Interferon regulatory factor 1 is an independent predictor of platinum resistance and survival in high-grade serous ovarian carcinoma

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HIGHLIGHTS

• IRF1 and its mechanistic pathway were found by global gene expression analysis to be linked to platinum resistance in OvCA.
• Using TCGA and GEO data sets, IRF1 is shown to be a prognostic marker for PFS and OS survival in HGSOC.

INTRODUCTION

Epithelial ovarian cancer is a leading cause of death from gynecologic malignancies in the United States. It is estimated that there will be more than 22,000 new cases of ovarian cancer and 14,000 deaths in 2013 [1]. This exceptionally high mortality rate is thought to result from delayed diagnosis owing to generally vague presenting symptoms, advanced stage at diagnosis, and adverse underlying biologic features of the tumor [2,3]. Although approximately 75% of patients enter clinical remission after primary treatment, the majority will relapse and 5-year survival is roughly 30% [4]. Even within the group of patients who respond to initial treatment with surgery and platinum-based chemotherapy, there is significant heterogeneity in disease-free interval. Current standard management of HGSOC includes combination platinum-taxane doublet chemotherapy [5]. The platinum-free interval is a clinically useful proxy for predicting overall survival in patients as...
well as guide for predicting that patient's future response to second-line chemotherapy, including non-platinum chemotherapy [6].

Based on the initial time interval to recurrence, patients are divided into a generally accepted classification system. Patients who relapse within 6 months of completing therapy are deemed platinum resistant and constitute approximately 20–30% of the total population. Those that experience disease recurrence after 6 months are classified as platinum sensitive [5,7]. Patients that experience recurrence within 6 months to one year constitute approximately 30–40% of all HGSOC patients. They are generally thought to have moderate platinum sensitivity and can be considered for platinum-based second-line chemotherapy [8]. Those with recurrence after one year are considered platinum sensitive and are good candidates for retreatment with platinum-based therapy [5]. Platinum-refractory disease is defined in those patients who progress with disease while still on initial treatment. This last group represents less than 10% of HGSOC patients and is associated with the poorest survival [8].

This challenging reality of platinum sensitivity and resistance as a major determinant of survival has driven the search for the molecular pathways that drive the biologic basis between these clinical groups. Factors that potentially affect these pathways, such as host immunogenetic responses have been the focus of recent study. The interaction between the immune system and ovarian cancer has led to clinical trials investigating whether the administration of modulating cytokines, such as interferon gamma (IFN-gamma), can influence the clinical disease course [9,10]. Included in the study of the IFN-gamma and other similar cytokines has been the study of the regulatory factors that influence them [11]. This includes the study of interferon regulatory factor 1, IRF1, a master regulatory protein of inflammatory response and recognized to function as a tumor suppressor regulator of cell cycle progression and apoptosis [12,13]. IRF1 expression has been the focus of study in a variety of malignancies and was reported to be increased in ovarian cancer cell-lines following the administration of cisplatin [14].

In our study, we began with the hypothesis that gene expression analysis could be used to identify genes and/or pathways that distinguished between platinum-sensitive and resistant ovarian tumors. We therefore explored the genetic differences between a set of tumor samples from platinum-sensitive and resistant patients, wherein PFS was highly divergent, using complete transcriptome (RNA-Seq) analysis coupled with IPA. Our goal was to identify candidate genes/pathways, which could distinguish between platinum-sensitive and resistant tumors and develop these as prognostic biomarkers. The impact of the identified candidate gene on patient survival was then determined using PFS and OS as outcome variables. In accord with our hypothesis, we identified a single gene whose intratumoral expression was directly correlated with survival.

Materials and methods

Patient and specimen collection

EOC tumor samples were collected at the time of primary debulking surgery at a single institution under an IRB-approved protocol. There were 7 patient tumor samples used for RNA-Seq as RNA-Seq analysis in the discovery set (Table 1) and 31 patient tumor samples used in the validation set (Table 2). All patients were staged according to the International Federation of Gynecology and Obstetrics (FIGO) and had advanced stage disease. All tumors were serous histology and grade 2 or 3. All patients received platinum and taxane-based adjuvant chemotherapy. Debulking surgery was defined as optimal versus suboptimal. Optimal was defined as ≤1 cm residual disease.

