FBXO30 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 FBXO30 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, FBXO30.

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^{6,7}. 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 FBXO30 as among
7 the most differentially expressed in HGSC tumors of the ovary. FBXO30 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.
10

11 **Methods**

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

24 A statistical test was performed to evaluate whether FBXO30 expression was
25 significantly different when comparing normal ovarian tissue from control subjects and primary
26 tumors from women diagnosed with HGSC using a two-tailed, unpaired t-test with Welch's
27 correction. Only p-values less than 0.05 were considered statistically significant. We used
28 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.

FBXO30 is differentially expressed in ovarian tumors from women diagnosed with HGSC.

We identified FBXO30 as among the genes whose expression was most different when comparing primary HGSC tumors to normal ovarian tissue² (Table 1). When sorting all of the transcripts measured by microarray based on change in expression between HGSC and the normal ovary, FBXO30 ranked 157 out of 41093 total transcripts (Table 1). Differential expression of FBXO30 in HGSC tumors was statistically significant (Table 1; $p=7.08E-06$).

We analyzed a second microarray dataset³ generated using normal ovarian tissues and tumors from women diagnosed with HGSC to determine whether differential expression of FBXO30 could be observed in tumors from a separate group of patients. We again found that FBXO30 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, FBXO30 ranked 3986 out of 29088 total transcripts (Table 2). Differential expression of FBXO30 in HGSC tumors in this second microarray dataset was statistically significant (Table 2; $p=7.81E-03$).

FBXO30 is expressed at significantly lower levels in HGSC when compared to the normal ovary.

We obtained exact mRNA expression levels for the differentially expressed FBXO30 transcript from both normal ovarian tissue and from high-grade serous ovarian tumors. FBXO30 was expressed at significantly lower levels in high-grade serous ovarian cancers when compared to the normal ovary (Figure 1; $p=0.0003$). We calculated a mean fold change of 0.8334 ± 0.0295 in FBXO30 expression when comparing HGSC tumors to normal ovarian tissue (Table 1).

Thus, we found using published microarray data that FBXO30 was among the genes most differentially expressed in tumors from women with high-grade serous carcinomas and that FBXO30 expression was significantly 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 mesenchymal⁷. We sought to continue to describe the transcriptional landscape of high-grade serous ovarian cancers⁷ 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, FBXO30 was among the genes whose expression changed most significantly when comparing the normal ovary to primary HGSC tumors.

Fbxo30 is a protein of the F-box family⁷, defined by the presence of the F-box motif^{8,9}, a protein-protein interaction motif. F-box proteins function as substrate adapters in the site-specific modification of proteins with ubiquitin for protein degradation at the proteasome in the context of SCF (Skp, Cullin, F-box) or CRL (Cullin RING Ligases) complexes^{10,11}. Incorporation of unique F-box members in a SCF complex enables distinct substrate specificity and thus degradation potential based on which F-box protein is utilized.

In developmental processes, Fbxo30 is important neural tube development, acting as a regulator of bone morphogenetic protein (BMP) signaling and ubiquitination of retinoid acid receptor γ ¹². Investigators used immunoprecipitation coupled with mass spectrometry to identify RAR γ interacting proteins, identifying Fbxo30 as an interaction partner of RAR γ in HEK293T cells¹². Retinoic acid (RA) treatment in a mouse model of neural tube defects (NTD) resulted in decreased expression of FBXO30; likewise, depletion of FBXO30 significantly decreased RA-induced BRE (BMP2-responsive element)-luciferase transcriptional activity¹². A separate study

investigating the BMP pathway in mice found that BMP signaling functioned through Smad1, Smad5 and Smad8 to support muscle growth¹³. Importantly, investigators identified the Fbxo30 gene as a target of transcriptional repression downstream of BMP-Smad1/5/8 signaling, naming Fbxo30 as the muscle ubiquitin ligase of the SCF complex in atrophy-1 (MUSA1)¹³. Fbxo30 expression is induced in denervated muscles and the atrophying muscles Smad4-deficient mice significantly up-regulate Fbxo30¹³. In the ovary Fbxo30 could regulate chromosome segregation during meiosis in oocytes, with peak expression in the germinal vesicle (GV) stage¹⁴. Depletion of FBXO30 in mouse oocytes using small interfering RNA (siRNA) results in halting of chromosome segregation and meiotic progress, and results in accumulation of proteins SBLP and H3¹⁴. H3 localization in FBXO30-depleted cells was disorganized, accompanied by loss of strip-like “chaotic” chromosome shape¹⁴.

A key developmental function of Fbxo30 is in the regulation of mammaryogenesis, exerting its control through dynamic targeting of the mitotic kinesin Eg5 for proteasomal degradation¹⁵. Fbxo30-deficient mice are born in normal mendelian ratios and fertility and growth rate were intact and indistinguishable from Fbxo30 +/+ mice¹⁵. However, while the survival rate of mice reared by Fbxo30+/+ mothers was 82-84%, for offspring reared by Fbxo30-/- mice this rate was 0-12%. Investigators found that mammary glands in Fbxo30-/- mice displayed decreased branching, and with alveoli development adjacent to large ducts¹⁵. Proliferation of mammary glands 2 days postpartum was significantly diminished relative to wild-type and few numbers of glandules contained milk droplets; most glandules in Fbxo30+/+ mice were filled with milk droplets¹⁵. Investigators subsequently found that Fbxo30 could exert control over cell proliferation in the mammary gland through ubiquitin ligase activity of the Eg5 ATPase motor protein¹⁵. There is otherwise limited literature describing roles for Fbxo30 in the ovary, in cancers, or in ovarian cancers.

Differential expression of FBXO30 in HGSC should be validated in larger and separate cohorts of women with HGSC. If validated, mouse models of high-grade serous ovarian

cancers¹⁶⁻¹⁸ can be utilized to assess the effect of conditional expression of FBXO30 in HGSC tumors post-development and on tumor cell proliferation and cancer progression *in vivo*.

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Rank	ID	p-value	t	B	FC	Gene	Gene name
157	A_23_P256231	7.08E-06	-7.6078166	4.20144	0.8334 ± 0.0295	FBXO30	F-box protein 30

Table 1: FBXO30 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 FBXO30 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
3986	8130032	7.81E-03	-2.7866992	-2.9582483	FBXO30	F-box protein 30

Table 2: FBXO30 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 FBXO30 in HGSC tumors when compared to the normal ovary, the gene and gene name are listed in this chart.

Figure 1: FBXO30 is expressed at significantly lower levels in HGSC tumors when compared to the normal ovary.

The mRNA expression of FBXO30 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 FBXO30 expression between the normal ovary and primary tumors from patients with HGSC, a p-value, listed above.