Enhanced GAB2 Expression Is Associated with Improved Survival in High-Grade Serous Ovarian Cancer and Sensitivity to PI3K Inhibition


Abstract

Identification of genomic alterations defining ovarian carcinoma subtypes may aid the stratification of patients to receive targeted therapies. We characterized high-grade serous ovarian carcinoma (HGSC) for the association of amplified and overexpressed genes with clinical outcome using gene expression data from 499 HGSC patients in the Ovarian Tumor Tissue Analysis cohort for 11 copy number amplified genes: ATRIP, BMPTB, CACNA1C, CCNE1, DYSK1B, GAB2, PAK4, RAD21, TPX2, ZFP36, and URI. The Australian Ovarian Cancer Study and The Cancer Genome Atlas datasets were also used to assess the correlation between gene expression, patient survival, and tumor classification. In a multivariate analysis, high GAB2 expression was associated with improved overall and progression-free survival ($P = 0.03$ and 0.02), whereas high BMP8B and ATP13A4 were associated with improved progression-free survival ($P = 0.004$ and 0.02). GAB2 overexpression and copy number gain were enriched in the AOCSS C4 subgroup. High GAB2 expression correlated with enhanced sensitivity in vitro to the dual PI3K/mTOR inhibitor PF-04691502 and could be used as a genomic marker for identifying patients who will respond to treatments inhibiting PI3K signaling.

Introduction

Ovarian cancer is the fifth most common cause of cancer death worldwide in women, contributing to 4.3% of all female cancer deaths (1). The poor prognosis of ovarian cancer is generally attributed to a combination of late-stage diagnosis and the inherent propensity of some subtypes to be intrinsically resistant or rapidly acquire resistance to chemotherapy (2). This poor prognosis is complicated by the heterogeneous nature of this disease at a cellular, molecular, and clinical level: intertumor heterogeneity has made it hard to predict response to therapy and to identify good novel molecular targets, whereas intratumor heterogeneity is likely to underpin resistance to current chemotherapies.

Ovarian cancer is defined histologically into the serous (low- and high-grade), clear cell, endometrioid, and mucinous tumor subtypes. The inherent propensity of some subtypes to be intrinsically resistant or rapidly acquire resistance to chemotherapy contributes to the heterogeneous nature of ovarian cancer.

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subtypes (3). Importantly, the histologic characteristics that define these ovarian tumor groups do not translate into similar clinical responses or outcomes between patients within subtypes, even when the same treatment regime is administered. Molecular analysis of these tumors has revealed distinct gene expression profiles both within and between the histologic subtypes (4, 5). Tothill and colleagues identified 6 ovarian cancer subtypes termed C1 to C6. C1, C2, C4, and C5 comprised mostly high-grade serous and endometrioid cases and were associated with distinct expression profiles summarized as high stromal response (C1), high immune response (C2), low stromal response (C4), and mesenchymal (C5). Low-grade serous carcinomas formed a distinct subgroup. A second study by The Cancer Genome Atlas (TCGA) found 4 related high-grade serous subtypes, comprising differentiated (D), immune (I), mesenchymal (M), and proliferative (P). Despite the histologic, molecular, and clinical diversity of these tumors, current treatment protocols for women with ovarian cancer are not histologic or subtype-specific.

High-grade serous ovarian carcinomas (HGSC) account for the majority of ovarian cancer incidence and mortality. These tumors are characterized by near-ubiquitous TP53 mutation and widespread copy number aberrations (5, 6). Approximately 80% of women with HGSC will respond to first-line chemotherapy (7, 8). However, the majority of women who respond will relapse within 12 months of the cessation of chemotherapy and no curative treatments are currently available for recurrent disease. The integration of targeted therapeutics into ovarian cancer treatment regimes has the potential to greatly improve patient outcomes. However, the genomic heterogeneity of HGSC makes the identification of appropriate molecular targets challenging.