RNA extraction

Immediately following surgical resection, tumor samples were frozen in liquid nitrogen and stored at –80 °C. RNA was extracted from the frozen tumor samples (QIAzol, Qiagen, Valencia, California). As previously described [15], RNA integrity number scores (RINs) were determined (Agilent Bioanalyzer, Agilent Technologies, Santa Clara, California) and only RNA that had RIN scores of ≥8.0 were submitted for RNA-Seq.

RNA-Seq

Tumor-derived transcriptomes were prepared for paired-end sequencing using the Illumina GAIL platform in accord with the manufacturer's protocols and with a second size selection step to reduce ligation artifacts, as we have previously described [15].

RNA was sequenced using different iterations of NGS technology, resulting in a variety of data formats and qualities. FASTQ samples were quality checked before alignment using FastQC (v0.10.1). All samples were aligned using TopHat (v2.0.4) [16] to the hg19 reference genome using the hg19 RefSeq transcriptome (version 10-17-2011) as a guide, and were quality checked after alignment using FastQC. The transcriptome of each sample was analyzed using Cufflinks, and samples were quantitated against a common transcriptome model using Cuffdiff [17]. The resulting distributions of TPM values required quantile normalization to compensate for the variety of initial data qualities. Additionally, batch correction was necessary to correct for an observed effect between FASTA and FASTQ format samples. Finally, group-wise fold changes and P-values were computed on the normalized, adjusted data using a moderated t-statistic [18]. This is similar to the analysis approach of Sun et al. [19]. Transcripts with a P-value < 0.05 and fold-change > 2.0 were deemed significantly differentially expressed.

Bioinformatic analysis

According to Ingenuity protocol, data sets containing RefSeq identifiers and corresponding expression values were uploaded into the application IPA. Each RefSeq identifier was mapped to its corresponding human splicing variant in the Ingenuity® Knowledge Base. An absolute fold change cutoff of 2 and P-value of 0.05 were set to identify isoforms whose expression was significantly differentially regulated (DEIs) and 1030 molecules were ready for analysis.

The IPA Downstream Effects Analysis (DEA) was used to identify the biological functions and/or diseases that were most significant to the data set. Right-tailed Fisher's Exact test was used to calculate a P-value determining the probability that each biological function and/or disease assigned to these data sets are due to chance alone. Furthermore, DEA was used to predict increases or decreases of these biological functions and/or diseases occurring in these ovarian cancer patients after cisplatin treatment by integrating the direction change of the DEGs into a z-score algorithm calculation. Functions and/or diseases with z-scores ≤−2 or ≥2 are considered significant. Further details of these calculations can be obtained at http://www.ingenuity.com/wp-content/themes/ingenuity-jqagen/pdf/iva/feature_highlight_upstream_downstream.pdf and http://pages.ingenuity.com/IngenuityDownstreamEffectsAnalysisipaWhitepaper.html.

Canonical pathway analysis identified the canonical pathways from the IPA library that were most significant to the data set. The significance of the association between the data set and the canonical pathway was measured in two ways: 1) A ratio of the number of molecules from the data set that map to the pathway divided by the total number of molecules that map to the canonical pathway. 2) Fisher's Exact test was used to calculate a P-value determining the probability that the association between the genes in the data set and the canonical pathway is explained by chance alone.

URA was used to identify the cascade of upstream transcriptional regulators (transcription factors, enzyme, cytokine, growth factor, miRNA, compound or drug) that could explain the observed gene expression changes in these data sets, by measuring an overlap P-value with Fisher's Exact test and by measuring the activation z-score as
well to infer the activation states of the predicted transcriptional regulators. Further details of these calculations can be obtained at: http://pages.ingenuity.com/IngenuityUpstreamRegulatorAnalysis.pdf
By taking the URA further, the mechanistic networks are generated by computationally generating plausible directional networks from these regulators. These connected upstream regulators might work together to elicit the gene expression changes observed in this data set.

