Over-expression of inositol 1,4,5-trisphosphate receptor type 3 in human endometrial cancer.

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Short Report

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Gynecologic cancers including cancers of the endometrium are a clinical problem[1-4]. 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 inositol 1,4,5-trisphosphate receptor type 3, encoded by ITPR3, as among the most differentially expressed genes, transcriptome-wide, in cancers of the endometrium. ITPR3 was expressed at significantly higher levels in endometrial tumor tissues as compared to the endometrium. Importantly, in human endometrial cancer, primary tumor expression of ITPR3 was correlated with overall survival in white patients with low mutational burden. ITPR3 may be a molecule of interest in understanding the etiology or progression of human endometrial cancer.

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Endometrial cancer is the most common gynecologic cancer in the developed world[1]. 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 inositol 1,4,5-trisphosphate receptor type 3 (ITPR3) in human endometrial cancer.

Methods

We utilized datasets GSE63678[5] and GSE115810[6] 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. GSE115810 was generated using Affymetrix Human Genome U133A Array technology with *n*=3 control endometrial tissues and *n*=24 endometrial cancers; analysis was performed using platform GPL96. 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 ITPR3 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 tool[7] for correlation of ITPR3 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.

ITPR3 is differentially expressed in endometrial cancer.

We identified inositol 1,4,5-trisphosphate receptor type 3, encoded by ITPR3, 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, ITPR3 ranked 139 out of 22273 transcripts, equating to 99.4% differential expression (Chart 1). Differential expression of ITPR3 in human endometrial cancers was statistically significant (Chart 1; *p*=1.52E-04).

We queried a second microarray data to validate differential expression of ITPR3 in endometrial cancer. Again, we observed differential expression of ITPR3 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, ITPR3 ranked 3030 out of 22283 transcripts, equating to 86.4% differential expression (Chart 2). Differential expression of ITPR3 in human endometrial cancers approached statistical significance (Chart 2; p=0.0554558).

ITPR3 is expressed at significantly higher levels in endometrial cancers as compared to benign endometrial tissue.

We obtained exact mRNA expression levels for ITPR3 in endometrial tumor tissues and from benign endometrial tissue to evaluate direction and statistical significance of change in expression of ITPR3 in human endometrial cancer. ITPR3 was expressed at higher levels in endometrial tissue as compared to normal endometrial tissue, and this difference was statistically significant (Figure 1; p=0.0011). We calculated a mean fold change of 1.24 in ITPR3 mRNA levels in human endometrial cancer, as ITPR3 was expressed at 8.33 $\pm$0.73 arbitrary units (A.U.) in control endometrial tissue but at 10.35 $\pm$0.66 A.U. in endometrial tumor tissue.

ITPR3 expression is correlated with patient survival outcomes in endometrial cancer.

We performed Kaplan-Meier survival analysis to evaluate correlation between ITPR3 primary tumor expression and survival outcomes in 543 patients with endometrial cancer. We observed a correlation between primary tumor expression of ITPR3 and overall survival in patients with endometrial cancer, in white patients with low mutational burden, in the upper survival tertile (Figure 2). ITPR3 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 ITPR3 possessed median OS of 103.73 months, while white patients with low mutational burden whose tumors expressed high levels of ITPR3 possessed median OS of 78.4 months. This difference in OS based on ITPR3 tumor expression in white patients with endometrial cancer with low mutational burden was statistically significant (Figure 2, Chart 3; logrank p-value: 0.02; hazard ratio: 2.18 (1.11-4.25)). ITPR3 primary endometrial tumor expression was not correlated with overall survival in white patients with high mutational burden (Figure 2, Chart 3; logrank p-value: 0.61; hazard ratio: 0.78 (0.31-1.98)), nor in black patients with high ((Figure 2, Chart 3; logrank p-value: 0.44; hazard ratio: 1.87 (0.38-9.3)) or low mutational burden (Figure 2, Chart 3; logrank p-value: 0.39; hazard ratio: 0.57 (0.16-2.08)).

