ASPM is differentially expressed in high-grade serous ovarian cancers.

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Ovarian cancer is the most lethal gynecologic cancer¹. We sought to identify genes associated with high-grade serous ovarian cancer (HGSC) by comparing global gene expression profiles of normal ovary with that of primary tumors from women diagnosed with HGSC using published microarray data^{2,3}. We found significant differential expression of the gene encoding ASPM in high-grade serous ovarian tumors.

Keywords: ovarian cancer, high-grade serous ovarian cancer, HGSC, targeted therapeutics in ovarian cancer, systems biology of ovarian cancer, ASPM.

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 ASPM as among the
7 most differentially expressed in HGSC tumors of the ovary. ASPM may be a gene of interest
8 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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16 **Methods**

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

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

11 12 **Results**

13 We mined published microarray data^{2,3} to identify differentially expressed genes in high-
14 grade serous ovarian cancer (HGSC), the type of ovarian cancer responsible for 70-80% of
15 deaths resulting from the most lethal gynecologic malignancy.

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

19 We identified ASPM as among the genes whose expression was most different when
20 comparing primary HGSC tumors to normal ovarian tissue² (Table 1). We found that ASPM was
21 differentially expressed in primary HGSC tumors (Table 1). When sorting all of the transcripts
22 measured by microarray based on change in expression between HGSC and the normal ovary,
23 ASPM ranked 234 out of 41093 total transcripts (Table 1). Differential expression of ASPM in
24 HGSC tumors was statistically significant (Table 1; $p=1.73E-05$).

27 We analyzed a second microarray dataset³ generated using normal ovarian tissues and
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tumors from women diagnosed with HGSC to determine whether differential expression of ASPM could be observed in tumors from a separate group of patients. We again found that ASPM 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, in this data set, ASPM ranked 581 out of 29088 total transcripts (Table 2). Differential expression of ASPM in HGSC tumors in this second microarray dataset was statistically significant (Table 2; $p=1.25E-05$).

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

We obtained exact mRNA expression levels for the differentially expressed ASPM transcript from both normal ovarian tissue and from high-grade serous ovarian tumors. ASPM was expressed at higher levels in high-grade serous ovarian cancers when compared to the normal ovary; this difference was statistically significant (Figure 1; $p=0.0002$). We calculated a mean fold change of 1.6297 ± 0.1577 in ASPM expression when comparing HGSC tumors to normal ovarian tissue (Table 1).

Thus, we found using published microarray data that ASPM was among the genes most differentially expressed in tumors from women with high-grade serous carcinomas and that ASPM expression was lower in HGSC tumors when compared to the normal ovary.

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, ASPM was among the genes whose expression changed most significantly when comparing the normal ovary to primary HGSC tumors.

ASPM, or abnormal spindle-like microcephaly associated, is the human ortholog of the *Drosophila* abnormal spindles (asp) gene⁷. Variations in ASPM are the most common mutations found in humans with autosomal recessive primary microencephaly (MCPH)⁸. *Drosophila* embryo extracts depleted of ASPM are defective in their ability to regenerate microtubule organizing centers after centrosome salt-stripping; authors concluded that ASPM was important to maintain the structural integrity of microtubule-nucleating γ -tubulin ring complexes, important for organization of the mitotic centrosome⁷. ASPM plays an important role in the nucleation of microtubules at centrosomes, localizing to spindle poles during mitosis. During interphase, ASPM is localized to the centrosome, and from prophase through telophase, to the spindle poles. Depletion of ASPM results in decreases in endogenous BRCA1 levels⁹. ASPM expression is most abundant in the developing brain but detected broadly throughout tissues. ASPM isoforms possess 74 or 81 IQ domains, organized into structures known as higher order repeats, in addition to amino-terminal calponin-homology (CH) domains, both of which have

1 actin-binding functions in the cytoskeleton.