**Quantitative Real-time Reverse Transcription PCR (qRT-PCR)**
Validation studies were performed using a second set of 31 EOC tumor samples. RNA was extracted, reverse transcribed using the BioRad Iscript system (BioRad, Hercules, California), and stored. qRT-PCR was performed using the ABI PRISM 7900HT sequence detection system (Applied Biosystems, Carlsbad, California). Real-time master mix (iQ Sybr Green Super Mix, BioRad, Hercules, California) was mixed with cDNA in a 1:1 ratio and all samples were run in triplicate. Cycle number values were normalized against the housekeeping gene GAPDH. Forward (F) and reverse (R) primers were used as follows: IRF1: CAAATCCCGGGGCTCATCTGG and CTGGCTCCTTTTCCCCTGGTT; GAPDH: AGAACGGGAAGCTTGTCATC and CATCGCCCCACTTGATTTTG. IRF1 expression values were obtained by averaging the triplicate, normalizing to a GAPDH standard and measuring the fold change over a specified set control sample.

**Statistical analysis**
Patients were divided into two groups based on IRF1 levels. Samples were defined as either $\geq 2.4$ fold change, which is the 75th percentile of the distribution, or $< 2.4$ fold change. The distributions of baseline patient characteristics were compared between high and low IRF1 groups with a Fisher’s Exact test for categorical variables and a t-test for continuous variables. PFS and OS distribution curves were estimated using the product-limit method of Kaplan–Meier. The log-rank test was utilized to compare PFS and OS curves between high and low IRF1 groups. A Cox proportional hazards model was used to estimate crude and age-adjusted hazard ratios for progression. All hypothesis testing was two-tailed and conducted at the 5% level of significance. Statistical analyses were performed using SAS Version 9.2.

Survival analyses were then expanded to include data from the KM-plot web application (http://kmplot.com/analysis/index.php?p=service&cancer=ovar), which includes data from a meta-analysis of global gene expression. This application represents gene expression data from eight total data sets, including The Cancer

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**Table 1**
Clinicopathologic profile of discovery set.

<table>
<thead>
<tr>
<th>N = 7</th>
<th>Platinum resistant</th>
<th>Platinum sensitive</th>
<th>Overall</th>
<th>P-value</th>
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<tbody>
<tr>
<td>Mean age (SD)</td>
<td>59.3 (1.53)</td>
<td>54.0 (11.92)</td>
<td>56.3 (8.9)</td>
<td>0.439</td>
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<td>FIGO stage II</td>
<td>2 (67%)</td>
<td>4 (100%)</td>
<td>6 (86%)</td>
<td>0.429</td>
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<tr>
<td>Grade 2/3</td>
<td>3 (100%)</td>
<td>4 (100%)</td>
<td>7 (100%)</td>
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</tr>
<tr>
<td>Histologic type Serous</td>
<td>3 (100%)</td>
<td>4 (100%)</td>
<td>7 (100%)</td>
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<tr>
<td>FIGO stage III</td>
<td>2 (67%)</td>
<td>4 (100%)</td>
<td>6 (86%)</td>
<td>0.05</td>
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<td>Grade 2/3</td>
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<td>4 (100%)</td>
<td>7 (100%)</td>
<td></td>
</tr>
<tr>
<td>Histologic type Serous</td>
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<td>4 (100%)</td>
<td>7 (100%)</td>
<td></td>
</tr>
<tr>
<td>FIGO stage IV</td>
<td>1 (33%)</td>
<td>0 (0%)</td>
<td>1 (14%)</td>
<td></td>
</tr>
<tr>
<td>Grade 2/3</td>
<td>3 (100%)</td>
<td>4 (100%)</td>
<td>7 (100%)</td>
<td></td>
</tr>
<tr>
<td>Histologic type Serous</td>
<td>3 (100%)</td>
<td>4 (100%)</td>
<td>7 (100%)</td>
<td></td>
</tr>
<tr>
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<td>4 (100%)</td>
<td>6 (86%)</td>
<td>0.05</td>
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<tr>
<td>Grade 2/3</td>
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<td>4 (100%)</td>
<td>7 (100%)</td>
<td></td>
</tr>
<tr>
<td>Histologic type Serous</td>
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<td>4 (100%)</td>
<td>7 (100%)</td>
<td></td>
</tr>
<tr>
<td>FIGO stage</td>
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<td>1 (14%)</td>
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<td>3 (100%)</td>
<td>4 (100%)</td>
<td>7 (100%)</td>
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</tr>
<tr>
<td>Histologic type Serous</td>
<td>3 (100%)</td>
<td>4 (100%)</td>
<td>7 (100%)</td>
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**Table 2**
Clinicopathologic profile of validation set.

