Over-expression of tissue specific transplantation antigen P35B 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 tissue specific transplantation antigen P35B, encoded by TSTA3, as among the most differentially expressed genes, transcriptome-wide, in cancers of the endometrium. TSTA3 was expressed at significantly higher levels in endometrial tumor tissues as compared to the endometrium. Importantly, in human endometrial cancer, primary tumor expression of TSTA3 was correlated with recurrence-free survival in white patients with low mutational burden. TSTA3 may be a molecule of interest in understanding the etiology or progression of human endometrial cancer.

Keywords: endometrial cancer, gynecologic cancers, endometrium, TSTA3, tissue specific transplantation antigen P35B, 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 tissue specific transplantation antigen P35B (TSTA3) 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 TSTA3 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 TSTA3 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.

TSTA3 is differentially expressed in endometrial cancer.

We identified tissue specific transplantation antigen P35B, encoded by TSTA3, 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, TSTA3 ranked 91 out of 22273 transcripts, equating to 99.6% differential expression (Chart 1). Differential expression of TSTA3 in human endometrial cancers was statistically significant (Chart 1; $p=8.16E-05$).

We queried a second microarray data to validate differential expression of TSTA3 in endometrial cancer. Again, we observed differential expression of TSTA3 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, TSTA3 ranked 246 out of 54675 transcripts, equating to 99.6% differential expression (Chart 2). Differential expression of TSTA3 in human endometrial cancers was statistically significant (Chart 2; $p=1.56E-06$).

TSTA3 is expressed at significantly higher levels in endometrial cancers as compared to benign endometrial tissue.

We obtained exact mRNA expression levels for TSTA3 in endometrial tumor tissues and from benign endometrial tissue to evaluate direction and statistical significance of change in expression of TSTA3 in human endometrial cancer. TSTA3 was expressed at higher levels in endometrial tissue as compared to normal endometrial tissue, and this difference was statistically significant (Figure 1; $p<0.0001$). We calculated a mean fold change of 1.15 in TSTA3 mRNA levels in human endometrial cancer, as TSTA3 was expressed at 7.92 ± 0.23 arbitrary units (A.U.) in control endometrial tissue but at 9.10 ± 0.42 A.U. in endometrial tumor tissue.

TSTA3 expression is correlated with patient survival outcomes in endometrial cancer.

We performed Kaplan-Meier survival analysis to evaluate correlation between TSTA3 primary tumor expression and survival outcomes in 543 patients with endometrial cancer. We observed a correlation between primary tumor expression of TSTA3 and recurrence-free survival in patients with endometrial cancer, in white patients with low mutational burden (Figure 2). TSTA3 primary tumor mRNA levels were a negative prognostic indicator in white endometrial cancer patients with low mutational burden. White patients with low mutational burden whose primary tumors expressed low levels of TSTA3 possessed median RFS of 112.17 months, while white patients with low mutational burden whose tumors expressed high levels of TSTA3 possessed median RFS of 78.4 months. This difference in RFS based on TSTA3 tumor expression in white patients with endometrial cancer with low mutational burden approached statistical significance (Figure 2, Chart 3; logrank p -value: 0.052; hazard ratio: 2 (0.98-4.08)). TSTA3 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: 0.78; hazard ratio: 0.89 (0.41-1.96)), nor in black patients with high ((Figure 2, Chart 3; logrank p -value: 0.3; hazard ratio: 0.42 (0.08-2.3)) or low mutational burden (Figure 2, Chart 3; logrank p -value: 0.94; hazard ratio: 1.05 (0.34-3.25)).

Thus, by mining published microarray data^{5,6} in an unbiased and systematic fashion, we identified tissue specific transplantation antigen P35B, encoded by TSTA3, 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

1 significantly increased expression of TSTA3 in endometrial tumor tissue as compared to benign
2 endometrial tissue. Further, we found a correlation between TSTA3 expression and patient
3 survival outcomes in human endometrial cancer, as recurrence-free survival was inferior in
4 patients whose tumors expressed higher levels of TSTA3 as compared to patients whose tumors
5 expressed lower levels of TSTA3, in white patients with low mutational burden, but not in white
6 patients with high mutational burden, nor in black patients with high or low mutational burden.

7 **Discussion**

8 We provided evidence here that tissue specific transplantation antigen P35B is among the
9 genes most differentially expressed in human endometrial cancer, that mRNA for TSTA3 is
10 present at significantly increased quantity in endometrial tumor tissue as compared to benign
11 endometrium, and that TSTA3 primary tumor expression is correlated with recurrence-free
12 survival in white endometrial cancer patients with low mutational burden. These data suggest
13 TSTA3 may be of importance to fundamental biological processes that underlie the initiation,
14 progression or maintenance of human endometrial cancer.
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Rank: 91
Probe ID: 201644_at
p-value: 8.16E-05
t: -5.7901664
B: 1.83315
Gene: TSTA3
Gene name: tissue specific transplantation antigen P35B

Chart 1: TSTA3 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: 246
probe ID: 36936_at
p-value: 1.56E-06
t: -5.1128846
B: 4.98025
Gene: TSTA3
Gene name: tissue specific transplantation antigen P35B

Chart 2: TSTA3 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: TSTA3 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 TSTA3 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 TSTA3 expression between benign endometrial tissue and primary tumors of the endometrial tissue is $p < 0.0001$.

Figure 2: Correlation between TSTA3 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 TSTA3 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 TSTA3 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 TSTA3 expression: 112.17 months

High TSTA3 expression: 78.4 months

Chart 3: Median recurrence-free survival is superior in endometrial cancer patients with low primary tumor expression of TSTA3, in white patients with low mutational burden.

The median RFS (recurrence-free survival) of white endometrial cancer patients with low mutational burden, with low primary tumor expression of TSTA3 and high primary tumor expression of TSTA3 is listed in this chart.