Fatty acid binding protein 5 (FABP5) is differentially expressed in high-grade serous ovarian cancers and based on survival outcomes.

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We used published and public microarray data to identify the most striking features of the transcriptional landscape of high-grade serous ovarian cancers (HGSC) (1-3). We identified fatty acid binding protein 5 (FABP5) among the genes whose expression changed most significantly in HGSC tumors when compared to normal ovarian tissue (1, 2). Analysis of a separate dataset revealed that FABP5 was expressed at significantly lower levels in the tumors of HGSC patients who survived greater than 1100 days as compared to patients with progression-free survival less than 100 days (3). Fatty acid binding protein 5 expression is pro-tumorigenic, correlated with worse prognosis, and supports metastasis and invasion of multiple solid tumors (4-9). These data suggest FABP5 might play a similar role in high-grade serous ovarian cancer.

Keywords: ovarian cancer, high-grade serous ovarian cancer, FABP5, HGSC, systems biology of ovarian cancer.

1 The five-year survival rate for women diagnosed with high-grade serous ovarian cancer
2 is between 30-40% and has not changed significantly in decades (10, 11). The development of
3 novel, targeted therapeutics to treat HGSC can be facilitated by an enhanced understanding of
4 the transcriptional behavior of ovarian tumors relative to that of the normal ovary. We mined
5 published microarray data (1, 2) to compare global gene expression profiles between HGSC
6 ovarian tumors and that of normal ovarian tissue. We identified the FABP5 gene as among the
7 most differentially expressed in HGSC tumors of the ovary. Differential FABP5 expression also
8 associated with patient survival outcomes (3). FABP5 is a gene of interest when prioritizing the
9 study of target genes and pathways for the development of novel therapeutic interventions in
10 high-grade serous ovarian cancers.
11

12 **Methods**

14 We used microarray data from datasets GSE124766 (1), GSE146556 (2) and
15 GSE140082 (3) for this differential gene expression analysis of high-grade serous carcinomas.
16 The Benjamini and Hochberg method of p-value adjustment was used for ranking of differential
17 expression but raw p-values were used for assessment of statistical significance of global
18 differential expression. Log-transformation of data was auto-detected, and the NCBI generated
19 category of platform annotation was used. GSE124766 (1) was generated using Agilent-014850
20 Whole Human Genome Microarray 4x44K G4112F with n=3 of for normal ovarian tissue and
21 n=8 for tumors from patients with high-grade serous ovarian cancer. GSE146556 (2) was
22 generated using Affymetrix Human Gene 1.0 ST Array with n=3 for normal ovarian tissue and
23 n=40 for tumors from patients with high-grade serous ovarian cancer. GSE140082 (3) was
24 generated using Illumina HumanHT-12 WG-DASL V4.0 R2 expression beadchip technology with
25 n=31 for tumors from patients with progression-free survival less than 100 days and n=36 for
26 tumors from patients with progression-free survival greater than 1100 days. GEO2R provides
27 mRNA expression levels only for the top 250 most differentially expressed genes.
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1 A statistical test was performed to evaluate whether FABP5 expression was significantly
2 different when comparing normal ovarian tissue from control subjects and primary tumors from
3 women diagnosed with HGSC using a two-tailed, unpaired t-test with Welch's correction. Only
4 p-values less than 0.05 were considered statistically significant. We used PRISM for all
5 statistical analyses (Version 8.4.0)(455).
6

7 **Results**

8 We mined published microarray data (1, 2) to identify differentially expressed genes in
9 high-grade serous ovarian cancer (HGSC), the type of ovarian cancer responsible for 70-80% of
10 deaths resulting from the most lethal gynecologic malignancy.
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12 FABP5 is differentially expressed in ovarian tumors from women diagnosed with HGSC.

