Over-expression of ADP ribosylation factor like GTPase 4C in human endometrial cancer.

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Gynecologic cancers including cancers of the endometrium are a clinical problem¹⁻⁴. We mined published and public 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 ADP ribosylation factor like GTPase 4C, encoded by ARL4C, as among the most differentially expressed genes, transcriptome-wide, in cancers of the endometrium. ARL4C was expressed at significantly higher levels in endometrial tumor tissues as compared to the endometrium. Importantly, in human endometrial cancer, primary tumor expression of ARL4C was correlated with recurrence-free survival in white patients with high mutational burden. ARL4C may be a molecule of interest in understanding the etiology or progression of human endometrial cancer.

Keywords: endometrial cancer, gynecologic cancers, endometrium, ARL4C, ADP ribosylation factor like GTPase 4C, 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 integrative microarray dataset analysis, published and public^{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 ADP ribosylation factor like GTPase 4C (ARL4C) in human endometrial cancer.

Methods

We utilized datasets GSE63678⁵ and GSE39099⁶ 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. GSE39099 was generated using Affymetrix Human Genome U133 Plus 2.0 Array technology with $n=2$ benign endometrial tissues ($n=1$ normal endometrium and $n=1$ atypical endometrium) and $n=2$ endometrial cancers ($n=1$ early stage endometrial cancer and $n=1$ advanced stage endometrial cancer); 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 ARL4C 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 ARL4C 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 and public microarray data^{5,6} to discover in an unbiased fashion genes associated with endometrial cancer in humans.

ARL4C is differentially expressed in endometrial cancer.

We identified ADP ribosylation factor like GTPase 4C, encoded by ARL4C, 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, ARL4C ranked 97 out of 22273 transcripts, equating to 99.6% differential expression (Chart 1).

Differential expression of ARL4C in human endometrial cancers was statistically significant (Chart 1; $p=8.74E-05$).

We queried a second, public microarray data to validate differential expression of ARL4C in endometrial cancer. Again, we observed differential expression of ARL4C 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, ARL4C ranked 1344 out of 54674 transcripts, equating to 97.5% differential expression (Chart 2). Differential expression of ARL4C in human endometrial cancers was statistically significant (Chart 2; $p=0.015402$).

ARL4C is expressed at significantly higher levels in endometrial cancers as compared to benign endometrial tissue.

We obtained exact mRNA expression levels for ARL4C in endometrial tumor tissues and from benign endometrial tissue to evaluate direction and statistical significance of change in expression of ARL4C in human endometrial cancer. ARL4C 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 1.42 in ARL4C mRNA levels in human endometrial cancer, as ARL4C was expressed at 7.04 ± 1.39 arbitrary units (A.U.) in control endometrial tissue but at 10.00 ± 0.50 A.U. in endometrial tumor tissue.

ARL4C expression is correlated with patient survival outcomes in endometrial cancer.

We performed Kaplan-Meier survival analysis to evaluate correlation between ARL4C primary tumor expression and survival outcomes in 543 patients with endometrial cancer. We observed a correlation between primary tumor expression of ARL4C and recurrence-free survival in patients with endometrial cancer, in white patients with high mutational burden (Figure 2). ARL4C 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 ARL4C possessed, on average, markedly greater median RFS as compared to white patients with high mutational burden whose tumors expressed high levels of ARL4C (Figure 2). This difference in RFS based on ARL4C tumor expression in white patients with endometrial cancer with high mutational burden was statistically significant (Figure 2, Chart 3; logrank p -value: 0.033; hazard ratio: 2.42 (1.04-5.62)). ARL4C 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: 0.42; hazard ratio: 1.31 (0.67-2.54)), nor in black patients with high ((Figure 2, Chart 3; logrank p -value: 0.32; hazard ratio: 2.34 (0.42-12.86)) or low mutational burden (Figure 2, Chart 3; logrank p -value: 0.45; hazard ratio: 1.53 (0.51-4.62)).

Thus, by mining published and public microarray data^{5,6} in an unbiased and systematic

1 fashion, we identified ADP ribosylation factor like GTPase 4C, encoded by ARL4C, as among
2 the genes whose expression was most different, transcriptome-wide, in the endometrial tumor
3 tissue of patients with endometrial cancer when compared to benign endometrial tissue; we
4 observed significantly increased expression of ARL4C in endometrial tumor tissue as compared
5 to benign endometrial tissue. Further, we found a correlation between ARL4C expression and
6 patient survival outcomes in human endometrial cancer, as recurrence-free survival was inferior
7 in patients whose tumors expressed higher levels of ARL4C as compared to patients whose
8 tumors expressed lower levels of ARL4C, in white patients with high mutational burden, but not
9 in white patients with low mutational burden, nor in black patients with high or low mutational
10 burden.

11 **Discussion**

12 We provided evidence here that ADP ribosylation factor like GTPase 4C is among the
13 genes most differentially expressed in human endometrial cancer, that mRNA for ARL4C is
14 present at significantly increased quantity in endometrial tumor tissue as compared to benign
15 endometrium, and that ARL4C primary tumor expression is correlated with recurrence-free
16 survival in white endometrial cancer patients with low mutational burden. These data suggest
17 ARL4C may be of importance to fundamental biological processes that underlie the initiation,
18 progression or maintenance of human endometrial cancer.
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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. GSE39099. Wei-Yun Huang, NCTU, Department of Biological Science and Technology. Hsin-Chu, Taiwan. <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE39099>.
7. Nagy, A., Munkacsy, G. and Gyorffy, B., 2020. Pancancer survival analysis of cancer hallmark genes. *bioRxiv*.

Rank: 97
Probe ID: 202207_at
p-value: 8.74E-05
t: -5.7466268
B: 1.76908
Gene: ARL4C
Gene name: ADP ribosylation factor like GTPase 4C

Chart 1: ARL4C 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: 1344
Probe ID: 202206_at
p-value: 0.015402
t: -3.7827839
B: -2.94
Gene: ARL4C
Gene name: ADP ribosylation factor like GTPase 4C

Chart 2: ARL4C 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: ARL4C 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 ARL4C 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 ARL4C expression between benign endometrial tissue and primary tumors of the endometrial tissue is $p<0.0001$.

Figure 2: Correlation between ARL4C 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 ARL4C 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 ARL4C 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.