Over-expression of ubiquinol-cytochrome c reductase complex III subunit VII 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 endometrium and endometrial tumors from humans. We identified ubiquinol-cytochrome c reductase complex III subunit VII, encoded by UQCRQ, as among the most differentially expressed genes, transcriptome-wide, in cancers of the endometrium. UQCRQ was expressed at significantly higher levels in endometrial tumor tissues as compared to the endometrium. Importantly, in human endometrial cancer, primary tumor expression of UQCRQ was correlated with overall survival in black patients with high mutational burden. UQCRQ may be a molecule of interest in understanding the etiology or progression of human endometrial cancer.

Keywords: endometrial cancer, gynecologic cancers, endometrium, UQCRQ, ubiquinol-cytochrome c reductase complex III subunit VII, 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 ubiquinol-cytochrome c reductase complex III subunit VII (UQCRQ) in human endometrial cancer.

Methods

We utilized datasets GSE63678⁵ and GSE32507⁶ 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. GSE32507 was generated using Agilent-014850 Whole Human Genome Microarray 4x44K G4112F technology with $n=24$ endometrioid adenocarcinomas and $n=14$ carcinosarcomas; analysis was performed using platform GPL6480. 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 UQCRQ 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 UQCRQ 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.

UQCRQ is differentially expressed in endometrial cancer.

We identified ubiquinol-cytochrome c reductase complex III subunit VII, encoded by UQCRQ, 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, UQCRQ ranked 129 out of 22273 transcripts, equating to 99.4% differential expression (Chart 1). Differential expression of UQCRQ in human endometrial cancers was

statistically significant (Chart 1; $p=1.28E-04$).

We queried a second microarray data to validate differential expression of UQCRQ in endometrial cancer, here comparing different endometrial cancer types - endometrioid adenocarcinomas with carcinosarcomas - as opposed to comparing benign tissues and tumor tissues. We observed significant differential expression of UQCRQ when comparing endometrioid adenocarcinomas with carcinosarcomas (Chart 2). When sorting each of the genes expressed in endometrioid adenocarcinomas based on significance of change in expression as compared to carcinosarcomas, UQCRQ ranked 12946 out of 41073 transcripts, equating to 68.5% differential expression (Chart 2). Differential expression of UQCRQ in human endometrial cancers trended towards statistical significance (Chart 2; $p=8.35E-02$).

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

We obtained exact mRNA expression levels for UQCRQ in endometrial tumor tissues and from benign endometrial tissue to evaluate direction and statistical significance of change in expression of UQCRQ in human endometrial cancer. UQCRQ was expressed at higher levels in endometrial tissue as compared to normal endometrial tissue, and this difference was statistically significant (Figure 1; $p=0.0007$). We calculated a mean fold change of 1.07 in UQCRQ mRNA levels in human endometrial cancer, as UQCRQ was expressed at 11.11 ± 0.25 arbitrary units (A.U.) in control endometrial tissue but at 11.84 ± 0.18 A.U. in endometrial tumor tissue.

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

We performed Kaplan-Meier survival analysis to evaluate correlation between UQCRQ primary tumor expression and survival outcomes in 543 patients with endometrial cancer. We observed a correlation between primary tumor expression of UQCRQ and overall survival in patients with endometrial cancer, in black patients with high mutational burden (Figure 2). UQCRQ 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 UQCRQ possessed, on average, markedly shorter median OS as compared to black patients with high mutational burden whose tumors expressed high levels of UQCRQ. This difference in OS based on UQCRQ tumor expression in black patients with endometrial cancer with high mutational burden approached statistical significance (Figure 2, Chart 3; logrank p -value: 0.068; hazard ratio: 0.17 (0.02-1.47)). UQCRQ primary endometrial tumor expression was not correlated with overall survival in black patients with low mutational burden (Figure 2, Chart 3; logrank p -value: 0.97; hazard ratio: 0.98 (0.33-2.94)), nor in white patients with high ((Figure 2, Chart 3; logrank p -value: 0.41; hazard ratio: 0.72 (0.32-1.59)) or low mutational burden (Figure 2, Chart 3; logrank p -value: 0.53; hazard ratio: 0.81 (0.42-1.57)).

Thus, by mining published microarray data^{5,6} in an unbiased and systematic fashion, we identified ubiquinol-cytochrome c reductase complex III subunit VII, encoded by UQCRQ, 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, and when comparing endometrial cancer types; we observed significantly increased expression of UQCRQ in endometrial tumor tissue as compared to benign endometrial tissue. Further, we found a correlation between UQCRQ expression and patient survival outcomes in human endometrial cancer, as overall survival was superior in patients whose tumors expressed higher levels of UQCRQ as compared to patients whose tumors expressed lower levels of UQCRQ, 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 ubiquinol-cytochrome c reductase complex III subunit VII is among the genes most differentially expressed in human endometrial cancer, that mRNA for UQCRQ is present at significantly increased quantity in endometrial tumor tissue as compared to benign endometrium, and that UQCRQ primary tumor expression is correlated with overall survival in black endometrial cancer patients with high mutational burden. These data suggest UQCRQ may be of importance to fundamental biological processes that underlie the initiation, progression or maintenance of human endometrial cancer.

References

1. Amant, F., Moerman, P., Neven, P., Timmerman, D., Van Limbergen, E. and Vergote, I., 2005. Endometrial cancer. *The Lancet*, 366(9484), pp.491-505.
2. Sorosky, J.I., 2008. Endometrial cancer. *Obstetrics & Gynecology*, 111(2), pp.436-447.
3. Morice, P., Leary, A., Creutzberg, C., Abu-Rustum, N. and Darai, E., 2016. Endometrial cancer. *The Lancet*, 387(10023), pp.1094-1108.
4. Sorosky, J.I., 2012. Endometrial cancer. *Obstetrics & Gynecology*, 120(2 Part 1), pp.383-397.
5. Pappa, K.I., Polyzos, A., Jacob-Hirsch, J., Amariglio, N., Vlachos, G.D., Loutradis, D. and Anagnou, N.P., 2015. Profiling of discrete gynecological cancers reveals novel transcriptional modules and common features shared by other cancer types and embryonic stem cells. *PLoS One*, 10(11), p.e0142229.
6. Chiyoda, T., Tsuda, H., Tanaka, H., Kataoka, F., Nomura, H., Nishimura, S., Takano, M., Susumu, N., Saya, H. and Aoki, D., 2012. Expression profiles of carcinosarcoma of the uterine corpus—are these similar to carcinoma or sarcoma?. *Genes, Chromosomes and Cancer*, 51(3), pp.229-239.
7. Nagy, A., Munkacsy, G. and Gyorffy, B., 2020. Pancancer survival analysis of cancer hallmark genes. *bioRxiv*.

Rank: 129
Probe ID: 201568_at
p-value: 1.28E-04
t: -5.5100702
B: 1.4156
Gene: UQCRQ
Gene name: ubiquinol-cytochrome c reductase complex III subunit VII

Chart 1: UQCRQ 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: 12946
probe ID: A_23_P213718
p-value: 8.35E-02
t: 1.77469964
B: -4.754227
Gene: UQCRQ
Gene name: ubiquinol-cytochrome c reductase complex III subunit VII

Chart 2: UQCRQ is differentially expressed in endometrial cancer when comparing different endometrial cancer types - endometrioid adenocarcinomas with carcinosarcomas.

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: UQCRQ 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 UQCRQ 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 UQCRQ expression between benign endometrial tissue and primary tumors of the endometrial tissue is $p=0.0007$.

Figure 2: Correlation between UQCRQ 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 UQCRQ 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 UQCRQ 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.