We previously identified putative oncogenes targeted by copy number amplification in HGSC and showed functional effects of gene knockdown (9). Here, we have sought to further characterize the clinical implications of expression of 11 of these genes using large clinical cohorts of HGSC from the Australian Ovarian Cancer Study (AOCS), the Ovarian Cancer Association Consortium (OCAC)/Ovarian Tumor Tissue Analysis Consortium (OTTA), and TCGA.

Materials and Methods

Clinical samples
Ovarian cancer biopsies (n = 499) from patients with HGSC were obtained as part of the OTTA study from women undergoing surgery for primary ovarian cancer at hospitals in the United States, Canada, United Kingdom, and Poland (10) as well as cases that were part of the Arbeitsgemeinschaft Gynäkologische Onkologie (AGO-OVAR3) randomized, controlled trial in Germany (11, 12). The AGO-OVAR3 cases were stage IIB–IV, underwent debulking surgery, and were randomized to either paclitaxel plus cisplatin or paclitaxel plus carboplatin, treated every 3 weeks until disease progression or a minimum of 6 cycles were achieved. The remaining cases were population-based and obtained retrospectively from hospitals and tissue banks. Specific treatment information for these cases is not available but is likely to also be surgery and platinum taxane-based chemotherapy. The central criterion for inclusion in this study was sufficient tissue to extract RNA.

Tumor RNA was extracted from formalin-fixed, paraffin-embedded tissue and used for the NanoString gene expression analysis described below. A small number of fresh-frozen samples were also included in this study. To enhance gene expression analysis, all tumors were evaluated on the frozen section to ensure they contained more than 50% epithelial tumor content. RNA was extracted from two to three 10-μm sections using the miRNeasy FFPE Kit (QIagen) following the manufacturer’s instructions with the addition of an elongated 45-minute Proteinase K digest. Appropriate institutional ethics committees approved the collection and use of tissues for this study. All participants gave written informed consent for their tissue to be used for research and publication. The Peter MacCallum Cancer Centre Human Research Ethics Committee approved this study (01/38).

NanoString gene expression analysis
ATP13A4, BMP8B, CACNA1C, CCNE1, DYRK1B, GAB2, PAK4, RAD21, TPX2, ZFP56, and URI1 were included in a custom NanoString nCounter CodeSet synthesized at NanoString Technologies as previously described (10, 13). Total RNA (~500 ng) was analyzed using the NanoString nCounter analysis system. mRNA quantification was carried out following the manufacturer’s instructions, including normalization by nSolver software v1.1 (NanoString Inc.), with ACTB, SDHA, RPL19, POLR1B, and PGK1 as control genes. Data were log2-transformed for analysis (Supplementary Table S1), thus each unit increase corresponds to a 2-fold increase in expression.

Cell lines and reagents
Ovarian cancer cell line data treated with small-molecule therapeutics were obtained as previously described (14). Cell lines were obtained in 2008 from Stephen Howell (University of California, Los Angeles, CA; 2008), European Collection of Cell Cultures (59M, A2780, OAW28, OAW42), National Cancer Institute (CAOV3, IGROV, OVCAR3, OVCAR4, OVCAR5, OVCAR8, SKOV3), Lloyd Kelland (Institute of Cancer Research, UK; CH1), Deutsche Sammlung von Mikroorganismen und Zellkulturen (EFO21, EFO27, FIO0V1), ATCC (ES2, OV90, TOV112D, TOV21G), RIKEN (JHOC5, JHOC7, JHOC9, JHOM1, JHOS3), Health Science Research Resources Bank (KIRIAMOCHI, MCAS, RMG1, RMGII), and Nuzhat Ahmed (Royal Women’s Hospital, Melbourne; OVC432, Supplementary Table S2). Cell line identity was routinely confirmed using a panel of 6 short tandem repeat loci at most 6 months before use.

