1 RBPMS is differentially expressed in high-grade serous ovarian cancers.

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7 Ovarian cancer is the most lethal gynecologic cancer¹. We sought to identify genes associated
8 with high-grade serous ovarian cancer (HGSC) by comparing global gene expression profiles of
9 normal ovary with that of primary tumors from women diagnosed with HGSC using published
10 microarray data^{2,3}. We found significant differential expression of RBPMS in high-grade serous
11 ovarian tumors.
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26 Keywords: ovarian cancer, high-grade serous ovarian cancer, HGSC, targeted therapeutics in
27 ovarian cancer, systems biology of ovarian cancer, RBPMS.
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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^{4,5}. 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^{2,3} to compare global gene expression profiles between HGSC ovarian
6 tumors and that of normal ovarian tissue. We identified the gene encoding RBPMS as among the
7 most differentially expressed genes in HGSC tumors of the ovary. RBPMS may be a gene of
8 interest when prioritizing the study of target genes and pathways for the development of novel
9 therapeutic interventions in high-grade serous ovarian cancers.
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14 **Methods**

15 We used microarray data from datasets GSE124766² and GSE146556³ for this
16 differential gene expression analysis of high-grade serous ovarian carcinomas. The Benjamini
17 and Hochberg method of p-value adjustment was used for ranking of differential expression but
18 raw p-values were used for assessment of statistical significance of global differential expression.
19 Log-transformation of data was auto-detected, and the NCBI generated category of platform
20 annotation was used. GSE124766 was generated using Agilent-014850 Whole Human Genome
21 Microarray 4x44K G4112F with n=3 of for normal ovarian tissue and n=8 for tumors from
22 patients with high-grade serous ovarian cancer. GSE146556 was generated using Affymetrix
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Human Gene 1.0 ST Array with n=3 for normal ovarian tissue and n=40 for tumors from patients with high-grade serous ovarian cancer. GEO2R provides mRNA expression levels only for the top 250 most differentially expressed genes.

A statistical test was performed to evaluate whether RBPMS expression was significantly different when comparing normal ovarian tissue from control subjects and primary tumors from women diagnosed with HGSC using a two-tailed, unpaired t-test with Welch's correction. Only p-values less than 0.05 were considered statistically significant. We used PRISM for all statistical analyses (Version 8.4.0)(455).

Results

We mined published microarray data^{2,3} 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.

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

We identified the gene encoding RBPMS as among the genes whose expression was most different when comparing primary HGSC tumors to normal ovarian tissue² (Table 1). We found that RBPMS was differentially expressed in primary HGSC tumors (Table 1). When sorting all of the transcripts measured by microarray based on change in expression between HGSC and the normal ovary, RBPMS ranked 132 out of 41093 total transcripts (Table 1). Differential

1 expression of RBPMS in HGSC tumors was statistically significant (Table 1; $p=5.05E-06$).

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3 We analyzed a second microarray dataset³ generated using normal ovarian tissues and
4 tumors from women diagnosed with HGSC to determine whether differential expression of
5 RBPMS could be observed in tumors from a separate group of patients. We again found that
6 RBPMS was differentially expressed in primary HGSC tumors (Table 2). When sorting all of the
7 transcripts measured by microarray based on change in expression between HGSC and the
8 normal ovary, in this data set, RBPMS ranked 2212 out of 29088 total transcripts (Table 2).
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10 Differential expression of RBPMS in HGSC tumors in this second microarray dataset was
11 statistically significant (Table 2; $p=1.23E-03$).
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14 RBPMS is expressed at lower levels in HGSC when compared to the normal ovary.

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16 We obtained exact mRNA expression levels for the differentially expressed RBPMS
17 transcript from both normal ovarian tissue and from high-grade serous ovarian tumors. RBPMS
18 was expressed at lower levels in high-grade serous ovarian cancers when compared to the normal
19 ovary; this difference was statistically significant (Figure 1; $p=0.0026$). We calculated a mean
20 fold change of 0.7338 ± 0.0466 in RBPMS expression when comparing HGSC tumors to normal
21 ovarian tissue (Table 1).
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24 Thus, we found using published microarray data that RBPMS was among the genes most
25 differentially expressed in tumors from women with high-grade serous carcinomas and that
26 RBPMS expression was lower in HGSC tumors when compared to the normal ovary.
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Discussion

Transcriptional profiling of HGSC in a Cancer Genome Atlas integrated genomic analysis classified HGSC into four subtypes based on gene clustering: immunoreactive, differentiated, proliferative, and mesenchymal⁶. We sought to continue to describe the transcriptional landscape of high-grade serous ovarian cancers⁶ and identify genes whose differential expression was associated with HGSC by using published microarray data from primary tumors of women diagnosed with HGSC compared to transcriptome data from normal ovarian tissue^{2,3}. In both datasets analyzed, RBPMS was among the genes whose expression changed most significantly when comparing the normal ovary to primary HGSC tumors.