Thus, by mining published microarray data[5,6] in an unbiased and systematic fashion, we identified inositol 1,4,5-trisphosphate receptor type 3, encoded by ITPR3, 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 significantly increased expression of ITPR3 in endometrial tumor tissue as compared to benign endometrial tissue. Further, we found a correlation between ITPR3 expression and patient survival outcomes in human endometrial cancer, as overall survival was inferior in patients whose tumors expressed higher levels of ITPR3 as compared to patients whose tumors expressed lower levels of ITPR3, in white patients with low mutational burden, but not in white patients with high mutational burden, nor in black patients with high or low mutational burden.

Discussion

We provided evidence here that inositol 1,4,5-trisphosphate receptor type 3 is among the genes most differentially expressed in human endometrial cancer, that mRNA for ITPR3 is present at significantly increased quantity in endometrial tumor tissue as compared to benign endometrium, and that ITPR3 primary tumor expression is correlated with overall survival in white endometrial cancer patients with low mutational burden. These data suggest ITPR3 may be of importance to fundamental biological processes that underlie the initiation, progression or maintenance of human endometrial cancer.

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Rank: 139
Probe ID: 201189_s_at
p-value: 1.52E-04
t: -5.4037036
B: 1.2537
Gene: ITPR3
Gene name: inositol 1,4,5-trisphosphate receptor type 3

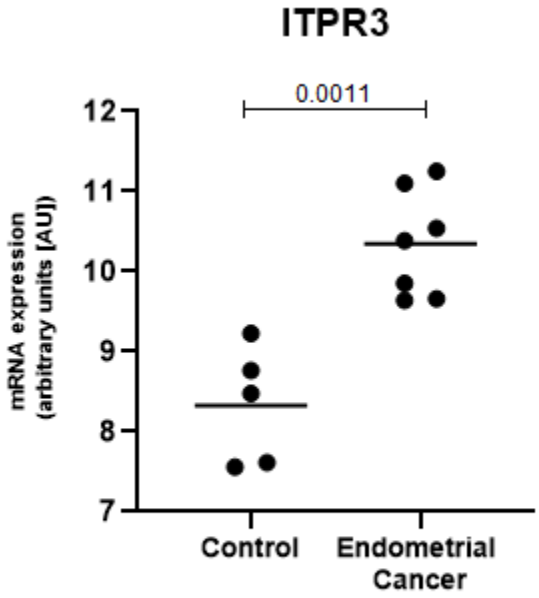
Chart 1: ITPR3 is differentially expressed in endometrial cancer when comparing primary endometrial tumors to benign endometrial tissue.

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: 3030
probe ID: 201187_s_at
p-value: 0.0554558
t: -1.99841
B: -4.04547
Gene: ITPR3
Gene name: inositol 1,4,5-trisphosphate receptor type 3