Statistical analysis
Patient survival of the NanoString dataset was determined by Cox regression analysis of continuous expression data and was performed in Partek Genomics Suite v6.5. Only high-grade serous cases from the OTTA cohort were included (n = 499). Tumor FIGO stage, residual disease (optimal/suboptimal), cohort subgroup (i.e., the centre from where the tissue was obtained), and age were assessed as potential confounders. A number of cases lacked stage (n = 40) and/or residual disease (n = 148) information. Data were assessed for violation of the proportionality assumption using the cox.zph function in R; none were significant. Stepwise Akaike Information Criterion model selection was performed in R using stepAIC.

Normalized gene expression data were obtained for the AOCS and TCGA patient cohorts (4, 5, 15). AOCS (Affymetrix U133Plus2, GSE9899) and TCGA (Agilent 244K arrays, from the TCGA Data Portal; https://tcga-data.nci.nih.gov/tcga/) data were preprocessed by log normalization and median centring. Expression comparisons were performed in Partek Genomics Suite.
Suite. Cases in the AOCS cohort were subtyped by the TCGA classifier as described (16). Copy number data as raw CEL files were obtained from TCGA and AOCS (refs. 6, 17; AOCS, GSE13813; TCGA, Data Portal; https://tcga-data.nci.nih.gov/tcga/) and normalized as previously described (18). Circular Binary Segmentation (19) was used to detect copy number alterations with thresholds of 0.3 for gain and –0.3 for losses. Copy number comparisons were performed using Nexus Copy Number 7.5 Discovery Edition (BioDiscovery, Inc.). Normalized (Level 3) Reverse Phase Protein Array (RPPA) data were downloaded from TCGA and were not further processed. The results published here are, in part, based upon data generated by TCGA pilot project established by the NCI and NHGRI. Information about TCGA and the investigators and institutions who constitute the TCGA research network can be found at http://cancergenome.nih.gov.

To perform a combined cohort survival analysis, patients from each assay group (AOCS, TCGA, AGO/OTTA) were classified into low- and high-expression subgroups using cutoff values of ±0.5 median absolute deviations (MAD). The low-expression group had values less than the median minus 0.5× MAD, whereas the high-expression group had values greater than the median plus 0.5× MAD. Cases with intermediate expression values were excluded. Association of gene expression with survival was analyzed using Cox regression and stage and cohort were again included in the model. The MAD classification was done for each data group independently and was also used to define groups for all other comparisons of high versus low expression of a particular gene in tumor samples. Other statistical analyses were performed using GraphPad Prism software (GraphPad Software) and were considered significant where P < 0.05. Multiple testing correction was performed using the p.adjust function in R, using “fdr” (20).

Results

Expression analysis of 11 putative oncogenes identifies GAB2 as correlating with improved patient survival

We previously conducted an siRNA screen that evaluated the effect of knocking down 272 genes on cell growth in 18 ovarian cancer cell lines (9). The genes were selected on the basis of their location in minimal regions of copy number amplification. The siRNA screen identified 11 potential driver genes: BMP8B, CACNA1C, Dyrk1B, GAB2, PAK4, URI, and ZFP36 all resulted in decreased cancer cell viability following gene knockdown that was more pronounced in cell lines with copy number amplification of the gene. Tpx2 showed an inverse correlation between amplification and reduction in cell viability after gene knockdown. ATP13A4 and RAD21 showed reduction in cell viability upon gene knockdown that was not correlated with copy number amplification and CCNE1 was added on the basis of previous data indicating an association with poor progression-free survival (17). All genes were analyzed using a custom codeset on the NanoString system in 499 HGSC from OTTA/AGO (Table 1, Supplementary Figs. S1 and S2, Supplementary Table S1). Genes showing an association with overall survival in univariate analyses (Supplementary Table S3). Stage, presence of residual disease, and the cohort source of the tumor were significant factors influencing overall survival. BMP8B, GAB2, and ATP13A4 were associated with progression-free survival and remained significant in a multivariate analysis (Table 2). We also performed stepwise Akaike Information Criterion model selection, which selected FIGO stage and GAB2 as predictors for overall survival, and FIGO stage, BMP8B, and GAB2 as predictors for progression-free survival (Supplementary Table S4).