13 We identified FABP5 as among the genes whose expression was most different when
14 comparing primary HGSC tumors to normal ovarian tissue (Table 1). When sorting all of the
15 transcripts measured by microarray based on change in expression between HGSC and the
16 normal ovary, FABP5 ranked 83 out of 41093 total transcripts (Table 1). Differential expression
17 of FABP5 in HGSC tumors was statistically significant (Table 1; $1.52E-06$).
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20 We analyzed a second microarray dataset encompassing transcriptome data from
21 normal ovarian tissues and from the tumors of women diagnosed with HGSC, in order to
22 determine whether differential expression of FABP5 could be observed in tumors from a
23 different group of patients. Here we identified two separate probe IDs, corresponding to FABP5
24 or FABP5 pseudogene 3 transcripts, among the most differentially expressed in HGSC patients
25 tumors, albeit less significantly so than in the previous dataset (Table 2). When sorting all of the
26 transcripts measured by microarray based on change in expression between HGSC and the
27 normal ovary, in this data set, FABP5/FABP5P3 ranked 4615 and 4658 out of 29088 total
28 transcripts (Table 2). Each of the two FABP5/FABP5P3 transcripts changed in quantity greater

than 83% of the HGSC transcriptome. Differential expression of FABP5/FABP5P3 in HGSC tumors was statistically significant (Table 2; $p=1.2E-02$ and $1.24E-02$, respectively).

FABP5 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 FABP5 transcript from both normal ovarian tissue and from high-grade serous ovarian tumors. FABP5 was expressed at significantly higher levels in high-grade serous ovarian cancers when compared to the normal ovary (Figure 1; $p=0.0019$). We calculated a mean fold change of 1.3117 ± 0.0724 in FABP5 expression when comparing HGSC tumors to normal ovarian tissue (Table 1).

FABP5 is differentially expressed when comparing HGSC tumor transcriptomes based on survival less than 100 days or greater than 1100 days

We used a third dataset to identify genes associated with greater patient survival in high-grade serous ovarian cancer, comparing tumors from patients surviving less than 100 days to that of patients surviving greater than 1100 days (3). This data was obtained from public microarray datasets generated using tumors from patients enrolled in the ICON7 trial (3). We again found that FABP5 was among the genes most differentially expressed in HGSC, but in this comparison, based on survival outcomes (Table 3 and Figure 2). When sorting all transcripts measured by microarray based on change in expression between the tumors of patients surviving less than 100 days and those surviving greater than 1100 days, FABP5 ranked 59 out of 29377 transcripts (Table 3). Differential expression of FABP5 in HGSC tumors based on patient progression-free survival less than 100 days or greater than 1100 days was statistically significant (Table 3; $p=0.00054048$). Consistent with higher expression of FABP5 in tumors when compared to the normal ovary, FABP5 was expressed at significantly lower levels in primary HGSC tumors from patients with progression-free survival greater than 1100 days when compared to that of patients surviving less than 100 days (Figure 2; $p=0.0005$).

Thus, we found using published microarray data that FABP5 was among the genes most differentially expressed in tumors from women with high-grade serous carcinomas relative to the normal ovary. FABP5 expression was significantly higher in HGSC tumors when compared to the normal ovary. Moreover, FABP5 was among the most differentially expressed genes when comparing primary HGSC tumors based on patient survival, expressed at significantly lower levels in the tumors of patients with progression-free survival greater than 1100 days when compared to that of patients with progression-free survival less than 100 days.

Discussion

To identify genes associated with high-grade serous ovarian cancer, we used published and public microarray data to compare global gene expression profiles of primary tumors from patients with HGSC to that of the normal ovary (1, 2). We found that HGSC tumors expressed significantly higher levels of FABP5 than normal ovarian tissue. Accordingly, using a separate HGSC patient tumor dataset with paired survival data (3), we found that the tumors of patients with better survival outcomes expressed significantly lower levels of FABP5 than those with the worst survival outcomes: progression-free survival less than 100 days.

