1 **Over-expression of the kayopherin α 2 gene (KPNA2) in high-grade serous ovarian cancers.**

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5 Ovarian cancer is the most lethal gynecologic malignancy and 70-80% of ovarian cancers are of
6 the high-grade serous type (1-3). To identify the most significant changes in gene expression in
7 high-grade serous ovarian cancer (HGSC), we compared global gene expression profiles of
8 tumors from patients with HGSC to that of normal ovary using published microarray datasets (4,
9 5). We found that the nuclear import receptor karyopherin α 2 (KPNA2) (6) was among the genes
10 whose expression changed most significantly when comparing HSGC tumors to the ovary.
11 Karyopherin α 2 may be relevant to the biology of high-grade serous ovarian tumors.

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27 **Keywords:** KPNA2, ovarian cancer, HGSC, high-grade serous ovarian cancer, systems biology of
28 ovarian cancer, targeted therapeutics in ovarian cancer.

1 The five-year survival rate for women diagnosed with high-grade serous ovarian cancer is
2 between 30-40% and has not changed significantly in decades (1-3). The development of novel,
3 targeted therapeutics to treat HGSC can be facilitated by an enhanced understanding of the
4 transcriptional behavior of ovarian tumors relative to that of the normal ovary. We mined
5 published microarray data (4, 5) to compare global gene expression profiles between HGSC
6 ovarian tumors and that of normal ovarian tissue. We identified the gene encoding karyopherin
7 $\alpha 2$, KPNA2, as among the most differentially expressed in HGSC tumors of the ovary. KPNA2
8 may be a gene of interest when prioritizing the study of target genes and pathways for the
9 development of novel therapeutic interventions in high-grade serous ovarian cancers.
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11 **Methods**

12 We used microarray data from datasets GSE146556 (4) and GSE124766 (5) for this
13 differential gene expression analysis of high-grade serous carcinomas. The Benjamini and
14 Hochberg method of p-value adjustment was used for ranking of differential expression but raw p-
15 values were used for assessment of statistical significance of global differential expression. Log-
16 transformation of data was auto-detected, and the NCBI generated category of platform
17 annotation was used. GSE146556 (4) was generated using Affymetrix Human Gene 1.0 ST Array
18 with $n=3$ for normal ovarian tissue and $n=40$ for tumors from patients with high-grade serous
19 ovarian cancer. GSE124766 (5) was generated using Agilent-014850 Whole Human Genome
20 Microarray 4x44K G4112F with $n=3$ of for normal ovarian tissue and $n=8$ for tumors from patients
21 with high-grade serous ovarian cancer. GEO2R provides mRNA expression levels only for the top
22 250 most differentially expressed genes.
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24 A statistical test was performed to evaluate whether KPNA2 expression was significantly
25 different when comparing normal ovarian tissue from control subjects and primary tumors from
26 women diagnosed with HGSC using a two-tailed, unpaired t-test with Welch's correction. Only
27 p-values less than 0.05 were considered statistically significant. We used PRISM for all
28 statistical analyses (Version 8.4.0)(455).

Results

We mined published microarray data (4, 5) to identify differentially expressed genes in high-grade serous ovarian cancer (HGSC), the type of ovarian cancer responsible for 70-80% of deaths resulting from the most lethal gynecologic malignancy.

KPNA2 is differentially expressed in ovarian tumors from women diagnosed with HGSC.

We identified the gene encoding karyopherin $\alpha 2$ (KPNA2) as among the genes whose expression was quantitatively most different in a published microarray dataset (4) when comparing primary HGSC tumors to normal ovarian tissue (Table 1). When sorting all of the transcripts measured by microarray based on change in expression between HGSC tumors and the normal ovary, KPNA2 ranked 184 out of 29088 total transcripts (Table 1). Differential expression of KPNA2 in HGSC tumors was statistically significant (Table 1; $p=1.31E-07$).