To extend the survival analysis in additional cohorts, GAB2, ATP13A4, and BMP8B expression data were obtained as described previously from 236 HG serous and endometrioid tumors from AOCS (4) and GAB2 and BMP8B data from 459 HGS tumors from TCGA (ref. 5; ATP13A4 data not available from TCGA). Significance testing was performed for key cohort characteristics.

### Table 1. Patient characteristics and clinicopathologic features of ovarian tumors relative to cohort

<table>
<thead>
<tr>
<th>Feature</th>
<th>AOCS</th>
<th>OTTA/AGO</th>
<th>TCGA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor cell nuclei (%)</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>&gt;70</td>
</tr>
<tr>
<td>RNA isolation method</td>
<td>T</td>
<td>Q</td>
<td>A</td>
</tr>
<tr>
<td>Analysis platform</td>
<td>Affymetrix U133 Plus 2.0</td>
<td>NanoString</td>
<td>Agilent 244K</td>
</tr>
<tr>
<td>Median follow-up (range), mo</td>
<td>36.2 (0–199)</td>
<td>36 (0–283)</td>
<td>35.8 (0–152)</td>
</tr>
<tr>
<td>Median PFS/OS, mo</td>
<td>14.8/42.4</td>
<td>16.5/45.4</td>
<td>16.9/44.5</td>
</tr>
<tr>
<td>Age at diagnosis</td>
<td>56.4</td>
<td>56.3</td>
<td>59.66</td>
</tr>
<tr>
<td>Range</td>
<td>23–80</td>
<td>29–90</td>
<td>30–89</td>
</tr>
<tr>
<td>Stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>11</td>
<td>24</td>
<td>11</td>
</tr>
<tr>
<td>II</td>
<td>18</td>
<td>40</td>
<td>20</td>
</tr>
<tr>
<td>III</td>
<td>196</td>
<td>327</td>
<td>357</td>
</tr>
<tr>
<td>IV</td>
<td>21</td>
<td>69</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>40</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Residual disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nil macroscopic</td>
<td>58</td>
<td>92</td>
<td>90</td>
</tr>
<tr>
<td>Macroscopic</td>
<td>135</td>
<td>259</td>
<td>317</td>
</tr>
<tr>
<td>Unknown</td>
<td>43</td>
<td>148</td>
<td>52</td>
</tr>
<tr>
<td>Total</td>
<td>226</td>
<td>499</td>
<td>459</td>
</tr>
</tbody>
</table>

**Abbreviations:** A, Allprep kit (Qiagen); Affy U133 Plus 2.0 expression array; Agilent, Agilent 244K; NanoString, NanoString nCounter Gene Expression Custom CodeSet; Q, miRNAseq FFPE kit (Qiagen); T, TRizol and column chromatography (Qiagen).

### Table 2. Multivariate overall and progression-free survival by Cox proportional hazards in the NanoString (OTTA/AGO) dataset

<table>
<thead>
<tr>
<th>Gene</th>
<th>Overall survival</th>
<th>Progression-free survival</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>P (corr)</td>
</tr>
<tr>
<td>ATPI3A4</td>
<td>0.91 (0.84–0.99)</td>
<td>0.022 (0.03)</td>
</tr>
<tr>
<td>BMP8B</td>
<td>0.84 (0.75–0.95)</td>
<td>0.004 (0.016)</td>
</tr>
<tr>
<td>CACNA1C</td>
<td>1.07 (0.97–1.19)</td>
<td>0.384 (0.28)</td>
</tr>
<tr>
<td>GAB2</td>
<td>1.06 (0.92–1.23)</td>
<td>0.428 (0.43)</td>
</tr>
<tr>
<td>PAK4</td>
<td>0.87 (0.77–0.99)</td>
<td>0.033 (0.099)</td>
</tr>
<tr>
<td></td>
<td>0.84 (0.72–0.97)</td>
<td>0.017 (0.03)</td>
</tr>
</tbody>
</table>

**NOTE:** Multivariate analysis combined each significant gene from the univariate analysis with stage, age, and cohort as cofactors (n = 458). The results were not different when residual disease was included as a factor (n = 350). Bold values are statistically significant at P < 0.05.