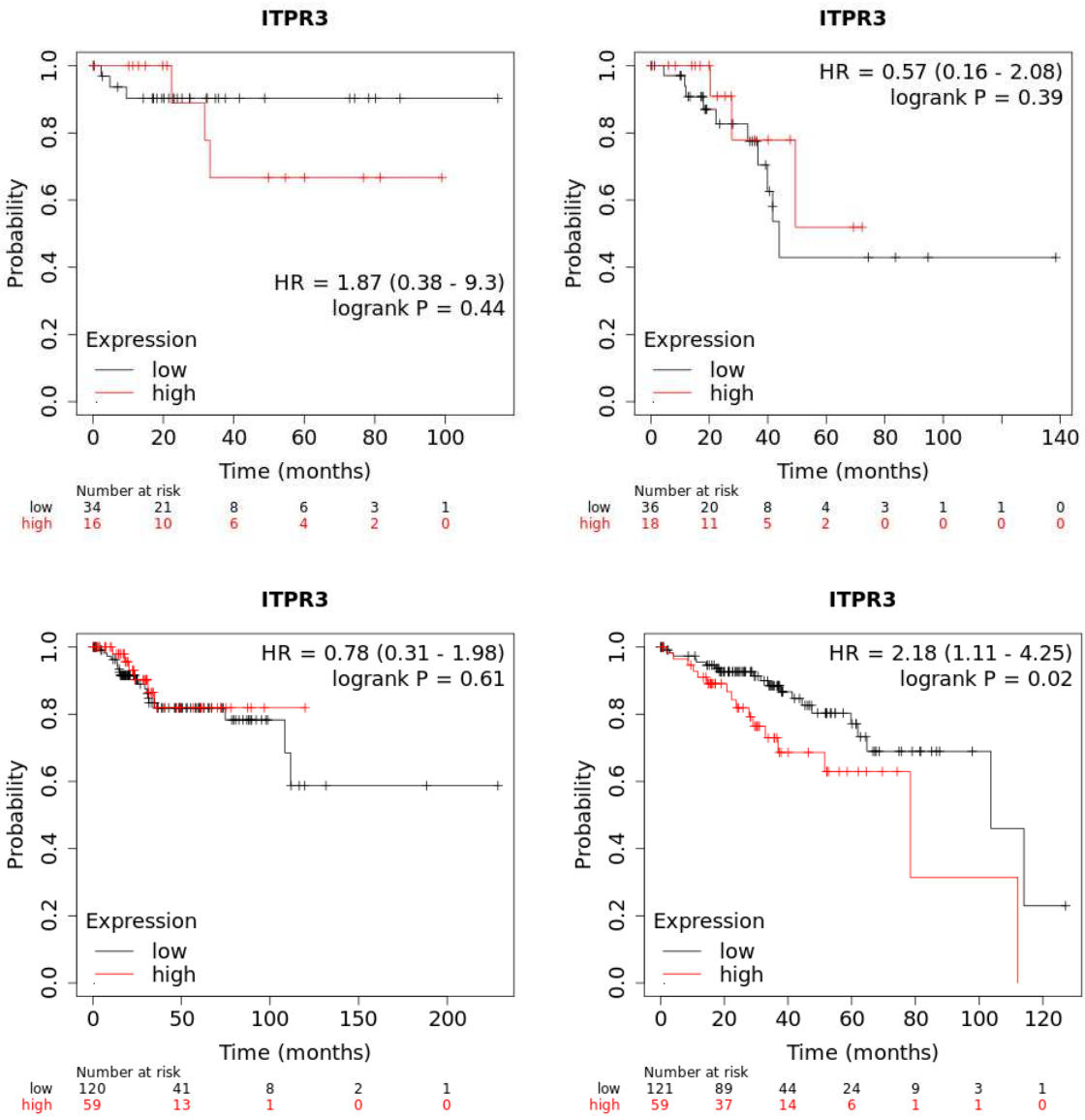
Chart 2: ITPR3 is differentially expressed in endometrial cancer when comparing primary endometrial tumors to benign endometrial tissue.

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.



<u>Figure 1:</u> ITPR3 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 ITPR3 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 ITPR3 expression between benign endometrial tissue and primary tumors of the endometrial tissue is p=0.0011.



<u>Figure 2:</u> Correlation between ITPR3 primary tumor expression and overall survival in endometrial cancer, in white patients with low 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 ITPR3 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 ITPR3 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 ITPR3 expression: 103.73 months
High ITPR3 expression: 78.4 months

Chart 3: Median overall survival is superior in endometrial cancer patients with low primary tumor expression of ITPR3, in white patients with low mutational burden.

The median OS (overall survival) of white endometrial cancer patients with low mutational burden, with low primary tumor expression of ITPR3 and high primary tumor expression of ITPR3 is listed in this chart, in the upper survival tertile.