Gene: CCNB2
Gene name: cyclin B2

Chart 2: CCNB2 is differentially expressed in endometrial cancer when comparing primary endometrial tumors to benign endometrial tissue.

The rank of global differential expression, probe/transcript ID, the *p*-value with respect to differential expression transcriptome-wide, *t*, a moderated *t*-statistic, *B*, the log-odds of differential expression between the groups compared, the gene and gene name are listed in this chart.

CCNB2: Cyclin B2

Figure 1: CCNB2 is expressed at significantly higher levels in the endometrial tumors of patients with endometrial cancer when compared to benign endometrium.

The mRNA expression level of CCNB2 in benign endometrial tissue (left) and in primary tumors of the endometrium (right) is graphically depicted; the result of a statistical test evaluating significance of difference in CCNB2 expression between benign endometrial tissue and primary tumors of the endometrial tissue is $p=0.000039$.

Figure 2: Correlation between CCNB2 primary tumor expression and overall survival in patients with endometrial cancer.

Depicted in this Kaplan-Meier plot is the probability of overall survival for $n=543$ total endometrial cancer patients stratified into two groups, based on low or high expression of CCNB2 in patient primary tumors, for patients in the lower survival quartile. 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 CCNB2 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.

Low CCNB2 expression: 78.4 months
High CCNB2 expression: 47.43 months

Chart 3: Median overall survival is inferior in endometrial cancer patients with high primary tumor expression of CCNB2.

The median OS (overall survival) of patients with low primary tumor expression of CCNB2 and high primary tumor expression of CCNB2 is listed in this chart, for $n=543$ endometrial cancer patients, in the lower survival quartile.