Abbreviations: CI, confidence interval; corr, corrected P value by the Benjamini and Hochberg method (20).
including age at diagnosis and stage (Table 1). No significant differences in age of onset were observed between the 3 patient cohorts. The proportion of samples in each stage significantly differed between cohorts ($\chi^2 = 30.1, P < 0.001$), driven by the higher proportion of stage I and II patients in the OTTA cohort. As described in Materials and Methods, cases were categorized as low expressors (38%–39% of each cohort) or high expressors (35%–38% of each cohort), and clinical data were combined across the 3 cohorts. The method of categorizing the cases depends on the distribution of the values about the median, and thus the proportions in each group vary slightly for each cohort. (This categorization is also used for analyses on the AOCS and TCGA expression array data described in subsequent sections.) In a multivariate model including stage, age, and cohort, $GAB2$ expression was significantly associated with overall survival ($P = 0.006, HR, 0.79$; Fig. 1A, Supplementary Fig. S3) and remained significant when residual disease was also factored in ($P = 0.02, HR, 0.80$). $BMP8B$ was significantly associated with progression-free survival ($P = 0.002, HR, 0.77$; Supplementary Figs. S1 and S4), but not when residual disease was also considered ($P = 0.08, HR 0.85$). $ATP13A4$ was no longer significant ($P = 0.27$). However, the signal for the latter genes came mostly from the Nanostring dataset, possibly because of low and therefore unreliable expression measurements in the microarray datasets (median log2 expression values AOCS: $BMP8B$, 5.5; $ATP13A4$, 3.4; TCGA, $BMP8B$ 3.1; cf. AOCS $GAB2$ 7.7; TCGA $GAB2$ 7.2; Supplementary Fig. S4).

Aberration of $GAB2$ is correlated with C4 and differentiated HGSC

We sought to further characterize patients with genomic aberrations of $GAB2$ using the ovarian tumor classifier proposed by Tothill and colleagues (4). We obtained classification data for the AOCS and TCGA cohorts with expression ($n = 642$), copy number data ($n = 452$), and those with both copy number and expression data available ($n = 445$). The distribution of subtypes across the combined expression cohort was C1: 28%, C2: 19%, C3: 4%, C4: 29%, C5: 18%, and C6: 1.4%. Excluding the rare C6 subtype, copy number gain ($>0.3 \log_2$ ratio) of $GAB2$ occurred in 33% of the tumors analyzed and showed a statistically significant different distribution across the subtypes ($P < 0.005, \chi^2$ test) with over-representation in the C4 subtype (41%) and underrepresentation in the C3 and C5 subtypes (13% and 21%). Samples highly expressing $GAB2$ mRNA as defined by the survival analysis above were also distributed nonrandomly ($P = 0.0002, \chi^2$ test), with bias toward the C4 subtype (49%), and underrepresentation in

![Figure 1](https://example.com/figure1.png)

**Figure 1.**
A, $GAB2$ expression is associated with overall patient outcome. Kaplan–Meier curve of overall patient survival of the combined AOCS, OTTA/AGO, and TCGA cohorts. HR shown is log-rank (GraphPad Prism). B, distribution of $GAB2$ genomic aberration (for cases with both copy number and expression data) across HGSC subtypes, combining AOCS with TCGA data and considering the Tothill classifier (left) and the TCGA classifier on TCGA cases only (right). C, differences in copy number profiles between $GAB2$ high- and low-expressing samples (TCGA data). Arrowheads, statistically significant regions.
the C1 and C5 subtypes (29% and 30%). When copy number and expression were combined, 50% of cases showed altered GAB2 by either copy number or expression and the distribution remained uneven \((P = 0.01, \chi^2\) test, Fig. 1B), with overrepresentation in the C4 subtype (39%), and underrepresentation the C3 and C5 subtypes (31% and 38%). Thus, the C4 subtype, which has to date been the least well-characterized, may be partly defined by increased aberration of GAB2.

