Over-expression of glyceraldehyde-3-phosphate dehydrogenase 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 glyceraldehyde-3-phosphate dehydrogenase, encoded by GAPDH, as among the most differentially expressed genes, transcriptome-wide, in cancers of the endometrium. GAPDH was expressed at significantly higher levels in endometrial tumor tissues as compared to the endometrium. Importantly, in human endometrial cancer, primary tumor expression of GAPDH was correlated with recurrence-free survival in white patients with low mutational burden. GAPDH may be a molecule of interest in understanding the etiology or progression of human endometrial cancer. These data argue that GAPDH should not be utilized as an internal reference or normalization gene for quantitative real-time polymerase chain reaction measurement of target depletion in loss-of-function genetic analyses in human endometrial cancer.

Keywords: endometrial cancer, gynecologic cancers, endometrium, GAPDH, glyceraldehyde-3-phosphate dehydrogenase, 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 glyceraldehyde-3-phosphate dehydrogenase (GAPDH) 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 GAPDH 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 GAPDH 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.

GAPDH is differentially expressed in endometrial cancer.

We identified glyceraldehyde-3-phosphate dehydrogenase, encoded by GAPDH, 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, GAPDH ranked 140 out of 22273 transcripts, equating to 99.4% differential expression (Chart 1). Differential expression of GAPDH in human endometrial cancers was statistically significant (Chart 1; $p=1.52E-04$).

We queried a second microarray data to validate differential expression of GAPDH in endometrial cancer. Again, we observed differential expression of GAPDH 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, GAPDH ranked 3603 out of 54675 transcripts, equating to 93.4% differential expression (Chart 2). Differential expression of GAPDH in human endometrial cancers was statistically significant (Chart 2; $p=4.20E-03$).

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

We obtained exact mRNA expression levels for GAPDH in endometrial tumor tissues and from benign endometrial tissue to evaluate direction and statistical significance of change in expression of GAPDH in human endometrial cancer. GAPDH was expressed at higher levels in endometrial tissue as compared to normal endometrial tissue, and this difference was statistically significant (Figure 1; $p=0.0032$). We calculated a mean fold change of 1.09 in GAPDH mRNA levels in human endometrial cancer, as GAPDH was expressed at 12.58 ± 0.46 arbitrary units (A.U.) in control endometrial tissue but at 13.68 ± 0.27 A.U. in endometrial tumor tissue.

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

We performed Kaplan-Meier survival analysis to evaluate correlation between GAPDH primary tumor expression and survival outcomes in 543 patients with endometrial cancer. We observed a correlation between primary tumor expression of GAPDH and recurrence-free survival in patients with endometrial cancer, in white patients with low mutational burden, in the lower survival tertile (Figure 2). GAPDH primary tumor mRNA levels were a positive prognostic indicator in white endometrial cancer patients with low mutational burden. White patients with low mutational burden whose primary tumors expressed low levels of GAPDH possessed, on average, markedly shorter median RFS as compared to white patients with low mutational burden whose tumors expressed high levels of GAPDH. This difference in RFS based on GAPDH tumor expression in white patients with endometrial cancer with low mutational burden approached statistical significance (Figure 2, Chart 3; logrank p -value: 0.063; hazard ratio: 0.45 (0.19-1.07)). GAPDH primary endometrial tumor expression was not correlated with recurrence-free survival in white patients with high mutational burden (Figure 2, Chart 3; logrank p -value: 0.68; hazard ratio: 0.82 (0.32-2.12)), nor in black patients with high ((Figure 2, Chart 3; logrank p -value: 0.54; hazard ratio: 1.95 (0.22-17.57)) or low mutational burden (Figure 2, Chart 3; logrank p -value: 0.55; hazard ratio: 0.64 (0.14-2.87)).

Thus, by mining published microarray data^{5,6} in an unbiased and systematic fashion, we identified glyceraldehyde-3-phosphate dehydrogenase, encoded by GAPDH, as among the genes whose expression was most different, transcriptome-wide, in the endometrial tumor tissue of

1 patients with endometrial cancer when compared to benign endometrial tissue; we observed
2 significantly increased expression of GAPDH in endometrial tumor tissue as compared to benign
3 endometrial tissue. Further, we found a correlation between GAPDH expression and patient
4 survival outcomes in human endometrial cancer, as recurrence-free survival was superior in
5 patients whose tumors expressed higher levels of GAPDH as compared to patients whose tumors
6 expressed lower levels of GAPDH, 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 glyceraldehyde-3-phosphate dehydrogenase is among the
10 genes most differentially expressed in human endometrial cancer, that mRNA for GAPDH is
11 present at significantly increased quantity in endometrial tumor tissue as compared to benign
12 endometrium, and that GAPDH primary tumor expression is correlated with recurrence-free
13 survival in white endometrial cancer patients with low mutational burden. These data suggest
14 GAPDH may be of importance to fundamental biological processes that underlie the initiation,
15 progression or maintenance of human endometrial cancer. Importantly, these data argue that
16 GAPDH should not be utilized as an internal reference or normalization gene for quantitative
17 real-time polymerase chain reaction measurement of target depletion in loss-of-function genetic
18 analyses in human endometrial cancer.
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Rank: 140
Probe ID: 217398_x_at
p-value: 1.52E-04
t: -5.4034706
B: 1.25334
Gene: GAPDH
Gene name: glyceraldehyde-3-phosphate dehydrogenase

Chart 1: GAPDH 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: 3603
probe ID: AFFX-HUMGAPDH/M33197_3_at
p-value: 4.20E-03
t: -2.9306618
B: -2.23129
Gene: GAPDH
Gene name: glyceraldehyde-3-phosphate dehydrogenase

Chart 2: GAPDH 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: GAPDH 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 GAPDH 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 GAPDH expression between benign endometrial tissue and primary tumors of the endometrial tissue is $p=0.0032$.

Figure 2: Correlation between GAPDH primary tumor expression and recurrence-free survival in endometrial cancer, in white 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 GAPDH 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), 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 GAPDH 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.