Over-expression of pyrophosphatase (inorganic) 1 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 pyrophosphatase (inorganic) 1, encoded by PPA1, as among the most differentially expressed genes, transcriptome-wide, in cancers of the endometrium. PPA1 was expressed at significantly higher levels in endometrial tumor tissues as compared to the endometrium. Importantly, in human endometrial cancer, primary tumor expression of PPA1 was correlated with recurrence-free survival in black patients with low mutational burden. PPA1 may be a molecule of interest in understanding the etiology or progression of human endometrial cancer.

Keywords: endometrial cancer, gynecologic cancers, endometrium, PPA1, pyrophosphatase (inorganic) 1, 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 integrative microarray dataset analysis, published and public^{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 pyrophosphatase (inorganic) 1 (PPA1) in human endometrial cancer.

Methods

We utilized datasets GSE63678⁵ and GSE39099⁶ 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. GSE39099 was generated using Affymetrix Human Genome U133 Plus 2.0 Array technology with $n=2$ benign endometrial tissues ($n=1$ normal endometrium and $n=1$ atypical endometrium) and $n=2$ endometrial cancers ($n=1$ early stage endometrial cancer and $n=1$ advanced stage endometrial cancer); 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 PPA1 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 PPA1 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.

PPA1 is differentially expressed in endometrial cancer.

We identified pyrophosphatase (inorganic) 1, encoded by PPA1, 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, PPA1 ranked 102 out of 22273 transcripts, equating to 99.5% differential expression (Chart 1). Differential expression of PPA1 in human endometrial cancers was statistically significant (Chart 1;

$p=9.22\text{E-}05$).

We queried a second microarray data to validate differential expression of PPA1 in endometrial cancer. Again, we observed differential expression of PPA1 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, PPA1 ranked 20937 out of 54674 transcripts, equating to 61.7% differential expression (Chart 2). Differential expression of PPA1 in human endometrial cancers was not deemed statistically significant (Chart 2; $p=0.333246$).

PPA1 is expressed at significantly higher levels in endometrial cancers as compared to benign endometrial tissue.

We obtained exact mRNA expression levels for PPA1 in endometrial tumor tissues and from benign endometrial tissue to evaluate direction and statistical significance of change in expression of PPA1 in human endometrial cancer. PPA1 was expressed at higher levels in endometrial tissue as compared to normal endometrial tissue, and this difference was statistically significant (Figure 1; $p=0.0002$). We calculated a mean fold change of 1.13 in PPA1 mRNA levels in human endometrial cancer, as PPA1 was expressed at 11.15 ± 0.29 arbitrary units (A.U.) in control endometrial tissue but at 12.57 ± 0.53 A.U. in endometrial tumor tissue.

PPA1 expression is correlated with patient survival outcomes in endometrial cancer.

We performed Kaplan-Meier survival analysis to evaluate correlation between PPA1 primary tumor expression and survival outcomes in 543 patients with endometrial cancer. We observed a correlation between primary tumor expression of PPA1 and recurrence-free survival in patients with endometrial cancer, in black patients with low mutational burden (Figure 2). PPA1 primary tumor mRNA levels were a positive prognostic indicator in black endometrial cancer patients with low mutational burden. Black patients with low mutational burden whose primary tumors expressed low levels of PPA1 possessed median RFS of 26.37 months, while black patients with low mutational burden whose tumors expressed high levels of PPA1 possessed median RFS of 48.43 months. This difference in RFS based on PPA1 tumor expression in black patients with endometrial cancer with low mutational burden approached statistical significance (Figure 2, Chart 3; logrank p -value: 0.081; hazard ratio: 0.18 (0.02-1.55)). PPA1 primary endometrial tumor expression was not correlated with recurrence-free survival in black patients with high mutational burden (Figure 2, Chart 3; logrank p -value: 0.95; hazard ratio: 0.94 (0.16-5.66)), nor in white patients with high ((Figure 2, Chart 3; logrank p -value: 0.3; hazard ratio: 0.61 (0.24-1.58)) or low mutational burden (Figure 2, Chart 3; logrank p -value: 0.58; hazard ratio: 1.27 (0.54-3.02)).

Thus, by mining published and public microarray data^{5,6} in an unbiased and systematic fashion, we identified pyrophosphatase (inorganic) 1, encoded by PPA1, 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 PPA1 in endometrial tumor tissue as compared to benign endometrial tissue. Further, we found a correlation between PPA1 expression and patient survival outcomes in human endometrial cancer, as recurrence-free survival was superior in patients whose tumors expressed higher levels of PPA1 as compared to patients whose tumors expressed lower levels of PPA1, in black patients with low mutational burden, but not in black patients with high mutational burden, nor in white patients with high or low mutational burden.

Discussion

We provided evidence here that pyrophosphatase (inorganic) 1 is among the genes most differentially expressed in human endometrial cancer, that mRNA for PPA1 is present at significantly increased quantity in endometrial tumor tissue as compared to benign endometrium, and that PPA1 primary tumor expression is correlated with recurrence-free survival in black endometrial cancer patients with low mutational burden. These data suggest PPA1 may be of importance to fundamental biological processes that underlie the initiation, progression or maintenance of human endometrial cancer.

References

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Rank: 102
Probe ID: 217848_s_at
p-value: 9.22E-05
t: -5.7129255
B: 1.71928
Gene: PPA1
Gene name: pyrophosphatase (inorganic) 1

Chart 1: PPA1 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: 20937
probe ID: 217848_s_at
p-value: 0.333246
t: -1.0823883
B: -4.83
Gene: PPA1
Gene name: pyrophosphatase (inorganic) 1

Chart 2: PPA1 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: PPA1 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 PPA1 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 PPA1 expression between benign endometrial tissue and primary tumors of the endometrial tissue is $p=0.0002$.

Figure 2: Correlation between PPA1 primary tumor expression and recurrence-free survival in endometrial cancer, in black patients with low mutational burden.

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 PPA1 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 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 PPA1 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 PPA1 expression: 26.37 months

High PPA1 expression: 48.43 months

Chart 3: Median recurrence-free survival is superior in endometrial cancer patients with high primary tumor expression of PPA1, in black patients with low mutational burden.

The median RFS (recurrence-free survival) of black endometrial cancer patients with low mutational burden, with low primary tumor expression of PPA1 and high primary tumor expression of PPA1 is listed in this chart.