<table>
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<tr>
<th>N = 31</th>
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<th>High IRF1</th>
<th>Overall</th>
<th>P-value</th>
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<td>Mean age (SD)</td>
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<td>60.5 (11.1)</td>
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<td>8 (100%)</td>
<td>26 (84%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Grade 2/3</td>
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<td>8 (100%)</td>
<td>31 (100%)</td>
<td></td>
</tr>
<tr>
<td>Histologic type Serous</td>
<td>23 (100%)</td>
<td>8 (100%)</td>
<td>31 (100%)</td>
<td></td>
</tr>
<tr>
<td>FIGO stage</td>
<td>19 (83%)</td>
<td>7 (87.5%)</td>
<td>26 (84%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Grade 2/3</td>
<td>23 (100%)</td>
<td>8 (100%)</td>
<td>31 (100%)</td>
<td></td>
</tr>
<tr>
<td>Histologic type Serous</td>
<td>23 (100%)</td>
<td>8 (100%)</td>
<td>31 (100%)</td>
<td></td>
</tr>
<tr>
<td>FIGO stage</td>
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<td>7 (87.5%)</td>
<td>22 (71.9%)</td>
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<tr>
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<td>8 (100%)</td>
<td>31 (100%)</td>
<td></td>
</tr>
<tr>
<td>FIGO stage</td>
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<td>7 (87.5%)</td>
<td>25 (81%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Grade 2/3</td>
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<td>8 (100%)</td>
<td>31 (100%)</td>
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<tr>
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<td>FIGO stage</td>
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<td>23 (100%)</td>
<td>8 (100%)</td>
<td>31 (100%)</td>
<td></td>
</tr>
</tbody>
</table>

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* P < 0.05

* Log-rank P-value comparing KM PFS and OS curves.

* 25th Kaplan–Meier percentile: the number of months by which 25% of patients have progressed (PFS) or died (OS) and 75% are progression free (PFS) or alive (OS).

* NE: Not estimable. The Kaplan–Meier percentile is not estimable because the 50% (for median) or 25% (for Q1) of patients did not die or progress.
Genome Atlas (TCGA) [20]. A total of 1287 ovarian cancer patients were represented in the meta-analysis.

Results

Clinicopathologic characteristics of discovery set patients and their tumors

RNA-Seq Analysis was performed on tumors from seven EOC patients that were selected based on whether they were platinum sensitive or platinum resistant/refractory. Four patients had chemosensitive disease and three patients were chemorefractory. The mean (SD) age for the patients in the discovery set overall was 56.3 (8.9) years and this did not differ significantly between the two groups (P = 0.439, Table 1). All patients had serious tumors and received platinum and taxane-based treatment (Table 1). Tumor grade was high grade across both groups. Of note, and central to our analysis, those in the platinum-sensitive group had a significantly longer PFS (probability of PFS within the first 12 months following surgery is 100% [95% CI: 100%–100%]) compared to those in the resistant group (probability of PFS within the first 12 months following surgery is 50% [95% CI: 0.6%–91%]; P = 0.018. This is in agreement with the idea that platinum sensitivity correlates with the PFS of an individual. The overall survival was not significantly different between the two groups (P = 0.070), perhaps owing to the short follow-up time interval, whereby only two death events have occurred to date. These two death events occurred in the platinum-resistant group.

