Over-expression of cyclin dependent kinase inhibitor 3 in human endometrial cancer.

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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 cyclin dependent kinase inhibitor 3, encoded by CDKN3, as among the most differentially expressed genes, transcriptome-wide, in cancers of the endometrium. CDKN3 was expressed at significantly higher levels in endometrial tumor tissues as compared to the endometrium. Importantly, in human endometrial cancer, primary tumor expression of CDKN3 was correlated with overall survival in white patients with low mutational burden. CDKN3 may be a molecule of interest in understanding the etiology or progression of human endometrial cancer.

Keywords: endometrial cancer, gynecologic cancers, endometrium, CDKN3, cyclin dependent kinase inhibitor 3, 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 inhibitor 3 (CDKN3) 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 CDKN3 gene expression was significantly different between control endometrial tissue and endometrial tumor tissue in humans using a two-tailed t-test. For Kaplan-Meier survival analysis, we used the Kaplan-Meier plotter tool⁷ for correlation of CDKN3 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.

CDKN3 is differentially expressed in endometrial cancer.

We identified cyclin dependent kinase inhibitor 3, encoded by CDKN3, 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, CDKN3 ranked 122 out of 22273 transcripts, equating to 99.5% differential expression (Chart 1). Differential expression of CDKN3 in human endometrial cancers was statistically significant (Chart 1; $p=1.22E-04$).

We queried a second microarray data to validate differential expression of CDKN3 in endometrial cancer. Again, we observed differential expression of CDKN3 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, CDKN3 ranked 459 out of 54675 transcripts, equating to 99.2% differential expression (Chart 2). Differential expression of CDKN3 in human endometrial cancers was statistically significant (Chart 2; $p=1.14E-05$).

CDKN3 is expressed at significantly higher levels in endometrial cancers as compared to benign endometrial tissue.

We obtained exact mRNA expression levels for CDKN3 in endometrial tumor tissues and from benign endometrial tissue to evaluate direction and statistical significance of change in expression of CDKN3 in human endometrial cancer. CDKN3 was expressed at higher levels in endometrial tissue as compared to normal endometrial tissue, and this difference was statistically significant (Figure 1; $p=0.0004$). We calculated a mean fold change of 1.38 in CDKN3 mRNA levels in human endometrial cancer, as CDKN3 was expressed at 5.51 ± 0.30 arbitrary units (A.U.) in control endometrial tissue but at 7.63 ± 0.88 A.U. in endometrial tumor tissue.

CDKN3 expression is correlated with patient survival outcomes in endometrial cancer.

We performed Kaplan-Meier survival analysis to evaluate correlation between CDKN3 primary tumor expression and survival outcomes in 543 patients with endometrial cancer. We observed a correlation between primary tumor expression of CDKN3 and overall survival in patients with endometrial cancer, in white patients with low mutational burden (Figure 2). CDKN3 primary tumor mRNA levels were a negative prognostic indicator in white endometrial cancer patients with low mutational burden. White patients with low mutational burden whose primary tumors expressed low levels of CDKN3 possessed median OS of 78.4 months, while white patients with low mutational burden whose tumors expressed high levels of CDKN3 possessed median OS of 32.97 months. This difference in OS based on CDKN3 tumor expression in white patients with endometrial cancer with low mutational burden was statistically significant (Figure 2, Chart 3; logrank p -value: 0.001; hazard ratio: 3.36 (1.56-7.24)). CDKN3 primary endometrial tumor expression was not correlated with overall survival in white patients with high mutational burden (Figure 2, Chart 3; logrank p -value: 0.7; hazard ratio: 1.17 (0.53-2.57)), nor in black patients with high ((Figure 2, Chart 3; logrank p -value: 0.58; hazard ratio: 1.61 (0.29-8.84)) or low mutational burden (Figure 2, Chart 3; logrank p -value: 0.22; hazard ratio: 2.02 (0.65-6.33)).

Thus, by mining published microarray data^{5,6} in an unbiased and systematic fashion, we identified cyclin dependent kinase inhibitor 3, encoded by CDKN3, as among the genes whose expression was most different, transcriptome-wide, in the endometrial tumor tissue of patients

1 with endometrial cancer when compared to benign endometrial tissue; we observed
2 significantly increased expression of CDKN3 in endometrial tumor tissue as compared to benign
3 endometrial tissue. Further, we found a correlation between CDKN3 expression and patient
4 survival outcomes in human endometrial cancer, as overall survival was inferior in patients
5 whose tumors expressed higher levels of CDKN3 as compared to patients whose tumors
6 expressed lower levels of CDKN3, in white patients with low mutational burden, but not in white
7 patients with high mutational burden, nor in black patients with high or low mutational burden.

8 **Discussion**

9 We provided evidence here that cyclin dependent kinase inhibitor 3 is among the genes
10 most differentially expressed in human endometrial cancer, that mRNA for CDKN3 is present at
11 significantly increased quantity in endometrial tumor tissue as compared to benign endometrium,
12 and that CDKN3 primary tumor expression is correlated with overall survival in white
13 endometrial cancer patients with low mutational burden. These data suggest CDKN3 may be of
14 importance to fundamental biological processes that underlie the initiation, progression or
15 maintenance of human endometrial cancer.
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Rank: 122
Probe ID: 209714_s_at
p-value: 1.22E-04
t: -5.5396956
B: 1.46036
Gene: CDKN3
Gene name: cyclin dependent kinase inhibitor 3

Chart 1: CDKN3 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: 459
probe ID: 209714_s_at
p-value: 1.14E-05
t: -4.6241487
B: 3.1355
Gene: CDKN3
Gene name: cyclin dependent kinase inhibitor 3

Chart 2: CDKN3 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: CDKN3 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 CDKN3 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 CDKN3 expression between benign endometrial tissue and primary tumors of the endometrial tissue is $p=0.0004$.

Figure 2: Correlation between CDKN3 primary tumor expression and overall survival in endometrial cancer, in white patients with low 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 CDKN3 in patient primary tumors, in black patients with high mutational burden (top left), black patients with low mutational burden (top right), white patients with high mutational burden (bottom left), and white patients with low mutational burden (bottom right). 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 CDKN3 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 CDKN3 expression: 78.4 months
High CDKN3 expression: 32.97 months

Chart 3: Median overall survival is superior in endometrial cancer patients with low primary tumor expression of CDKN3, in white patients with low mutational burden.

The median OS (overall survival) of white endometrial cancer patients with low mutational burden, with low primary tumor expression of CDKN3 and high primary tumor expression of CDKN3 is listed in this chart.