2 During mitosis, ASPM localizes to the spindle poles¹⁰. In *C. Elegans*, positioning of the
3 spindle apparatus requires machinery including LIN-5/GPR-1/2/G α . ASPM was identified as an
4 interaction partner of LIN-5, and in conjunction with calmodulin (CMD-1), ASPM supported
5 organization of the meiotic spindle and LIN-5 accumulation at the spindle poles during both
6 mitosis and meiosis. As LIN-5 can engage dynein, these data suggested that CMD-1 and ASPM
7 were important for recruitment of LIN-5 to the meiotic and mitotic spindle poles¹¹.
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11 ASPM is essential for normal cell cycle kinetics. Mice engineered to express a truncated
12 ASPM mutant have low brain-to-body weight ratios, characteristic of microencephaly, and the
13 outer layers of the cortex are thinner, with an expansion of deep layers and reduction in
14 superficial layers II-IV. The brains of ASPM mutant mice manifest cortical thinning that could
15 be attributed to premature neural progenitor cell (NPC) exhaustion as a result of increases in
16 NPCs exiting the cell cycle - the Quiescence fraction - accompanied by reductions in daughter
17 cells re-entering the cell cycle - the Proliferative fraction¹². ASPM can influence the localization
18 and abundance of phosphorylated Cyclin E; delivery of Cyclin E by lentivirus transduction could
19 correct elongated cell cycle phenotypes found in the brains of ASPM mutant mouse embryos¹¹.
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22 Mechanistically, ASPM could interact with and maintain the stability of Cyclin E by limiting
23 interactions between Cyclin E and both Fbw7 and Skp2 E3 ligases, and thus Cyclin E
24 degradation by ubiquitination¹². Depletion of ASPM by small interfering RNA increases the
25 sensitivity of normal human fibroblasts and glioblastoma cell lines to ionizing radiation, with a
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1 concomitant increase in DNA double-strand breaks in irradiated cells¹³. IR sensitization was not
2 observed in DNA-PK-deficient glioblastoma cells, suggesting that ASPM was able to exert this
3 influence on the DNA damage response through DNA-PK¹³.
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5 ASPM is implicated in a variety of human cancers. In epithelial ovarian cancer, lower
6 expression of ASPM was generally associated with improved survival probability in a small
7 group of patients, and highest levels of ASPM expression in the cytoplasm were associated with
8 Grade 1 tumors in epithelial ovarian cancer¹⁴. A separate study using a larger sample of tissues
9 from patients with EOC demonstrated that in the serous type, as tumor grade and tumor stage
10 increased, cytoplasmic ASPM expression decreased¹⁵. Tumor invasiveness and lymph node
11 involvement was also significantly associated with decreased cytoplasmic ASPM¹⁵.
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15 Outside of epithelial ovarian cancer, roles for ASPM have been described in prostate
16 cancer, in medulloblastoma and glioblastoma, and in breast cancer. In prostate cancer, higher
17 expression of ASPM is associated with tumor progression and worst outcome¹⁶; a study found
18 that ASPM was able to support stem-like properties in prostate cancers through Wnt-Dvl3- β
19 catenin signaling pathways, interacting with disheveled-3 and enhancing its stability by
20 preventing its proteasomal degradation¹⁷. Depletion of ASPM in prostate cancer cells impaired
21 proliferation and colony formation, and ASPM co-localized with aldehyde dehydrogenase
22 (ALDH)¹⁷. In mice with constitutively active Smoothened-driven medulloblastoma, deletion of
23 ASPM resulted in increased DNA damage and reduced tumor growth¹⁸. While deletion of
24 ASPM reduced normal cerebellar growth in mice, it concurrently resulted in increased mitosis in
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1 cerebellar granule neuron progenitors¹⁸.

2 Scientists using gene expression data to identify gene expression signatures shared
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4 between glioblastoma and breast cancers found a co-expression module within which ASPM was
5 deemed a key member¹⁹. ASPM was found to be over-expressed in glioblastomas and depletion
6 of ASPM using siRNA impaired proliferation of both glioblastoma tumor cells as well as neural
7 stem cells¹⁸. Similarly, scientists using gene expression datasets to identify biomarkers in breast
8 cancer found that ASPM was associated with worse prognosis in patient datasets and that highest
9 expression of ASPM was correlated with advanced tumor grade²⁰. ASPM was over-expressed in
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11 66% of hepatocellular carcinomas (HCC), and its over-expression was significantly correlated
12 with tumor grade, tumor stage, as well as early tumor recurrence. ASPM expression was the
13 single most significant factor associated with early tumor recurrence, and patients with
14 ASPM over-expression possessed significantly lower 5-year survival in both p53-mutated and
15 non-p53-mutated HCC²¹. Thus, ASPM is over-expressed in multiple tumor tissues, and its
16 expression is associated with poor outcomes in multiple cancers.

17 Differential expression of ASPM in HGSC should be validated in larger and separate
18 cohorts of women with HGSC. If validated, mouse models of high-grade serous ovarian
19 cancers²²⁻²⁴ can be utilized to assess the effect of conditional expression of ASPM in HGSC
20 tumor growth post-development and on survival *in vivo*.

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Rank	ID	p-value	t	B	FC	Gene	Gene name
234	A_23_P52017	1.73E-05	6.9409796	3.33288	1.6297 ± 0.1577	ASPM	abnormal spindle microtubule assembly

Table 1: ASPM 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 ASPM 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
581	7923086	1.25E-05	4.9157912	3.0742901	ASPM	abnormal spindle microtubule assembly

Table 2: ASPM 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: ASPM is expressed at significantly higher levels in HGSC tumors when compared to the normal ovary.

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