IRF1 is an activated upstream regulator in chemosensitive ovarian cancer

Using the RNA-Seq data generated from these samples, IPA was first used to identify possible differences in alternative splicing. No differentially alternatively spliced isoforms were found to be significantly altered between the two sample sets. Next we used the URA feature of IPA. URA allowed the identification and determination of the state of activation of upstream regulators that might be responsible for the observed gene expression changes between the chemosensitive/chemoresistant patient groups. We focused our attention by analyzing the potential transcription factors responsible for expression changes. In total, ten transcriptional regulators were predicted to be activated in the chemosensitive group of tumors (Supplemental Table 1). The two transcriptional regulators with the highest z-scores were IRF7 (z = 4.15; P = 0.0006) and IRF1 (z = 3.09; P = 0.0017). Examination of the target molecules of each allowed us to focus our attention on IRF1, since IRF7 is itself regulated by IRF1. The IRF1 mechanistic network and its direction of activation are shown in Fig. 1. A number of the other identified factors, including MYCN, MYC, STAT3, GATA4 and SMARCA4, have been previously associated with ovarian cancer growth and proliferation but their calculated z-scores were lower than IRF1 and IRF7 and so we felt confident in focusing our initial attention on IRF1.

IRF1 expression levels are associated with survival in HGSOC: single institution validation set

To test the transcriptome-based finding that IRF1 is a candidate gene associated with platinum-sensitivity and hence, survival, quantitative RT-PCR was performed on tumor samples from an independent validation set of 31 patients (Table 2). The mean (SD) age was 60.5 (11.1) years for the patients in this validation set. There was a significant difference in PFS between low and high IRF1 groups (Fig. 2A; log rank P = 0.048). Patients with low IRF1 had a 61% [95% CI: 42%–80%] probability of recurrence within the same 12-month time period. Patients in the high IRF1 group had only a 12.5% [95% CI: 2%–61%] probability of recurrence within the first 12 months following surgery. At 36 months following surgery, the probability of recurrence in low IRF1 patients increased to 85% [95% CI: 65%–97%]. In the high IRF1 group, the probability of recurrence was 44% less than half that of the low IRF1 group [95% CI: 16%–85%].

While there was no significant difference in OS between low and high IRF1 groups (Fig. 2B; log rank P = 0.125), there was a trend toward increased survival in the high expression group. At 12 months post primary surgery, the probability of survival in patients with low IRF1 was 96% [95% CI: 73%–99%] and in patients with high IRF1 it was 100%. At 36 months, the probability of survival in the low expression group dropped to 71% [95% CI: 47%–86%] but remained 100% among patients in the high IRF1 group. There was a significant difference in the proportion of platinum-sensitive patients in the low IRF1 group (35%) compared to the high IRF1 group (87.5%); (P = 0.016).

Of interest, patients with low levels of IRF1 tumor expression were on average 11.9 years older than patients with high IRF1 expression (P = 0.007). Since there was a significant difference in age between IRF1 groups, a Cox proportion hazards model was used to estimate an age-adjusted hazard ratio representing the risk of recurrence in high IRF1 patients versus low IRF1 patients. The unadjusted hazard ratio for recurrence comparing patients with high IRF1 to patients with low IRF1 was 0.31 ([95% CI: 0.09–1.06]; P = 0.06) representing a 69% reduced risk of recurrence in high IRF1 patients. Once adjusted for age, the hazard risk for recurrence increased to 0.33 ([0.09–1.27]; P = 0.108) representing a 67% reduced risk of recurrence in high IRF1 patients. Neither the unadjusted nor the age adjusted hazard ratios achieved statistical significance.

IRF1 expression levels are associated with PFS and OS in HGSOC: multi-institution validation set

Given the significant differences noted in the unadjusted PFS analysis and the trend demonstrated in OS for the single study center set of samples, we sought to analyze IRF1 expression in a larger, independent and multi-center data set. To do so, we accessed the KM-plot web application that provides access to data from a meta-analysis of global gene expression [20]. This publicly accessible online tool provides microarray data on a global scale, representing 22,227 genes from 1287 ovarian cancer patients. Data was only included in this data set if raw microarray gene expression data and clinical survival information were available [20].

