Over-expression of KPNA2 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 the nuclear import adaptor protein karyopherin α 2, encoded by KPNA2, as among the most differentially expressed genes, transcriptome-wide, in cancers of the endometrium. KPNA2 was expressed at significantly higher levels in endometrial tumor tissues as compared to the endometrium. Importantly, high primary tumor expression of KPNA2 was correlated with worse overall survival in patients with endometrial cancer. The data suggest that nuclear import pathways, either broadly or of a specific cargo, are hyperactive in endometrial cancer.

Keywords: endometrial cancer, gynecologic cancers, endometrium, KPNA2, karyopherin α 2, 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 karyopherin α 2 (KPNA2) 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 KPNA2 gene expression was significantly different between primary tumors of the breast and brain metastases in humans with breast cancer using a two-tailed t-test. For Kaplan-Meier survival analysis, we used the Kaplan-Meier plotter tool⁷ for correlation of KPNA2 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.

KPNA2 is differentially expressed in endometrial cancer.

We identified karyopherin α 2, encoded by KPNA2, 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, KPNA2 ranked 59 out of 22273 transcripts, equating to 99.7% differential expression (Chart 1). Differential expression of KPNA2 in human endometrial cancers was statistically significant (Chart 1; $p=4.69\text{e-}05$).

We queried a second microarray data to validate differential expression of KPNA2 in endometrial cancer. Again, we observed differential expression of KPNA2 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, KPNA2 ranked 11 out of 54675 transcripts, equating to 99.98% differential expression (Chart 2). Differential

expression of KPNA2 in human endometrial cancers trended was statistically significant (Chart 2; $p=1.03e-10$).

KPNA2 is expressed at significantly higher levels in endometrial cancers as compared to benign endometrial tissue.

We obtained exact mRNA expression levels for KPNA2 in endometrial tumor tissues and from benign endometrial tissue to evaluate direction and statistical significance of change in expression of KPNA2 in human endometrial cancer. We observed significantly higher expression of KPNA2 in the endometrial tumor tissues of patients with endometrial cancer as compared to control endometrial tissues: KPNA2 was expressed at 8.96 ± 0.46 arbitrary units (AU) in endometrial tumor tissues, while it was expressed at 10.71 ± 0.56 AU in control endometrial tissues (Figure 1). The difference in KPNA2 mRNA levels between endometrial tumor tissues and control endometrial tissues was statistically significant (Figure 1; $p=0.000181$).

KPNA2 expression is correlated with patient survival outcomes in endometrial cancer.

We performed Kaplan-Meier survival analysis to evaluate correlation between KPNA2 primary tumor expression and survival outcomes in 543 patients with endometrial cancer. We observed a correlation between primary tumor expression of KPNA2 and overall survival in patients with endometrial cancer, in the upper survival quartile (Figure 2). The difference in overall survival based on KPNA2 tumor expression in patients with endometrial cancer was statistically significant (Figure 2, Chart 3; logrank p -value: 0.04; hazard ratio: 1.58 (1.02-2.46)).

Thus, by mining published microarray data^{5,6} in an unbiased and systematic fashion, we identified karyopherin $\alpha 2$, encoded by KPNA2, 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 KPNA2 in endometrial tumor tissue as compared to benign endometrial tissue. Further, we found a statistically significant correlation between KPNA2 expression and patient survival outcomes in human endometrial cancer, as overall survival was superior in patients whose tumors expressed lower levels of KPNA2 as compared to patients whose tumors expressed higher levels of KPNA2.

Discussion

We provided evidence here that the adapter protein for the canonical nuclear import receptor complex, karyopherin $\alpha 2$, is among the genes most differentially expressed in human endometrial cancer transcriptome-wide, that mRNA for KPNA2 is present at significantly increased quantity in endometrial tumor tissue as compared to benign endometrium, and that increased KPNA2 primary tumor expression is correlated with worse overall survival in patients with endometrial cancer. These data allude to potential kinetic activation of nuclear trafficking mediated by karyopherin $\alpha 2$ in 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. Day, R.S., McDade, K.K., Chandran, U.R., Lisovich, A., Conrads, T.P., Hood, B.L., Kolli, V.K., Kirchner, D., Litzi, T. and Maxwell, G.L., 2011. Identifier mapping performance for integrating transcriptomics and proteomics experimental results. *BMC bioinformatics*, 12(1), p.213.
7. Nagy, A., Munkacsy, G. and Gyorffy, B., 2020. Pancancer survival analysis of cancer hallmark genes. *bioRxiv*.

Rank: 59
Probe ID: 211762_s_at
p-value: 4.69e-05
t: -6.1454585
B: 2.34438
Gene: KPNA2
Gene name: karyopherin alpha 2

Chart 1: KPNA2 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: 11
probe ID: 201088_at
p-value: 1.03e-10
t: -7.2335576
B: 13.96311
Gene: KPNA2
Gene name: karyopherin alpha 2

Chart 2: KPNA2 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: KPNA2 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 KPNA2 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 KPNA2 expression between benign endometrial tissue and primary tumors of the endometrial tissue is $p=0.000181$.

Figure 2: Significant correlation between KPNA2 primary tumor expression and overall survival in patients with endometrial cancer.

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 KPNA2 in patient primary tumors, for patients in the upper survival quartile. 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 KPNA2 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 KPNA2 expression: 78.4 months
High KPNA2 expression: 34.83 months

Chart 3: Median overall survival is inferior in endometrial cancer patients with high tumor expression of KPNA2.

The median overall survival of endometrial cancer patients expressing low levels of KPNA2 or high levels of KPNA2 in endometrial cancer tissue is listed in this chart, for $n=543$ endometrial cancer patients, in the upper survival quartile.