We also assessed membership of the TCGA classification system, comprising differentiated (D), immune (I), mesenchymal (M), and proliferative (P) using TCGA data only (Fig. 1B). Combining copy number and expression data as above, we found a borderline statistically significantly different distribution of GAB2 status \((P = 0.057, \chi^2\) test) with overrepresentation in the D subtype (60%) and underrepresentation in the P subtype (41%). The D subtype is related to the C4 subtype above (76% of D cases were also classified C4) and comprises a group of tumors with generally better survival outcome, reinforcing our observation of correlation of GAB2 expression with better survival.

Correlation of GAB2 expression with copy number events

We previously observed that GAB2 mRNA expression was statistically significantly correlated with its copy number in 3 datasets \((r^2, 0.48–0.7; \text{ref. 9})\). As particular genomic aberrations have been shown to have significant associations with mRNA expression–based ovarian cancer subtypes (5), we evaluated whether GAB2 expression was correlated with any other copy number events, by using the GAB2-high and -low designations from the survival analysis and assessing differences in copy number events using TCGA data (Fig. 1C). We found that GAB2 high-expressing cases were enriched for 11q gain (as expected) and also 8q gain, 16q loss, and Xq loss \((P < 0.001, \text{at least } 20\% \text{ difference in frequency})\). This result is consistent with the observation that 8q gain at MYC is most common in the differentiated TCGA subtype (49%; \text{ref. 5}), in which we have found GAB2 overexpression to be more frequent. In addition, GAB2 low-expressing cases showed enrichment for gain on 2p and 20q. There was no difference in the percentage of the genome altered by copy number.

GAB2 copy number and overexpression correlates to protein levels

We used the TCGA reverse-phase protein array (RPPA) data to evaluate the effect of GAB2 aberration on protein levels (Fig. 2A). GAB2 protein was correlated with both GAB2 copy number and RNA \((P < 0.0001 \text{ for both, Pearson } r^2 = 0.31 \text{ for copy number and } 0.49 \text{ for RNA})\). GAB2 protein levels were significantly different between both Tothill and TCGA subtypes \((P = 0.006 \text{ and } P = 0.009, \text{1-way ANOVA})\), with elevated protein in the C2 and C4 subtypes and immune and differentiated subtypes (Fig. 2B and C). Consistent with the increase in copy number gain at 8q observed in GAB2 overexpressing tumors, the protein with the most significant association with GAB2 overexpression was c-Myc \((P = 3.9 \times 10^{-5}, \text{ANOVA of RPPA data using GAB2 high- versus low-expressing tumors as defined above})\).

Previous studies have suggested that GAB2 functions as a scaffold to facilitate activation of pathways downstream of a receptor tyrosine kinase, such as the PI3K pathway (21). We assessed whether elevated GAB2 expression correlated with downstream activation of the PI3K pathway by TCGA RPPA protein levels of pAKT-473, pAKT-308, and pPRAS40-246. pAKT-473 was weakly negatively correlated with GAB2 protein expression \((P = 0.02, \text{Pearson } r = -0.11)\). Copy number change at GAB2 did not affect pAKT or p-PRAS40 expression. Thus, increased expression of GAB2 does not strongly correlate with activation of the PI3K pathway as measured by this assay.
Data mining identifies protein networks associated with GAB2