We analyzed a second microarray dataset generated using normal ovarian tissues and tumors from women diagnosed with HGSC (5) to determine whether differential expression of KPNA2 could be observed in a separate group of patients. We again found that KPNA2 was differentially expressed in primary HGSC tumors (Table 2). When sorting all of the transcripts measured by microarray based on change in expression between HGSC and the normal ovary, in this data set, KPNA2 ranked 4418 out of 41093 total transcripts (Table 2). This means the change in KPNA2 expression when comparing HGSC tumors to the ovary was greater than the change in expression of 89% of the remaining transcripts measured, globally. Differential expression of KPNA2 in HGSC tumors was statistically significant (Table 2; $1.11E-02$).

KPNA2 is expressed at significantly higher levels in HGSC when compared to the normal ovary.

We then obtained exact mRNA expression levels for the differentially expressed KPNA2 transcript from both normal ovarian tissue and from high-grade serous ovarian tumors. KPNA2 was expressed at significantly higher levels in high-grade serous ovarian cancers when compared

1 to the normal ovary (Figure 1; $p < 0.0001$). We calculated a mean fold change of 8.4478 ± 4.4836
2 in KPNA2 expression when comparing HGSC tumors to normal ovarian tissue (Table 1).

3 Thus, we found using published microarray data (4, 5) that KPNA2 was among the genes
4 most differentially expressed in tumors from women with high-grade serous carcinomas and that
5 KPNA2 expression was significantly higher in HGSC tumors when compared to the normal ovary.
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7 **Discussion**

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9 We sought to continue to describe the transcriptional landscape of ovarian cancer (7) by
10 mining published microarray data (4, 5) comparing the normal ovary to tumors from patients with
11 the most common type of epithelial ovarian cancer, high-grade serous ovarian cancer (HGSC).
12 We identified the nuclear import receptor karyopherin $\alpha 2$ (KPNA2) as among the genes most
13 differentially expressed in HGSC tumors.
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15 Transport of cargoes into the nucleus, for molecules above a molecular weight threshold,
16 is a receptor- and ATP-mediated process (8). KPNA2 is part of the cellular nuclear import
17 machinery used by these cargoes (6). Also known as Importin $\alpha 2$, the product encoded by the
18 KPNA2 gene functions as an adapter, interacting with proteins containing a classical nuclear
19 localization signal (NLS) rich in basic amino acids (lysine and arginine) through interactions
20 between the armadillo domain of KPNA2 and the NLS-containing cargo (6, 8). In the presence of
21 the G-protein Ran in its GTP-bound form, the importin- β binding (IBB) domain of KPNA2 can, at
22 the same time, interact with the Kap β nuclear import receptor through structural elements known
23 as HEAT repeats that are the hallmark of the karyopherin β family (9). RanGTP-bound Kap $\beta 1$ can
24 then facilitate transport through the nuclear pore complex through hydrophobic interactions with
25 FG-repeat containing nucleoporins (Nups) (9). Thus, KPNA2 functions in the transport of protein
26 cargoes containing a classical NLS into the nucleus and through the nuclear pore complex by
27 forming dual contacts with the cargo and RanGTP-bound KPN $\beta 1$ (6, 8, 9).
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1 KPNA2 has been described broadly as a biomarker in multiple cancers (10) and cited as a
2 uniform marker of poor prognosis in cancer (11). In ovarian cancer, KPNA2 has been reported as
3 a serum biomarker and correlated with worse prognosis (12). KPNA2 was found to promote the
4 proliferation of EFO-21 and SK-OV3 ovarian cancer cell lines *in vitro* and *in vivo*; depletion of
5 KPNA2 caused cell cycle arrest at G1/S phase (13). Investigators cited up-regulation of Myc,
6 down-regulation of FOXO3a, activation of protein kinase B (Akt), as well as effects on genes of
7 the cell cycle - up-regulation of p21Cip1 and p21Kip1 and down-regulation of Cyclin D1 - as
8 mechanisms by which KPNA2 might exert its function in epithelial ovarian cancers (13).
9 Depletion of KPNA2 lead to an increased fraction of Myc in the cytoplasm but investigators did not
10 demonstrate transport of Myc by KPNA2 (13). A separate study of epithelial ovarian cancers cited
11 interactions between the microRNA-26b and KPNA2 as important for limiting the progression of
12 ovarian cancer, demonstrating that KPNA2 was a direct target of miR-26b and that miR-26b
13 levels were significantly decreased in epithelial ovarian cancer tissues (14).

