Over-expression of NADH:ubiquinone oxidoreductase subunit A7 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 and hyperplastic endometrium to endometrial tumors from humans. We identified NADH:ubiquinone oxidoreductase subunit A7, encoded by NDUF7, as among the most differentially expressed genes, transcriptome-wide, in cancers of the endometrium. NDUF7 was expressed at significantly higher levels in endometrial tumor tissues as compared to the endometrium. Importantly, in human endometrial cancer, primary tumor expression of NDUF7 was correlated with overall survival in black patients with high mutational burden. NDUF7 may be a molecule of interest in understanding the etiology or progression of human endometrial cancer.

Keywords: endometrial cancer, gynecologic cancers, endometrium, NDUF7, NADH:ubiquinone oxidoreductase subunit A7, 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 NADH:ubiquinone oxidoreductase subunit A7 (NDUFA7) in human endometrial cancer.

Methods

We utilized datasets GSE63678⁵ and GSE106191⁶ 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. GSE106191 was generated using Affymetrix Human Genome U133 Plus 2.0 Array technology with $n=33$ hyperplastic endometrial tissues and $n=28$ endometrial cancer tissues; 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 NDUFA7 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 NDUFA7 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.

NDUFA7 is differentially expressed in endometrial cancer.

We identified NADH:ubiquinone oxidoreductase subunit A7, encoded by NDUFA7, 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, NDUFA7 ranked 161 out of 22273 transcripts, equating to 99.3% differential expression (Chart 1). Differential expression of NDUFA7 in human endometrial cancers was statistically significant (Chart 1; $p=1.82E-04$).

We queried a second microarray data to validate differential expression of NDUFA7 in endometrial cancer. Here, we observed differential expression of NDUFA7 when comparing endometrial tumor tissue to hyperplastic 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 hyperplastic endometrial tissue, NDUFA7 ranked 16671 out of 54675 transcripts, equating to 69.5% differential expression (Chart 2). Differential expression of NDUFA7 in human endometrial cancers was not deemed statistically significant (Chart 2; $p=1.73E-01$).

NDUFA7 is expressed at significantly higher levels in endometrial cancers as compared to benign endometrial tissue.

We obtained exact mRNA expression levels for NDUFA7 in endometrial tumor tissues and from benign endometrial tissue to evaluate direction and statistical significance of change in expression of NDUFA7 in human endometrial cancer. NDUFA7 was expressed at higher levels in endometrial tissue as compared to normal endometrial tissue, and this difference was statistically significant (Figure 1; $p=0.0005$). We calculated a mean fold change of 1.10 in NDUFA7 mRNA levels in human endometrial cancer, as NDUFA7 was expressed at 8.64 ± 0.28 arbitrary units (A.U.) in control endometrial tissue but at 9.53 ± 0.29 A.U. in endometrial tumor tissue.

NDUFA7 expression is correlated with patient survival outcomes in endometrial cancer.

We performed Kaplan-Meier survival analysis to evaluate correlation between NDUFA7 primary tumor expression and survival outcomes in 543 patients with endometrial cancer. We observed a correlation between primary tumor expression of NDUFA7 and overall survival in patients with endometrial cancer, in black patients with high mutational burden, in the upper survival tertile (Figure 2). NDUFA7 primary tumor mRNA levels were a positive prognostic indicator in black endometrial cancer patients with high mutational burden. Black patients with high mutational burden whose primary tumors expressed low levels of NDUFA7 possessed, on average, markedly shorter median OS as compared to black patients with high mutational burden whose tumors expressed high levels of NDUFA7. This difference in OS based on NDUFA7 tumor expression in black patients with endometrial cancer with high mutational burden trended towards statistical significance (Figure 2, Chart 3; logrank p -value: 0.08; hazard ratio: 0 (0- ∞)). NDUFA7 primary endometrial tumor expression was not correlated with overall survival in black patients with low mutational burden (Figure 2, Chart 3; logrank p -value: 0.47; hazard ratio: 0.65 (0.2-2.11)), nor in white patients with high ((Figure 2, Chart 3; logrank p -value: 0.75; hazard ratio: 1.14 (0.5-2.58)) or low mutational burden (Figure 2, Chart 3; logrank p -value: 0.47; hazard ratio: 1.28 (0.66-2.5)).

Thus, by mining published microarray data^{5,6} in an unbiased and systematic fashion, we identified NADH:ubiquinone oxidoreductase subunit A7, encoded by NDUFA7, 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 and hyperplastic endometrial tissue; we observed significantly increased expression of NDUFA7 in endometrial tumor tissue as compared to benign endometrial tissue. Further, we found a correlation between NDUFA7 expression and patient survival outcomes in human endometrial cancer, as overall survival was superior in patients whose tumors expressed higher levels of NDUFA7 as compared to patients whose tumors expressed lower levels of NDUFA7, in black patients with high mutational burden, but not in black patients with low mutational burden, nor in white patients with high or low mutational burden.

Discussion

We provided evidence here that NADH:ubiquinone oxidoreductase subunit A7 is among the genes most differentially expressed in human endometrial cancer, that mRNA for NDUFA7 is present at significantly increased quantity in endometrial tumor tissue as compared to benign endometrium, and that NDUFA7 primary tumor expression is correlated with overall survival in black endometrial cancer patients with high mutational burden. These data suggest NDUFA7 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: 161
Probe ID: 202785_at
p-value: 1.82E-04
t: -5.293289
B: 1.08372
Gene: NDUFA7
Gene name: NADH:ubiquinone oxidoreductase subunit A7

Chart 1: NDUFA7 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: 16671
probe ID: 202785_at
p-value: 1.73E-01
t: 1.3791463
B: -5.0506841
Gene: NDUFA7
Gene name: NADH:ubiquinone oxidoreductase subunit A7

Chart 2: NDUFA7 is differentially expressed in endometrial cancer when comparing primary endometrial tumors to hyperplastic 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: NDUFA7 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 NDUFA7 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 NDUFA7 expression between benign endometrial tissue and primary tumors of the endometrial tissue is $p=0.0005$.

Figure 2: Correlation between NDUFA7 primary tumor expression and overall survival in endometrial cancer, in black patients 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 NDUFA7 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 upper survival tertile. 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 NDUFA7 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.

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