To identify any other pathways that could illuminate the role of GAB2 in ovarian carcinoma, we analyzed the AOCS and TCGA expression datasets. Normalized data were first filtered for the most variable genes (SD > 0.5) that had median expression levels >3 (TCGA Agilent) or >4 (AOCS Affy Plus2). Each dataset was used to compare GAB2 high-versus low-expressing cases based on the stratification performed above for the survival analysis. The Trottoli C subtypes were included as a factor in the ANOVA. Differentially expressed genes (n = 772 for TCGA and n = 211 for AOCS) were imported to Metacore v6.19 (Thomson Reuters). Enrichment analysis on each gene list consistently identified cytoskeleton remodeling as the most enriched pathway, encompassing the upregulated genes PAK1 and LIMK2; however, the significance was limited (AOCS FDR P = 0.16, TCGA FDR P = 0.007; Supplementary Table S5). We also used Metacore to undertake network building, to identify relevant pathways where proteins whose mRNA were not differentially expressed could still be included as intermediary nodes to link genes that were differentially expressed. Networks were first built independently for each dataset by using the 2-step building algorithm with GAB2 as the starting node (Supplementary Fig. S5) and second using the commonly differentially expressed genes (n = 40) to build a network agnostic to GAB2 (Supplementary Fig. S5). The first analysis identified STAT3 as a hub in both datasets. The second analysis found a complex network of 206 objects that was enriched for EGFR signaling pathway members (FDR: P = 4.4 × 10−21; Supplementary Table S5). These interrelated networks could be explained by the physical interaction of GAB2 with STAT3 (22, 23), leading to downstream changes in transcription and the involvement of GAB2 in the receptor tyrosine kinase signaling transduction pathway.

Cell lines with high expression of GAB2 are specifically sensitive to PI3K inhibition

Given that our clinical and genomic data indicated that aberrant GAB2 expression is characteristic of a subset of patients with HGSC that have improved overall survival, we sought to explore the possibility that enhanced GAB2 expression may provide a marker for sensitivity to targeted therapeutic intervention. As previous studies have implicated GAB2 in the activation of PI3K signaling through the recruitment of p85 and GAB2 (21, 24), we assessed the relationship between GAB2 expression and sensitivity of ovarian tumor cell lines to the dual PI3K/mTOR inhibitor, PF-04691502. Using the 50% growth inhibition (GI50) values generated across a panel of 30 ovarian tumor cell lines (14), the response to PF-04691502 was correlated with GAB2 gene expression (Fig. 3A and Table 3). We observed a statistically significant correlation between GAB2 gene expression and GI50 (Pearson R = −0.48, P = 0.003) that suggests cell lines with high expression of GAB2 are more sensitive to PI3K/mTOR inhibition. We repeated the analysis with only those cell lines that had a high probability of being HGSC in origin [classification value >1 in Domcke and colleagues (ref. 25) or HGSC in Anglesio and colleagues (ref. 10)]. Only 10 cell lines fulfilled these criteria (59M, CaOv3, Kuramochi, OAW28, OVCAR3, OVCAR4, OVCAR5, OVCAR8, FLJOV1, and OV90). The correlation between GI50 and GAB2 expression was still negative, but no longer statistically significant, perhaps due to the smaller sample size (Pearson R = −0.49, P = 0.12). To evaluate whether the response observed using PF-04691502 was specific to the PI3K-dependent function of GAB2, we also assessed the role of the RAS/ERK pathway in the same panel of 30 ovarian tumor cell lines using the selective MEK inhibitor, PD-0325901 (14). No significant correlation between GAB2 expression and GI50 was observed (Fig. 3B).

We have previously assessed P-PRAS40 across the panel of cell lines (14), and we used this as a marker of AKT activity in the cell lines. Cell lines were defined as either high or low expressors of GAB2 and P-PRAS40 (Supplementary Table S6). There was a trend toward an association between GAB2 high expressors and low P-PRAS40 (P = 0.099, Fisher exact test), indicating that high GAB2-expressing cells may have lower AKT signaling.

Discussion

The success of molecular targeted therapeutics for the treatment of HGSC will be dependent upon the identification of the appropriate drug targets and the stratification of patient cohorts that will respond to such therapies. It is widely recognized that ovarian cancer is a highly heterogeneous disease, with a diverse range of histologic and molecular subtypes. As a consequence, the refinement of tumor subtyping will be an important step in the design
of clinical trials and downstream integration of molecular targeted agents into treatment regimes of patients with ovarian cancer. Our expression analysis of 11 oncogene candidates in a large clinically annotated dataset of HGSC identified 3 with correlation to patient outcome: GAB2, ATP13A4, and BMP8B. Surprisingly, CCNE1 and JUN, which have previously been reported to show a strong correlation to outcome (17, 26), were not significant in a multivariate analysis. Our analysis was able to take into account the known prognostic factors of age, stage, and residual disease, but not BRCA mutation status, which may have contributed to the difference observed with CCNE1, as amplification of this gene tends to be exclusive of BRCA1 mutation (5).

