Over-expression of cathepsin Z 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 cathepsin Z, encoded by CTSZ, as among the most differentially expressed genes, transcriptome-wide, in cancers of the endometrium. CTSZ was expressed at significantly higher levels in endometrial tumor tissues as compared to the endometrium. Importantly, in human endometrial cancer, primary tumor expression of CTSZ was correlated with overall survival in black patients with high mutational burden, in white patients with high and low mutational burden, but not in black patients with low mutational burden. CTSZ may be a molecule of interest in understanding the etiology or progression of human endometrial cancer.

Keywords: endometrial cancer, gynecologic cancers, endometrium, CTSZ, cathepsin Z, 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 cathepsin Z (CTSZ) 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 CTSZ 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 CTSZ 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.

CTSZ is differentially expressed in endometrial cancer.

We identified cathepsin Z, encoded by CTSZ, 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, CTSZ ranked 113 out of 22273 transcripts, equating to 99.5% differential expression (Chart 1). Differential expression of CTSZ in human endometrial cancers was statistically significant (Chart 1; $p=1.12E-04$).

We queried a second microarray data to validate differential expression of CTSZ in endometrial cancer. Here, we observed differential expression of CTSZ 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, CTSZ ranked 7493 out of 54675 transcripts, equating to 86.3% differential expression (Chart 2). Differential expression of CTSZ in human endometrial cancers approached statistical significance (Chart 2; $p=5.04E-02$).

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

We obtained exact mRNA expression levels for CTSZ in endometrial tumor tissues and from benign endometrial tissue to evaluate direction and statistical significance of change in expression of CTSZ in human endometrial cancer. CTSZ was expressed at higher levels in endometrial tissue as compared to normal endometrial tissue, and this difference was statistically significant (Figure 1; $p=0.0006$). We calculated a mean fold change of 1.43 in CTSZ mRNA levels in human endometrial cancer, as CTSZ was expressed at 6.79 ± 0.25 arbitrary units (A.U.) in control endometrial tissue but at 9.68 ± 1.23 A.U. in endometrial tumor tissue.

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

We performed Kaplan-Meier survival analysis to evaluate correlation between CTSZ primary tumor expression and survival outcomes in 543 patients with endometrial cancer. We observed a correlation between primary tumor expression of CTSZ and overall survival in patients with endometrial cancer, in black patients with high mutational burden (Figure 2). CTSZ 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 CTSZ possessed, on average, markedly shorter median OS as compared to black patients with high mutational burden whose tumors expressed high levels of CTSZ (Figure 2). This difference in OS based on CTSZ tumor expression in black patients with endometrial cancer with high mutational burden approached statistical significance (Figure 2, Chart 3; logrank p -value: 0.089; hazard ratio: 0.19 (0.02-1.62)). We also observed a correlation between primary tumor expression of CTSZ and overall survival in patients with endometrial cancer, in white patients with high mutational burden (Figure 2). CTSZ primary tumor mRNA levels were a positive prognostic indicator in white endometrial cancer patients with low mutational burden. White patients with high mutational burden whose primary tumors expressed low levels of CTSZ possessed median OS of 36.03 months, while white patients with high mutational burden whose tumors expressed high levels of CTSZ possessed median OS of 111.63 months. This difference in OS based on CTSZ tumor expression in white patients with endometrial cancer with high mutational burden was statistically significant (Figure 2, Chart 3;

logrank *p*-value: 0.016; hazard ratio: 0.37 (0.16-0.86)). We also observed a correlation between primary tumor expression of CTSZ and overall survival in patients with endometrial cancer, in white patients with low mutational burden (Figure 2). CTSZ 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 CTSZ possessed median OS of 64.8 months, while white patients with low mutational burden whose tumors expressed high levels of CTSZ possessed median OS of 114.1 months. This difference in OS based on CTSZ tumor expression in white patients with endometrial cancer with low mutational burden was statistically significant (Figure 2, Chart 3; logrank *p*-value: 0.01; hazard ratio: 0.4 (0.2-0.83)). CTSZ primary endometrial tumor expression was not correlated with overall survival in black patients with low mutational burden (Figure 2, Chart 3; logrank *p*-value: 0.81; hazard ratio: 1.15 (0.37-3.54)).

Thus, by mining published microarray data^{5,6} in an unbiased and systematic fashion, we identified cathepsin Z, encoded by CTSZ, 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 CTSZ in endometrial tumor tissue as compared to benign endometrial tissue. Further, we found a correlation between CTSZ expression and patient survival outcomes in human endometrial cancer, as overall survival was superior in patients whose tumors expressed higher levels of CTSZ as compared to patients whose tumors expressed lower levels of CTSZ, in black patients with high mutational burden, and in white patients with high and low mutational burden, but not in black patients with low mutational burden.

Discussion

We provided evidence here that cathepsin Z is among the genes most differentially expressed in human endometrial cancer, that mRNA for CTSZ is present at significantly increased quantity in endometrial tumor tissue as compared to benign endometrium, and that CTSZ primary tumor expression is correlated with overall survival in white endometrial cancer patients with high and low mutational burden, as well as in black endometrial cancer patients with high mutational burden. These data suggest CTSZ 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. Sugiyama, Y., Gotoh, O., Fukui, N., Tanaka, N., Hasumi, K., Takazawa, Y., Noda, T. and Mori, S., 2020. Two distinct tumorigenic processes in endometrial endometrioid adenocarcinoma. *The American journal of pathology*, 190(1), pp.234-251.
7. Nagy, A., Munkacsy, G. and Gyorffy, B., 2020. Pancancer survival analysis of cancer hallmark genes. *bioRxiv*.

Rank: 113
Probe ID: 210042_s_at
p-value: 1.12E-04
t: -5.5886779
B: 1.53407
Gene: CTSZ
Gene name: cathepsin Z

Chart 1: CTSZ 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: 7493
probe ID: 210042_s_at
p-value: 5.04E-02
t: 1.9955485
B: -4.11408579
Gene: CTSZ
Gene name: cathepsin Z

Chart 2: CTSZ 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: CTSZ 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 CTSZ 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 CTSZ expression between benign endometrial tissue and primary tumors of the endometrial tissue is $p=0.0006$.

Figure 2: Correlation between CTSZ primary tumor expression and overall survival in endometrial cancer, in white patients with high and low mutational burden, and 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 CTSZ 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 CTSZ 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.

White patients with low mutational burden

Low CTSZ expression: 64.8 months

High CTSZ expression: 114.1 months

White patients with high mutational burden

Low CTSZ expression: 36.03 months

High CTSZ expression: 111.63 months

Chart 3: Median overall survival is superior in endometrial cancer patients with high primary tumor expression of CTSZ, in white patients with high and low mutational burden.

The median OS (overall survival) of white endometrial cancer patients with high and low mutational burden, with low primary tumor expression of CTSZ and high primary tumor expression of CTSZ is listed in this chart.