BIRC5 is differentially expressed in epithelial ovarian cancer.

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Epithelial ovarian cancer (EOC) is the most lethal gynecologic cancer¹. We sought to identify genes associated with epithelial ovarian cancer and the high-grade serous ovarian cancer (HGSC) subtype by comparing global gene expression profiles of normal ovary with that of primary tumors from women diagnosed with epithelial cancer and HGSC in specific using published microarray data^{2,3}. We identified the gene encoding the baculoviral inhibitor of apoptosis repeat (IAP) containing 5, BIRC5 as among the genes whose expression was most different in epithelial ovarian cancer and in HGSC ovarian tumors. BIRC5 expression was significantly higher in ovarian tumors relative to normal ovary. Correlation of BIRC5 expression with survival outcomes in patients with ovarian cancer was complex, with low expression favorable early in disease and high expression favorable later in disease. These data indicate that expression of BIRC5 is perturbed in epithelial ovarian cancers broadly and in ovarian cancers of the HGSC subtype. BIRC5 may be relevant to pathways underlying ovarian cancer progression.

Keywords: ovarian cancer, high-grade serous ovarian cancer, HGSC, targeted therapeutics in ovarian cancer, systems biology of ovarian cancer, BIRC5.

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^{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
6 ovarian tumors and that of normal ovarian tissue. We identified the gene encoding BIRC5 as
7 among the most differentially expressed in HGSC tumors of the ovary and in epithelial ovarian
8 cancer broadly. BIRC5 may be a gene of interest when prioritizing the study of target genes
9 and pathways for the development of novel therapeutic interventions in epithelial ovarian
10 cancer and specifically in high-grade serous ovarian cancers.

11 **Methods**

12 We used microarray data from datasets and GSE124766² and GSE146556³ for this
13 differential gene expression analysis of epithelial ovarian cancer. The Benjamini and Hochberg
14 method of *p*-value adjustment was used for ranking of differential expression but raw *p*-values
15 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. GSE124766² was generated using Agilent-014850 Whole Human
18 Genome Microarray 4x44K G4112F with *n*=3 of for normal ovarian tissue and *n*=8 for tumors
19 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 epithelial ovarian cancer: *n*=26 high-grade serous ovarian cancer; *n*=3
22 carinosarcoma, *n*=2 clear cell, *n*=1 endometrioid, *n*=1 granulosa cell, *n*=1 immature teratoma,
23 *n*=4 mixed, and *n*=2 mucinous. GEO2R provides mRNA expression levels only for the top 250
24 most differentially expressed genes.

25 A statistical test was performed to evaluate whether BIRC5 expression was significantly
26 different when comparing normal ovarian tissue from control subjects and primary tumors from
27 women diagnosed with HGSC using a two-tailed, unpaired t-test with Welch's correction. Only
28 *p*-values less than 0.05 were considered statistically significant. We used PRISM for all
statistical analyses (Version 8.4.0)(455). For Kaplan-Meier survival analysis, we used the
Kaplan-Meier plotter online tool⁶ for correlation of BIRC5 mRNA expression levels with
progression-free survival (PFS) in *n*=1435 ovarian cancer patients, and overall survival (OS) in
n=135 stage I & II and *n*=1220 stage III & IV ovarian cancer patients.

Results

We mined published microarray data^{2,3} to identify differentially expressed genes in epithelial ovarian cancer and specifically 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.

BIRC5 is differentially expressed in ovarian tumors from women diagnosed with HGSC

We identified BIRC5 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, in this data set, BIRC5 ranked 206 out of 41093 total transcripts (Table 1), equating to 99.5% differential expression. Differential expression of BIRC5 in HGSC tumors was statistically significant (Table 1; $p=1.34E-05$).

We analyzed a second microarray dataset³ generated using normal ovarian tissues and tumors from women diagnosed with epithelial ovarian cancer (EOC) to determine whether differential expression of BIRC5 could be observed in tumors from a different group of patients, which included HGSC tumors. We found that BIRC5 was differentially expressed in epithelial ovarian cancers broadly (Table 2). When sorting all of the transcripts measured by microarray based on change in expression between tumors from patients with epithelial ovarian cancers and the normal ovary, BIRC5 ranked 5640 out of 29088 total transcripts (Table 2), equating to 80.6% differential expression. Differential expression of BIRC5 in EOC tumors was statistically significant (Table 2; $p=2.16E-02$).

BIRC5 is expressed at significantly higher levels in HGSC when compared to the normal ovary.

We then extracted exact mRNA expression levels for the differentially expressed BIRC5 transcript from both normal ovarian tissue and from high-grade serous ovarian tumors. BIRC5 was expressed at significantly higher levels in high-grade serous ovarian cancers when compared to the normal ovary (Figure 1; $p=0.0233$). We calculated a mean fold change of 1.6807 ± 0.1310 in BIRC5 expression when comparing HGSC tumors to normal ovarian tissue (Table 1).

The relationship between BIRC5 tumor expression and survival outcomes in patients with ovarian cancer is complex.