As a starting point, we selected clinical inclusion parameters that were consistent with our originally defined cohort of patients in both the discovery and validation sets of our study. Specifically, we only selected cases for analysis with high-grade serous ovarian cancer, including patients with both optimal and suboptimal debulking, and those who received doublet platinum and taxane-based chemotherapy. Based on these parameters, a total of 346 patients were represented in the PFS analysis. In accord with our original hypothesis and finding in our own patient cohort, in this larger and independent data set increased expression of IRF1 was associated with increased PFS (Fig. 3A; P = 0.043).

We next evaluated IRF1 levels in the context of OS. Using the same selection criteria outlined above, a total of 365 patients were included in this analysis. As shown in Fig. 3, increased levels of IRF1 were strongly associated with increased OS (Fig. 3B; P = 0.019).

Since the patient data accessed in the KM-plot web application represents a collection of eight different studies, we noted the potential for possible heterogeneity in regard to chemotherapy, specifically that some of the treatments likely predated the routine use of taxane-based chemotherapy [21]. We therefore investigated if the survival advantage would be maintained when selecting patients that received platinum-based treatment, regardless of taxane. A total of 615 patients were represented in this analysis. In this grouping there was an even more significant association between increased IRF1 expression and overall survival (Fig. 4A; P = 0.004).

Finally, since surgical debulking status is one of the most significant contributors to overall survival [22], we next evaluated the
association of IRF1 levels with survival in those patients who re-
ceived platinum-based treatment, regardless of taxane, and were ei-
ther optimally or suboptimally debulked. Patients received optimal 
surgery, had HGSOC, advanced-stage disease, and grade 3 tumors, 
(n = 351). There was a significant association between increased 
IRF1 expression and overall survival (Fig. 4B; P = 0.025). This sur-
vival advantage was again maintained in the suboptimally debulked 
group (Fig. 4C; n = 169; P = 0.041).

Discussion

Taken together, our findings suggest that IRF1 expression is an in-
dependent predictor of survival in HGSOC. Classifying patients based 
on platinum response in EOC provides a clinically useful guide to under-
standing the overall behavior of patients but falls short of a personalized 
prognostic or predictive marker. The duration of PFS is variable between 
patients and defining the biology behind this variability, and how it re-
lates to platinum response, is a unique challenge. In this current study, 
we selected a discovery set of tumors that differed markedly in their 
platinum-response and PFS for complete transcriptome analysis. RNA-
Seq analysis, as interpreted by IPA, highlighted genes previously de-
scribed as being differentially expressed based on survival thus provid-
ing confidence in our ability to detect bona fide candidates and our 
approach. The goal of our study was to identify candidate genes that 
were differentially expressed between clinically distinct groups. We 
identified IRF1, in part through it being an upstream regulator within 
a pathway, as a potential gene target associated with predictive treat-
ment response.

IRF1 expression levels were demonstrated to be associated with PFS 
and OS in both single and multi-center data sets. Our discovery data set, 
with which we identified higher IRF1 levels in platinum-sensitive pa-
tients, divided patients based on the timing of recurrence relative to 
chemotherapy. Therefore, it is not surprising that patients with high 
IRF1 would have a longer time-to-progression. Platinum resistance or 
sensitivity status correlates with prognosis and is used to dictate treat-
ment strategy at the time of recurrence. In this way PFS, as an outcome 
measure relative to platinum response is a meaningful outcome 
measure.

Little is known about the role of IRF1 in the setting of ovarian cancer 
and indeed, this was one of the criteria in our initial selection strategy. In
one of the first studies to explore its possible association with chemotherapy resistance, three ovarian cancer cell lines were examined and IRF1 expression was shown to be increased following their treatment with cisplatin [14]. Within these different cell-lines, SKOV-3, NIH:OVCAR-3, and TOV-21G, which have inherent varying response to platinum, IRF1 silencing resulted in cells becoming more susceptible to cisplatin treatment. Intriguingly, these results, based on highly passaged immortalized cell lines appear to be contrary to our results obtained using primary tumor samples. Ultimately, it will be necessary to assess the effects of cisplatin treatment on primary patient-derived tumor cell lines and/or patient derived xenograft (PDX) models to determine if the discrepancy arises from an artifact of the cell culture system.

