Over-expression of La ribonucleoprotein domain family member 4B 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 La ribonucleoprotein domain family member 4B, encoded by LARP4B, as among the most differentially expressed genes, transcriptome-wide, in cancers of the endometrium. LARP4B was expressed at significantly higher levels in endometrial tumor tissues as compared to the endometrium. Importantly, in human endometrial cancer, primary tumor expression of LARP4B was correlated with recurrence-free survival in white patients with high mutational burden. LARP4B may be a molecule of interest in understanding the etiology or progression of human endometrial cancer.

Keywords: endometrial cancer, gynecologic cancers, endometrium, LARP4B, La ribonucleoprotein domain family member 4B, 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 La ribonucleoprotein domain family member 4B (LARP4B) 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 LARP4B 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 LARP4B 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.

LARP4B is differentially expressed in endometrial cancer.

We identified La ribonucleoprotein domain family member 4B, encoded by LARP4B, 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, LARP4B ranked 188 out of 22273 transcripts, equating to 99.2% differential expression (Chart 1). Differential expression of LARP4B in human endometrial cancers was statistically significant (Chart 1; $p=2.33E-04$).

We queried a second microarray data to validate differential expression of LARP4B in endometrial cancer. Again, we observed differential expression of LARP4B 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, LARP4B ranked 628 out of 54675 transcripts, equating to 98.9% differential expression (Chart 2). Differential expression of LARP4B in human endometrial cancers was statistically significant (Chart 2; $p=2.98E-05$).

LARP4B is expressed at significantly higher levels in endometrial cancers as compared to benign endometrial tissue.

We obtained exact mRNA expression levels for LARP4B in endometrial tumor tissues and from benign endometrial tissue to evaluate direction and statistical significance of change in expression of LARP4B in human endometrial cancer. LARP4B was expressed at higher levels in endometrial tissue as compared to normal endometrial tissue, and this difference was statistically significant (Figure 1; $p=0.0017$). We calculated a mean fold change of 1.11 in LARP4B mRNA levels in human endometrial cancer, as LARP4B was expressed at 7.17 ± 0.31 arbitrary units (A.U.) in control endometrial tissue but at 7.97 ± 0.22 A.U. in endometrial tumor tissue.

LARP4B expression is correlated with patient survival outcomes in endometrial cancer.

We performed Kaplan-Meier survival analysis to evaluate correlation between LARP4B primary tumor expression and survival outcomes in 543 patients with endometrial cancer. We observed a correlation between primary tumor expression of LARP4B and recurrence-free survival in patients with endometrial cancer, in white patients with high mutational burden, in the lower survival tertile (Figure 2). LARP4B primary tumor mRNA levels were a negative prognostic indicator in white endometrial cancer patients with high mutational burden. White patients with high mutational burden whose primary tumors expressed low levels of LARP4B possessed, on average, significantly greater median RFS as compared to white patients with high mutational burden whose tumors expressed high levels of LARP4B (Figure 2). This difference in RFS based on LARP4B tumor expression in white patients with endometrial cancer with high mutational burden approached statistical significance (Figure 2, Chart 3; logrank p -value: 0.071; hazard ratio: 2.98 (0.86-10.33)). LARP4B primary endometrial tumor expression was not correlated with recurrence-free survival in white patients with low mutational burden (Figure 2, Chart 3; logrank p -value: 0.47; hazard ratio: 0.73 (0.31-1.73)), nor in black patients with high ((Figure 2, Chart 3; logrank p -value: 0.87; hazard ratio: 0.86 (0.14-5.14)) or low mutational burden (Figure 2, Chart 3; logrank p -value: 0.38; hazard ratio: 0.54 (0.13-2.18)).

Thus, by mining published microarray data^{5,6} in an unbiased and systematic fashion, we identified La ribonucleoprotein domain family member 4B, encoded by LARP4B, 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 LARP4B in endometrial tumor tissue as compared to benign endometrial tissue. Further, we found a correlation between LARP4B expression and patient survival outcomes in human endometrial cancer, as recurrence-free survival was inferior in patients whose tumors expressed higher levels of LARP4B as compared to patients whose tumors expressed lower levels of LARP4B, in white patients with high mutational burden, but not in white patients with low mutational burden, nor in black patients with high or low mutational burden.

Discussion

We provided evidence here that La ribonucleoprotein domain family member 4B is among the genes most differentially expressed in human endometrial cancer, that mRNA for LARP4B is present at significantly increased quantity in endometrial tumor tissue as compared to benign endometrium, and that LARP4B primary tumor expression is correlated with recurrence-free survival in white endometrial cancer patients with high mutational burden. These data suggest LARP4B 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: 188
Probe ID: 208952_s_at
p-value: 2.33E-04
t: -5.1447341
B: 0.85197
Gene: LARP4B
Gene name: La ribonucleoprotein domain family member 4B

Chart 1: LARP4B 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: 628
probe ID: 208952_s_at
p-value: 2.98E-05
t: -4.3785845
B: 2.25225
Gene: LARP4B
Gene name: La ribonucleoprotein domain family member 4B

Chart 2: LARP4B 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: LARP4B 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 LARP4B 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 LARP4B expression between benign endometrial tissue and primary tumors of the endometrial tissue is $p=0.0017$.

Figure 2: Correlation between LARP4B primary tumor expression and recurrence-free survival in endometrial cancer, in white patients with high 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 LARP4B 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 lower survival tertile. 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 LARP4B 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.