Over-expression of CDK5 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 the cyclin-dependent kinase CDK5, encoded by CDK5, as among the most differentially expressed genes, transcriptome-wide, in cancers of the endometrium. CDK5 was expressed at significantly higher levels in endometrial tumor tissues as compared to the endometrium. Importantly, high primary tumor expression of CDK5 was correlated with worse overall survival in white endometrial cancer patients with high mutational burden. The data illustrate a molecular mechanism by which the endometrial cell cycle is dysregulated in patients with endometrial cancer.

Keywords: endometrial cancer, gynecologic cancers, endometrium, CDK5, cyclin-dependent kinase 5, 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-dependent kinase 5 (CDK5) in human endometrial cancer.

Methods

We utilized datasets GSE63678⁵ and GSE17025⁶ 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. GSE17025 was generated using Affymetrix Human Genome U133 Plus 2.0 Array technology with $n=5$ normal endometrial tissues (inactive endometrium) and $n=91$ endometrial cancers; analysis was performed using platform GPL570. 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 CDK5 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 CDK5 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.

CDK5 is differentially expressed in endometrial cancer.

We identified cyclin-dependent kinase 5, encoded by CDK5, 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, CDK5 ranked 136 out of 22273 transcripts, equating to 99.4% differential expression (Chart 1). Differential expression of CDK5 in human endometrial cancers was statistically significant (Chart 1; $p=1.43\text{e-}04$).

We queried a second microarray data to validate differential expression of CDK5 in endometrial cancer. Again, we observed differential expression of CDK5 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, CDK5 ranked 5556 out of 54675 transcripts, equating to 89.8% differential expression (Chart 2). Differential expression of CDK5 in human endometrial cancers was statistically significant (Chart 2; $p=1.22\text{e-}02$).

CDK5 is expressed at significantly higher levels in endometrial cancers as compared to benign endometrial tissue.

We obtained exact mRNA expression levels for CDK5 in endometrial tumor tissues and from benign endometrial tissue to evaluate direction and statistical significance of change in expression of CDK5 in human endometrial cancer. We observed significantly higher expression of CDK5 in the endometrial tumor tissues of patients with endometrial cancer as compared to control endometrial tissues: CDK5 was expressed at 6.63 ± 0.57 arbitrary units (AU) in endometrial tumor tissues, while it was expressed at 7.82 ± 0.22 AU in control endometrial tissues (Figure 1). The difference in CDK5 mRNA levels between endometrial tumor tissues and control endometrial tissues was statistically significant (Figure 1; $p=0.001035$).

CDK5 expression is correlated with patient survival outcomes in endometrial cancer.

We performed Kaplan-Meier survival analysis to evaluate correlation between CDK5 primary tumor expression and survival outcomes in 543 patients with endometrial cancer. We observed a correlation between primary tumor expression of CDK5 and overall survival in patients with endometrial cancer, in white women with high mutational burden (Figure 2). The difference in overall survival based on CDK5 tumor expression in patients with endometrial cancer trended was statistically significant (Figure 2, Chart 3; logrank p -value: 0.013; hazard ratio: 2.89 (1.2-6.93)).

Thus, by mining published microarray data^{5,6} in an unbiased and systematic fashion, we identified cyclin dependent kinase 5 encoded by CDK5, 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 CDK5 in endometrial tumor tissue as compared to benign endometrial tissue. Further, we found a statistically significant correlation between CDK5 expression and patient survival outcomes in human endometrial cancer, as overall survival was superior in white patients with high mutational burden whose tumors expressed lower levels of CDK5 as compared to patients whose tumors expressed higher levels of CDK5.

Discussion

We provided evidence here that the cyclin dependent kinase CDK5 is among the genes most differentially expressed in human endometrial cancer transcriptome-wide, that mRNA for CDK5 is present at significantly increased quantity in endometrial tumor tissue as compared to benign endometrium, and that increased CDK5 primary tumor expression is correlated with worse overall survival in patients with endometrial cancer. We have previously demonstrated transcriptome-wide differential and increased expression of CDK1⁸, cyclin B1⁹ and cyclin B2¹⁰ in human endometrial cancer. These data illustrate specific molecular mechanisms by which the cell cycle is dysregulated in human endometrial cancer.

References

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Rank: 136
Probe ID: 204247_s_at
p-value: 1.43e-04
t: -5.4409029
B: 1.31053
Gene: CDK5
Gene name: cyclin dependent kinase 5

Chart 1: CDK5 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: 5556
probe ID: 204247_s_at
p-value: 1.22e-02
t: -2.5544867
B: -3.15918
Gene: CDK5
Gene name: cyclin dependent kinase 5

Chart 2: CDK5 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: CDK5 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 CDK5 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 CDK5 expression between benign endometrial tissue and primary tumors of the endometrial tissue is $p=0.001035$.

Figure 2: Significant correlation between CDK5 primary tumor expression and overall survival in patients with endometrial cancer, in white women with high mutational burden.

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 CDK5 in patient primary tumors, for white patients with high mutational burden. 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 CDK5 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 CDK5 expression: 108.37 months
High CDK5 expression: 34.83 months

Chart 3: Median overall survival is inferior in endometrial cancer patients with high tumor expression of CDK5.

The median overall survival of endometrial cancer patients expressing low levels of CDK5 or high levels of CDK5 in endometrial cancer tissue is listed in this chart, for $n=543$ endometrial cancer patients, in white patients with high mutational burden.