Zeimet et al. evaluated IRF1 expression in tissue samples collected at primary surgery for a heterogeneous mix of stages and histologic types in ovarian cancer [11]. Twenty-nine percent of the samples were early primary surgery for a heterogeneous mix of stages and histologic types together does not have direct relevance to biomarker application in a clinical setting.

While a mechanistic explanation of why increased intratumoral levels of IRF1 are associated with platinum sensitivity and increased survival at this time has yet to be determined, it is important to note that the link between cancer progression and immune system function represents one evolving area of research in regard to treatment response and disease-free survival in EOC. Again, it is also important to note that IRF1 was identified in our study because of its increased expression and the activation of its downstream mediators. Ovarian cancer is immunogenic [24] and the presence of tumor-infiltrating T cells correlates with survival [25,26]. Furthermore, differential expression of cytokines that modulate the immune system, such as interferons (IFNs), has been correlated to disease outcome. IFN-gamma has antiproliferative effects on neoplastic cells and its expression has been associated with delayed recurrence in advanced EOC. [9,26] Anti-neoplastic mechanisms of IFN-gamma are thought to potentially include the initiation of antibodies and T cell response, the down-regulation of the HER-2/neu proto-oncogene, and the stimulation of NK cells and macrophages. This particular cytokine has been shown to collaborate with lymphocytes to provide immune surveillance in the setting of tumor development. In this role it is part of a tumor-suppressor response.

Critical to the function and understanding of IFN-gamma and the IFN family, is the study of the proteins that regulate them, specifically the interferon regulatory factors (IRFs) [11]. IRF1 is a transcription factor marked by functional diversity. Depending on the stimulus and cell type IRF1 responds selectively, including a role in cell cycle arrest and apoptosis [27]. Initially discovered and characterized as a regulator of the IFN-beta gene, IRF1 may induce IFNs in the context of ovarian cancer to activate the immune response and delay progression survival. This, along with precisely how IRF1 and IFN-gamma interact with platinum agents, remains to be investigated. Further study demonstrated IRF1’s function as a tumor suppressor [12]. MEFs derived from overtly normal IRF1+/−/− mice were oncogenically transformed by HRAS oncogene, whereas wild-type MEFs were not [12,28]. Furthermore, normal IRF1+/−/− mice, expressing activated HRAS, were exposed to DNA-damaging ionizing radiation, and underwent cell death via apoptosis. This supports the idea that functioning IRF1 contributes to an apoptotic event in the face of DNA damage, and increased expression can promote a clinical response to chemotherapy, translating into platinum-sensitivity.

Taken together, our transcriptome-driven findings identify and demonstrate IRF1 to be a prognostic marker for distinguishing platinum response and survival in ovarian cancer. Increased levels of IRF1 were shown to be associated with both increased PFS and OS in high-grade serous ovarian cancer. Based on its known biology, we believe IRF1 represents not only a candidate biomarker, but also a potential therapeutic candidate in HGSOC.
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Conflict of interest statement
The authors declare that there are no conflicts of interest.

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References

Fig. 4. Survival analysis setting parameters included IRF1 (Affymetrix ID: 202531_at), as the gene of interest, and HGSOC. (A) Overall survival analysis in a platinum-only treatment group. Setting parameters also included surgical debulking with optimal or suboptimal outcome, platinum-based adjuvant chemotherapy, and no limit to the follow-up threshold. There was a significant difference in OS between low and high IRF1 expression groups (P = 0.0043). (B) Overall survival analysis in patients with optimal surgical debulking. Setting parameters also included surgical debulking with optimal outcome, platinum-based adjuvant chemotherapy, and no limit to the follow-up threshold. There was a significant difference in OS between low and high IRF1 expression groups (P = 0.025). (C) Overall survival analysis in patients with suboptimal debulking. Setting parameters also included surgical debulking with suboptimal outcome, platinum-based adjuvant chemotherapy, and no limit to the follow-up threshold. There was a significant difference in OS between low and high IRF1 expression groups; (P = 0.041). (D) Elevated IRF1 expression is a strong predictor of overall survival across all stages and grades of ovarian cancer regardless of debulking status.