FABP5 can interact with both linoleic acid arachidonic acid, and this interaction leads to nuclear translocation of FABP5 and activation of PPAR β transcriptional activity (12). The adipose tissues of transgenic mice over-expressing FABP5 display significant increases in basal and hormone-stimulated lipolysis (13). Experiments using viral infection models in mice demonstrated that tissue-resident memory CD8⁺ T-cells generated in the skin during viral infection require uptake of lipids and that these cells differentially expressed FABP5 (14). While central memory T-cells were intact in FABP4/FABP5-deficient mice, T_{RM} cells were impaired functionally impaired in uptake of free fatty acids and possessed impaired survival. Inhibiting mitochondrial β -oxidation of free fatty acids (FFA) lead to decreases in persistence of T_{RM} in the skin, together demonstrating a role for FABP5 in lipid uptake and survival of T_{RM} generated

1 during viral infection, and involving mitochondrial FFA β -oxidation (14). There is a wide body of
2 literature regarding roles for FABP5 in an array of cancer types. One study documented
3 dysregulated expression of FABP5 in prostate cancer involving epigenetic mechanisms (4), and
4 another showed that inactivation of FABP5 disfavored tumor growth by activating the
5 peroxisome proliferator-activated receptor gamma (PPAR γ) (5). Accordingly, expression of
6 FABP5 has been demonstrated to promote metastasis in cervical cancer (6) and gastric cancer
7 (7), and support the metastatic potential of triple negative breast cancer cells (8). In gastric
8 cancer, enhancement of metastatic through FABP5 involved palmitic acid and UCA1/SP1 (7).
9 FABP5 is over-expressed at the protein level in skin tissue from patients with mycosis fungoides
10 (9).
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13 FABP5 differential expression in high-grade serous ovarian cancers should be validated
14 in separate cohorts of patient and at the protein level. We found using public and published
15 microarray datasets that the tumors of HGSC patients with progression-free survival (PFS)
16 greater than 1100 days expressed significantly lower levels of FABP5 mRNA in their primary
17 tumors than patients with PFS less than 100 days. Thus, it would be of interest to determine
18 whether depletion or reduction of FABP5 can alter survival in a mouse model of high-grade
19 serous ovarian cancer (15). FABP5 could be one factor that cooperates with many to promote
20 tumor progression in patients with high-grade serous ovarian cancer.
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Rank	ID	p-value	t	B	Fold change	Gene	Gene name
83	A_32_P204676	1.52E-06	8.8567484	5.66831	1.3117 ± 0.0724	FABP5	fatty acid binding protein 5

Table 1: FABP5 is differentially expressed in tumors from patients with 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 FABP5 in the dorsolateral prefrontal cortex of the patient population when compared to that of control subjects, the gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
4615	7948420	1.2E-02	2.6197701	-3.3448868	FABP5P3///FABP5	fatty acid binding protein 5 pseudogene 3///fatty acid binding protein 5
4658	8147049	1.24E-02	2.607308	-3.3730627	FABP5P3///FABP5	fatty acid binding protein 5 pseudogene 3///fatty acid binding protein 5

Table 2: Two FABP5/FABP5P3 transcripts are differentially expressed in tumors from a separate group of patients 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 FABP5 in the dorsolateral prefrontal cortex of the patient population when compared to that of control subjects, the gene and gene name are listed in this chart.

Rank	ID / GB_ACC	p-value	t	B	Gene
59	ILMN_2146766/ NM_001444.1	0.00054048	-4.0112674	-0.22432	FABP5

Table 3: FABP5 is among the genes most differentially expressed when comparing the tumors of HGSC patients surviving greater than 1100 days with that of patients surviving less than 100 days.

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 FABP5 in the tumors of patients surviving great than 1100 days when compared to that of patients surviving less than 100 days, and the gene are listed in this chart.

Figure 1: FABP5 is expressed at significantly higher levels in HGSC tumors when compared to the normal ovary.

The mRNA expression of FABP5 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 FABP5 expression between the ovary of control subjects and primary tumors from patients with HGSC, a p-value, listed above.

Figure 2: FABP5 is expressed at significantly lower levels in HGSC tumors of patients surviving greater than 1100 days when compared to that of patients surviving less than 100 days.

The mRNA expression of FABP5 in tumors from HGSC patients surviving less than 100 days (left) and in the primary tumors of HGSC patients surviving greater than 1100 days (right) is represented with mean mRNA expression level marked and the result of a statistical test, a p-value, listed above.