Over-expression of Cyclin D1 in human endometrial cancer.

Shahan Mamoor, MS¹

¹shahanmamoor@gmail.com

East Islip, NY USA

Gynecologic cancers including cancers of the endometrium are a clinical problem¹⁻⁴. 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 cell cycle machinery cyclin D1, encoded by CCND1, as among the most differentially expressed genes, transcriptome-wide, in cancers of the endometrium. CCND1 was expressed at significantly higher levels in endometrial tumor tissues as compared to the endometrium. Importantly, primary tumor expression of CCND1 was correlated with overall survival in patients with endometrial cancer. The data, in conjunction with our previous identification of cyclin B1⁷, cyclin B2⁸, CDK1⁹ and CDK5¹⁰ are over-expressed in endometrial cancer, illustrate grossly aberrant activation of the cell cycle in endometrial cancer.

Keywords: endometrial cancer, gynecologic cancers, endometrium, CCND1, cyclin D1, 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 cyclin D1 (CCND1) in human endometrial cancer.

Methods

We utilized datasets GSE63678⁵ and GSE115810⁶ 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. GSE115810 was generated using Affymetrix Human Genome U133A Array technology with $n=3$ control endometrial tissues and $n=24$ endometrial cancers; analysis was performed using platform GPL96. 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 CCND1 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 tool¹¹ for correlation of CCND1 mRNA expression levels with overall survival in $n=543$ endometrial cancer patients, with auto cut-off selected.

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.

CCND1 is differentially expressed in endometrial cancer.

We identified cyclin D1, encoded by CCND1, 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, CCND1 ranked 195 out of 22273 transcripts, equating to 99.1% differential expression (Chart 1). Differential expression of CCND1 in human endometrial cancers was statistically significant (Chart 1; $p=2.38e-04$).

We queried a second microarray data to validate differential expression of CCND1 in endometrial cancer. Again, we observed differential expression of CCND1 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, CCND1 ranked 4353 out of 22283 transcripts, equating to 80.5% differential expression (Chart 2). Differential expression of CCND1 in human endometrial cancers trended towards statistical significance (Chart 2; $p=0.0927016$).

CCND1 is expressed at significantly higher levels in endometrial cancers as compared to benign endometrial tissue.

We obtained exact mRNA expression levels for CCND1 in endometrial tumor tissues and from benign endometrial tissue to evaluate direction and statistical significance of change in expression of CCND1 in human endometrial cancer. We observed significantly higher expression of CCND1 in the endometrial tumor tissues of patients with endometrial cancer as compared to control endometrial tissues: CCND1 was expressed at 7.74 ± 0.61 arbitrary units (AU) in endometrial tumor tissues, while it was expressed at 9.72 ± 0.78 AU in control endometrial tissues (Figure 1). The difference in CCND1 mRNA levels between endometrial tumor tissues and control endometrial tissues was statistically significant (Figure 1; $p=0.000791$).

CCND1 expression is correlated with patient survival outcomes in endometrial cancer.

We performed Kaplan-Meier survival analysis to evaluate correlation between CCND1 primary tumor expression and survival outcomes in 543 patients with endometrial cancer. We observed a correlation between primary tumor expression of CCND1 and overall survival in patients with endometrial cancer, in the upper survival quartile (Figure 2). The difference in overall survival based on CCND1 tumor expression in patients with endometrial cancer was statistically significant, but with high false discovery rate (Figure 2, Chart 3; logrank p -value: 0.014; hazard ratio: 0.6 (0.39-0.9); FDR: >0.5).

Thus, by mining published microarray data^{5,6} in an unbiased and systematic fashion, we identified cyclin D1 encoded by CCND1, 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 CCND1 in endometrial tumor tissue as compared to benign endometrial tissue. Further, we found a statistically significant correlation between CCND1 expression and patient survival outcomes in human endometrial cancer, as overall survival was superior in patients whose tumors expressed higher levels of CCND1 as compared to patients whose tumors expressed lower levels of CCND1.

Discussion

We provided evidence here that the D1 cyclin is among the genes most differentially expressed in human endometrial cancer transcriptome-wide, that mRNA for CCND1 is present at significantly increased quantity in endometrial tumor tissue as compared to benign endometrium, and that increased CCND1 primary tumor expression is correlated with worse overall survival in patients with endometrial cancer. These data, together with our previous demonstrations that cyclin B1⁷, cyclin B2⁸, CDK1⁹ and CDK5¹⁰ are over-expressed in endometrial cancer, and among the genes whose expression is most significantly different transcriptome-wide between normal and malignant endometrial tissues, illustrate grossly aberrant activation of the cell cycle in endometrial cancer.

References

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Rank: 195
Probe ID: 208712_at
p-value: 2.38e-04
t: -5.1323846
B: 0.83255
Gene: CCND1
Gene name: cyclin D1

Chart 1: CCND1 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: 4353
probe ID: 208711_s_at
p-value: 0.0927016
t: -1.740716
B: -4.44379
Gene: CCND1
Gene name: cyclin D1

Chart 2: CCND1 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.

Figure 1: CCND1 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 CCND1 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 CCND1 expression between benign endometrial tissue and primary tumors of the endometrial tissue is $p=0.000791$.

Figure 2: Significant correlation between CCND1 primary tumor expression and overall survival in patients with endometrial cancer, but with high false discovery rate.

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 CCND1 in patient primary tumors, for patients in the upper 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 CCND1 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; the false discovery rate is >0.5 .

Low CCND1 expression: 36.87 months
High CCND1 expression: 108.37 months

Chart 3: Median overall survival is inferior in endometrial cancer patients with low tumor expression of CCND1.

The median overall survival of endometrial cancer patients expressing low levels of CCND1 or high levels of CCND1 in endometrial cancer tissue is listed in this chart, for $n=543$ endometrial cancer patients.