15 Investigators have identified dysregulated expression of KPNA2 in multiple other solid
16 tumors types: breast (15, 16), endometrial (17), gastric (18), bladder (19), colorectal (20),
17 hepatocellular (21) and non-small cell lung cancer (22-23). Mechanistically, whether karyopherin
18 $\alpha 2$ can support tumorigenesis or is simply a reflection of the tumor environment is unclear.
19 Changes in localization and expression of the pluripotency factor Oct4 (24) have been reported as
20 correlated with dysregulated expression of KPNA2 in epithelial ovarian (13), bladder (19), and
21 non-small cell lung cancer (23). In bladder cancer, increased levels of Oct4 in the nucleus
22 resulting from up-regulation of KPNA2 were found to be an important mechanism in driving
23 cancer cell proliferation, as silencing KPNA2 in T24 and J82 bladder cancer cell lines inhibited
24 proliferation (19); this same study demonstrated a direct interaction between Oct4 and KPNA2
25 (19). A study of hepatocellular carcinomas (21) described changes in expression of cell cycle
26 genes CCNB2 and CDK1 coincident with changes in KPNA2, and found significant correlation
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1 between KPNA2, CCNB2 and CDK1 levels, but it is unclear how these changes relate to the
2 function of karyopherin α 2 as a nuclear import receptor.

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4 We found here that the nuclear import receptor KPNA2 was expressed at significantly
5 higher levels in tumors from a group of patients with high-grade serous ovarian cancer when
6 compared to the normal ovary, using published microarray data (4, 5). This change in KPNA2
7 expression was among the highest transcriptome-wide when comparing HGSC tumors to benign
8 ovarian tissue. A small molecule targeting the nuclear export receptor CRM1, XPOVIO (formerly
9 Selinexor) (25) was recently approved by the FDA for use in relapsed and refractory multiple
10 myeloma. A specific requirement for KPNA2 in high-grade serous ovarian cancer can be
11 assessed by measuring tumor cell proliferation in HGSC cell lines *in vitro* (26) and on survival
12 outcomes *in vivo* using HGSC xenografts and genetically engineered mouse models of HGSC
13 (27-29). KPNA2 mediates the transport of many cargoes, and toxicities resulting from off-target
14 effects might be limited by identifying specific cargoes of KPNA2 that support tumor growth and
15 by targeting the interface between KPNA2 and those cargoes rather than the general function of
16 KPNA2.
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Rank	ID	p-value	t	B	Fold change	Gene	Gene name
184	8009417	1.31E-07	6.2696404	7.4456848	8.4478 ± 4.4836	KPNA2	karyopherin subunit alpha 2

Table 1: KPNA2 is differentially expressed in high-grade serous ovarian carcinomas.

The rank of differential expression, the probe/transcript ID, the p-value with respect to global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the two groups compared, the fold change of KPNA2 in the patient population when compared to control subjects, the gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
4418	A_23_P125265	1.11E-02	3.009041	-3.04454	KPNA2	karyopherin subunit alpha 2

Table 2: KPNA2 is differentially expressed in high-grade serous ovarian carcinomas.

The rank of differential expression, the probe/transcript ID, the p-value with respect to global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the two groups compared, the gene and gene name are listed in this chart.

Figure 1: KPNA2 expression is significantly elevated in HGSC tumors.

The mRNA expression of KPNA2 in normal ovarian tissues from control subjects (left) and in the primary tumors of patients with HGSC (right) is represented with mean mRNA expression level marked and the result of a statistical test evaluating the significance of difference in KPNA2 expression between the ovary of control subjects and primary tumors from patients with HGSC, a *p*-value, listed above.