Over-expression of RNA polymerase II subunit L 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 RNA polymerase II subunit L, encoded by POLR2L, as among the most differentially expressed genes, transcriptome-wide, in cancers of the endometrium. POLR2L was expressed at significantly higher levels in endometrial tumor tissues as compared to the endometrium. Importantly, primary tumor expression of POLR2L was correlated with recurrence-free survival in patients with endometrial cancer. POLR2L may be a molecule of interest in understanding the etiology or progression of human endometrial cancer.

Keywords: endometrial cancer, gynecologic cancers, endometrium, POLR2L, RNA polymerase II subunit L, 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 RNA polymerase II subunit L (POLR2L) 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 POLR2L 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 POLR2L mRNA expression levels with recurrence-free 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.

POLR2L is differentially expressed in endometrial cancer.

We identified RNA polymerase II subunit L, encoded by POLR2L, 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, POLR2L ranked 53 out of 22273 transcripts, equating to 99.8% differential expression (Chart 1).

Differential expression of POLR2L in human endometrial cancers was statistically significant (Chart 1; $p=3.97E-05$).

We queried a second microarray data to validate differential expression of POLR2L in endometrial cancer. Again, we observed differential expression of POLR2L 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, POLR2L ranked 7515 out of 22283 transcripts, equating to 66.3% differential expression (Chart 2). Differential expression of POLR2L in human endometrial cancers was not deemed statistically significant (Chart 2; $p=0.2067676$).

POLR2L is expressed at significantly higher levels in endometrial cancers as compared to benign endometrial tissue.

We obtained exact mRNA expression levels for POLR2L in endometrial tumor tissues and from benign endometrial tissue to evaluate direction and statistical significance of change in expression of POLR2L in human endometrial cancer. POLR2L was expressed at higher levels in endometrial tissue as compared to normal endometrial tissue, and this difference was statistically significant (Figure 1; $p<0.0001$). We calculated a mean fold change of 1.08 in POLR2L mRNA levels in human endometrial cancer, as POLR2L was expressed at 10.35 ± 0.08 arbitrary units (A.U.) in control endometrial tissue but at 11.16 ± 0.25 A.U. in endometrial tumor tissue.

POLR2L expression is correlated with patient survival outcomes in endometrial cancer.

We performed Kaplan-Meier survival analysis to evaluate correlation between POLR2L primary tumor expression and survival outcomes in 543 patients with endometrial cancer. We observed a correlation between primary tumor expression of POLR2L and recurrence-free survival (RFS) in patients with endometrial cancer, in the lower survival tertile (Figure 2). POLR2L mRNA levels were a positive prognostic indicator in patients with endometrial cancer. Patients whose primary tumors expressed low levels of POLR2L possessed, on average, markedly shorter median RFS as compared to patients whose tumors expressed high levels of POLR2L (Figure 2). This difference in RFS based on POLR2L tumor expression in patients with endometrial cancer was statistically significant (Figure 2, Chart 3; logrank p -value: 0.021; hazard ratio: 0.54 (0.32-0.92)).

Thus, by mining published microarray data^{5,6} in an unbiased and systematic fashion, we identified RNA polymerase II subunit L, encoded by POLR2L, 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 POLR2L in endometrial tumor tissue as compared to benign endometrial tissue. Further, we found a correlation between POLR2L expression and patient survival outcomes in human endometrial cancer, as recurrence-free survival was superior in patients

1 whose tumors expressed higher levels of POLR2L as compared to patients whose tumors
2 expressed lower levels of POLR2L.

3 **Discussion**

4 We provided evidence here that RNA polymerase II subunit L is among the genes most
5 differentially expressed in human endometrial cancer, that mRNA for POLR2L is present at
6 significantly increased quantity in endometrial tumor tissue as compared to benign endometrium,
7 and that POLR2L primary tumor expression is correlated with recurrence-free survival in patients
8 with endometrial cancer. These data suggest POLR2L may be of importance to fundamental
9 biological processes that underlie the initiation, progression or maintenance of human
10 endometrial cancer.
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Rank: 53
Probe ID: 211730_s_at
p-value: 3.97E-05
t: -6.2556815
B: 2.49881
Gene: POLR2L
Gene name: RNA polymerase II subunit L

Chart 1: POLR2L 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: 7515
probe ID: 202586_at
p-value: 0.2067676
t: -1.292412
B: -5.02963
Gene: POLR2L
Gene name: RNA polymerase II subunit L

Chart 2: POLR2L 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: POLR2L 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 POLR2L 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 POLR2L expression between benign endometrial tissue and primary tumors of the endometrial tissue is $p < 0.0001$.

Figure 2: Correlation between POLR2L primary tumor expression and recurrence-free survival in patients with endometrial cancer.

Depicted in this Kaplan-Meier plot is the probability of recurrence-free survival for $n=543$ total endometrial cancer patients stratified into two groups, based on low or high expression of POLR2L in patient primary tumors, in the lower survival tertile. The log rank p -value denoting statistical significance of difference in recurrence-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 POLR2L 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.