We performed Kaplan Meier survival analysis⁶ to evaluate whether BIRC5 tumor expression correlated with survival outcomes in patients with ovarian cancer. For patients in the upper but not lower survival quartile, BIRC5 tumor expression was significantly correlated with progression-free survival (PFS) (Figure 2). While median PFS was 18.37 months for ovarian cancer patients with low tumor expression of BIRC5, median PFS was 27 months for ovarian cancer patients with high tumor expression of BIRC5 (Table 3). Correlation of PFS with BIRC5 tumor expression in patients with ovarian cancer was statistically significant in the upper survival quartile (Figure 2: log rank p -value: 0.004; hazard ratio: 0.81 (0.71 - 0.94)).

These data came as a surprise as previous reports have demonstrated positive correlation between BIRC5 expression and patient survival. We further analyzed survival data, now assessing overall survival (OS) and segregating patients based on stage of disease. Interestingly, while overall survival was greater in stage I and II ovarian cancer patients with low BIRC5 tumor expression in the lower but not upper survival quartile, overall survival was greater in stage III and IV ovarian cancer patients with high BIRC5 tumor expression, in the upper but not lower survival quartile (Figure 3). Correlation of OS with low BIRC5 tumor expression in patients with stage I and II ovarian cancer in the lower survival quartile was statistically significant (Figure 3A: log rank p -value: 0.0033; hazard ratio: 10.99 (1.48 - 81.37)) as was correlation of OS with high BIRC5 tumor expression in patients with stage III and IV ovarian cancer in the upper survival quartile (Figure 3D: log rank p -value: 0.011; hazard ratio: 0.8 (0.67 - 0.95)). Thus, these data suggested that in ovarian cancer, BIRC5 could exert context-dependent influence on patient survival based on stage of disease, with low BIRC5 expression favorable early in disease and high BIRC5 expression favorable late in disease.

Thus, we found using published microarray data^{2,3} that BIRC5 was among the genes most differentially expressed in tumors from women with high-grade serous carcinomas, and in epithelial ovarian cancer broadly. BIRC5 expression was significantly higher in HGSC tumors when compared to the normal ovary, and while BIRC5 tumor expression was significantly correlated with progression-free survival in patients with ovarian cancer in the upper survival quartile, with high expression portending greater PFS, overall survival analysis was more nuanced, with low BIRC5 expression favorable early in disease and high BIRC5 expression somewhat more favorable later in disease.

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 epithelial ovarian cancers as well as ovarian cancers of the high-grade serous type, identifying genes whose differential expression was associated with EOC and 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^{2,3}, BIRC5 was among the genes whose expression changed most significantly when comparing the normal ovary to ovarian tumors, from women with epithelial ovarian cancer as well as specifically from women with HGSC.

BIRC5, also known as survivin or survivin B, has been described to possess tumor-specific expression and is a member of the IAP or inhibitor of apoptosis family proteins with a BIR domain. The anti-apoptotic functions of BIRC5 have been studied in detail: survivin expression can confer varying degrees of protection from cell death resulting from a number of apoptotic triggers. Expression of survivin can partially protect human cancer cell lines from cell death in the context of Fas or Bax-triggered apoptosis, and can also protect 293 cells from caspase-3- and caspase-7-induced cell death. Survivin can also reduce caspase-8 function *in vitro*⁸. In this role, BIRC5 can interact with SMAC/diablo, and BIRC5-diablo interaction has been demonstrated in ovarian carcinoma cells⁹. BIRC5 is remarkable in that while being an IAP family protein, it also possesses functions in mitotic cell division: survivin has the ability to read phosphorylation of threonine 3 on Histone H3 to activate Aurora B kinase¹⁰. Binding of the BIRC5 BIR domain to phosphorylated threonine 3 of H3 recruits the chromosomal passenger complex (CPC), composed of Aurora B, INCENP, Borealin/Dasra and BIRC5; as H3 phosphorylation at T3 occurs in M phase, BIRC5 binding to phosphoT3 on H3 permits phase-specific recruitment of CPC to chromatin and activate Aurora B kinase to enable cell cycle progression during M phase¹⁰. Thus, BIRC5 has functions in regulation of cell death and in cell cycle dynamics, either or both of which could be important in the context of a cancer cell. Consistent with these features, localization of BIRC5 has been reported at the centrosome, mitotic spindle, mid body and kinetochore and cleavage furrow¹¹. Importantly, expression of BIRC5 was correlated with protection from cell death after treatment with taxol, a spindle-disrupting chemotherapeutic¹².