RBPMS, or RNA-binding protein with multiple splicing, is an RNA-binding protein and the human homologue of *Xenopus* Hermes⁷. RBPMS is one member of a large family of RRM (RNA Recognition Motif) proteins that possess one RRM motif. Highest expression of RBPMS is in the developing heart⁸. In the vegetal cortex of the *Xenopus* oocyte, Hermes is degraded within hours during oocyte maturation, and depletion of Hermes results in cleavage defects of vegetal blastomeres⁹. In stage I oocytes, Hermes interacts with nanos and this interaction requires RNA localization signals in the nanos-3'-UTR. hnRNP I also interacts with Hermes and may be a co-factor required for the interaction between nanos and Hermes within germinal granules¹⁰. In the central nervous system, RBPMS has exclusive expression in retinal ganglion cells, and an established function of RBPMS is in the regulation of axon arbor formation in RGC¹¹. Deficiency of Hermes protein *in vivo* leads to defects in axon arbor complexity in the

1 optic tectum in both *Xenopus* and zebrafish and defects in granule localization¹¹.

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3 In differentiated vascular smooth muscle cells, RBPMS is a regulator of mRNA splicing.
4 RBPMS expression is down-regulated during switching of SMCs from contractile to motile
5 phenotypes; 20% of the alternative splicing (AS) changes during this phenotypic switch could be
6 attributed to RBPMS¹². RBPMS regulated alternative splicing of cytoskeletal elements including
7 actin and focal adhesion machinery, as well as splicing of the SMC transcription factor
8 Myocardin, suggesting RBPMS is a master regulator of AS in SMCs¹².
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12 Investigators used PAR-CLIP (photo-activatable-ribonucleoside-enhanced cross linking
13 and immunoprecipitation) in HEK293 cells to identify RNA interaction partners of RBPMS and
14 found that RBPMS bound tandem CAC motifs separated by spacers variable in length; *in vitro*,
15 RBPMS could bind (AC)₉ and (CAC)₆ motifs¹³. Specific RNA messages bound by RBPMS in
16 PAR-CLIP included NDUFA6, UBE2V1, SRM and ETF1¹³. In the free state, RBPMS forms a
17 symmetrical dimer, and mutations in the dimerization and RNA-binding interface of RRM motif
18 result in disruption of RBPMS dimerization and decreased RNA-binding affinity¹⁴.
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22 Multiple studies implicate RBPMS in some aspect of ovarian cancer pathogenesis and in
23 the biology of other human cancers. RBPMS is over-expressed in the A2780 cervical cancer cell
24 line and depletion of RBPMS in A2780 cells using small interfering RNA (siRNA) resulted in an
25 increase in invasive potential, while over-expression of RBPMS in cisplatin-resistant
26 A2780CP20 cells resulted in decreases in cell proliferation¹⁵. A study identifying differentially
27 expressed genes between recurrent and non-recurrent ovarian cancer found that RBPMS was
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1 among the most differentially expressed genes (DEGs) between these groups and formed a node
2 when generating a protein-protein interaction network based on interaction between DEGs (16).
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4 miR-375 was a differentially expressed microRNA in this analysis, and RBPMS was identified
5 as a target gene of miR-375¹⁶. Investigators studying miR-21-3p in ovarian cancer found that
6 depletion of miR-21-3p resulted in decreases in proliferation and invasion *in vitro*; luciferase
7 assays identified RBPMS as a target of miR-21-3p¹⁷. In cell culture, depletion of RBPMS
8 reduced sensitivity to cisplatin treatment¹⁷. In a group of Korean patients with stage IIIC HGSC,
9 a one of 27 genes located in a region of copy number loss on 8p21.1-p12¹⁸. In drug-resistant
10 multiple myeloma (MM) cells, polycomb group protein EZH2 was identified as over-expressed,
11 and associated with worse prognosis in MM patients; using chromatin immunoprecipitation of
12 the H3K27me3 mark, investigators identified RBPMS as a target of EZH2¹⁹. miR-138 was
13 identified as an EZH2-targeting microRNA, and over-expression of EZH2, like depletion of
14 RBPMS, resulted in higher resistance of MM cells to anti-myeloma therapies¹⁹. miR-138 over-
15 expression restored RBPMS and restored sensitivity to MM chemotherapeutics *in vitro*¹⁹.
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17 RBPMS has also been described as up-regulated in dedifferentiated hepatocellular carcinomas
18 (HCC) and in HCC with loss of 13q²⁰.
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23 Differential expression of RBPMS in HGSC should be validated in larger and separate
24 cohorts of women with HGSC. If validated, mouse models of high-grade serous ovarian
25 cancers²¹⁻²³ can be utilized to assess the effect of conditional expression of RBPMS in HGSC
26 tumor growth post-development and on survival *in vivo*.
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Rank	ID	p-value	t	B	FC	Gene	Gene name
132	A_24_P930418	5.05E-06	-7.8692125	4.52513	0.7338 ± 0.0466	RPMS	RNA binding protein with multiple splicing

Table 1: RPMS 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 RPMS in HGSC tumors when compared to the normal ovary, the gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
2212	8145669	1.23E-03	-3.4529684	-1.2615066	RPMS	RNA binding protein with multiple splicing

Table 2: RBPMS 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 RBPMS in HGSC tumors when compared to the normal ovary, the gene and gene name are listed in this chart.

Figure 1: RBPMS is expressed at significantly lower levels in HGSC tumors when compared to the normal ovary.

The mRNA expression of RBPMS 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 RBPMS expression between the normal ovary and primary tumors from patients with HGSC, a p-value, listed above.