BIRC5 over-expression has been reported in neuroblastoma, and lower expression of BIRC5 was correlated with increased survival probability¹³. In neuroblastoma cancer cell lines, the anti-apoptotic effect of BIRC5 was attributed to its role in the CPC during the cell cycle and independent of its functions in the intrinsic apoptotic pathway¹³. Accordingly, a specific inhibitor of BIRC5, YM155, could induce cell death in some but not all neuroblastoma cell lines, with expression of the multi-drug resistant pump ABC1 being the most significant predictor of YM155 resistance¹⁴. In a study of B-cell lymphomas, expression of BCL2 and BIRC5 was generally found to be mutually exclusive, and with BCL2 and BIRC5 distinguishing small from aggressive B-cell lymphomas, respectively; NF- κ B could induce expression of both of these molecules¹⁵. BIRC5 has been described as a target of the microRNA miR-203 in multiple cancer types including leukemia, hepatocellular carcinoma, and ovarian cancer¹⁶⁻¹⁸. High

1 expression of BIRC5 has been reported in ovarian cancers, and BIRC5 could control
2 expression of the epithelial to mesenchymal gene expression program, with BIRC5 deletion in
3 SKOV3 and OVCAR3 cell lines resulting in induction of cytokeratin 7 and repression of snail 2,
4 β -catenin and vimentin¹⁹. Accordingly, use of the BIRC5 inhibitor YM155 in SKOV3 and
5 OVCAR3 ovarian cancer cell lines inhibited EMT, resulted in decreased cell migration and
6 invasion *in vitro*, and induced cell death by apoptosis. Depletion of BIRC5 using small hairpin
7 RNA in one study increased sensitivity of the OVCAR3 cell line to paclitaxel by 15-fold but had
8 not effect on carboplatin resistance²⁰. However, positive BIRC5 expression was associated
9 with platinum resistance in patients with ovarian cancer²⁰. We suggest this indicates value of
10 the use of the YM155 BIRC5 inhibitor as an adjunct, to augment response to standard
11 paclitaxel-carboplatin chemotherapy in patients with chemotherapy, potentially in conjunction
12 with verapamil or experimental ABCB1 inhibitors like HM30181^{21,22}.

9 We found that survivin, also known as BIRC5, was among the genes whose expression
10 was most significantly different transcriptome-wide when comparing primary tumors from
11 women with epithelial ovarian cancer and high-grade serous ovarian cancer with the normal
12 ovary. Differential expression of BIRC5 in EOC and HGSC should be validated in larger and
13 separate cohorts of women. If validated, mouse models of high-grade serous ovarian cancers
14 can be utilized to assess the effect of conditional deletion of BIRC5 in HGSC tumors post-
15 development and on tumor cell proliferation and cancer progression *in vivo*²³.

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Rank	ID	p-value	t	B	FC	Gene	Gene name
206	A_23_P118815	1.34E-05	7.1308546	3.5866	1.6807 ± 0.1310	BIRC5	baculoviral IAP repeat containing 5

Table 1: BIRC5 is differentially expressed in high-grade serous ovarian carcinomas (HGSC).

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 BIRC5 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
5640	8010260	2.16E-02	2.3821627	-3.8648244	BIRC5	baculoviral IAP repeat containing 5

Table 2: BIRC5 is differentially expressed in epithelial ovarian cancers, including high-grade serous ovarian carcinomas (HGSC).

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: BIRC5 is expressed at significantly higher levels in HGSC tumors when compared to the normal ovary.

The mRNA expression of BIRC5 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 BIRC5 expression between the ovary of control subjects and primary tumors from patients with HGSC, a *p*-value, listed above.

Figure 2: BIRC5 expression correlates with progression-free survival in patients with ovarian cancer in the upper survival quartile.

Depicted in this Kaplan-Meier plot is the probability of progression-free survival (PFS) for $n=1435$ total patients stratified into two groups, based on low or high expression of BIRC5 in patient primary tumors, in the lower survival quartile (left, 2A) and in the upper survival quartile (right, 2B). The log rank p -value denoting statistical significance of difference in progression-free survival when comparing the two groups, as well as hazard ratio for this comparison is listed above. Listed below is the number of patients at risk (number of patients alive) per interval, after stratification based on BIRC5 expression; in the first interval, number at risk is number of patients alive; in each subsequent interval, number at risk is the number at risk less those who have expired or are censored.

Low expression cohort (months)	High expression cohort (months)
18.37	27

Table 3: Progression-free survival is greater in ovarian cancer patients with high tumor expression of BIRC5, in the upper survival quartile.

The median progression-free survival of $n=1435$ ovarian cancer patients based on stratification into low or high tumor expression of BIRC5 is listed in this chart, for patients in the upper survival quartile.

Figure 3: Correlation of BIRC5 tumor expression with overall survival in patients with ovarian cancer is dependent on stage of disease.

Depicted in this Kaplan-Meier plot is the probability of overall survival (OS) for $n=135$ patients with stage I and II disease and $n=1220$ patients with stage III and IV disease stratified into two groups each, based on low or high expression of BIRC5 in patient primary tumors, in the lower survival quartile and in the upper survival quartile. The log rank p -value denoting statistical significance of difference in overall survival when comparing the patients based on degree of BIRC5 expression, as well as hazard ratio for this comparison is listed above. Listed below each Kaplan-Meier survival analysis is the number of patients at risk (number of patients alive) per interval, after stratification based on BIRC5 expression; in the first interval, number at risk is number of patients alive; in each subsequent interval, number at risk is the number at risk less those who have expired or are censored.