ATP13A4 is a calcium ion transporter acting in the endoplasmic reticulum and overexpression leads to elevated levels of intracellular calcium (27). The gene is located on 3q29 and has not previously been associated with cancer. However, deregulated calcium signaling is considered an important tumorigenic pathway (28). BMP8B (bone morphogenetic protein 8B) is located on 1p and encodes a secreted signaling molecule that is important in early embryonic development (29) and may regulate adipogenesis (30). A role in ovarian cancer could be related to the promotion of peritoneal spread by adipocytes, which provide an energy source to the cancer cells (31). In gastric cancer, expression in the bone marrow was associated with metastasis and elevated expression in the primary tumor was related to poor patient outcome (32), in contrast to the results here where higher expression was related to longer progression-free survival.

GAB2 has previously been implicated as an oncogene in multiple cancer types, including breast cancer (33, 34), ovarian cancer (5, 35, 36), leukemia (37), and melanoma (38). We previously reported that GAB2 is a putative cancer driver gene that is copy number amplified in approximately 15% of HGSC (9, 18), and we extend that analysis here by exploring the correlation of GAB2 expression with improved overall survival and ovarian cancer subtypes. The gene encodes the scaffolding adaptor GRB2-associated binding protein, a large protein located at the internal cell membrane where it forms a dock for multiple proteins to mediate signals from transmembrane protein kinases (21). GAB2 binds to the p85 regulatory subunit of PI3K to stimulate PI3K signaling, and overexpression of GAB2 has been demonstrated to potentiate ovarian tumorigenesis via the activation of PI3K signaling in an mTOR-dependent manner (36). Other overexpression studies have also linked GAB2 to enhanced epithelial–mesenchymal transition, cell migration, and invasiveness (39) and response to follicle-stimulating hormone (FSH) in ovarian granulosa cells (40), again through the PI3K pathway. The PI3K signaling pathway is recognized to be a key driver in the development of a diverse range of epithelial tumors (41–43). Genetic alterations to pathway members such as PIK3CA, PIK3CB, AKT1, AKT3, AKT4, PTEN, and PDK1 result in the activation of the pathway, which enhances cellular proliferation and survival. Large-scale genomic studies have indicated that 45% of HGSC exhibit activation of the PI3K signaling pathway (3), suggesting this pathway could be an ideal target for the development of targeted therapeutic strategies for the treatment of HGSC. Our data from both the tumor samples and cell lines suggest there is no relationship between high GAB2 expression and elevated PI3K pathway signaling, in contrast to what has been described in studies that have induced overexpression of GAB2. Nonetheless, we found that high GAB2 expression was an indicator of sensitivity to a PI3K/mTOR inhibitor, whereas our previous studies failed to demonstrate a correlation of PF-04691502 sensitivity with genetic aberrations in PI3K pathway genes (PIK3CA and PTEN; ref. 14). The mechanism for this sensitivity is unclear but bears further investigation given the dozens of clinical trials featuring various PI3K and PI3K/mTOR inhibitors (44).

In summary, we have used gene expression data from 3 clinically annotated patient cohorts to identify a correlation between GAB2 gene expression and improved patient survival. Elevated GAB2 expression was also correlated with the C4 and differentiated ovarian cancer subtypes, with differentially expressed genes in relevant signaling pathways. GAB2 expression can be upregulated by copy number, but it is likely that other mechanisms are also involved. We further correlated GAB2 gene expression in ovarian tumor cell lines with response to the dual PI3K/mTOR inhibitor, PF-04691502. Collectively, these data led us to postulate that aberrant GAB2 expression may provide a molecular marker for the characterization of a subset of HGSC that will be responsive to the targeted inhibition of PI3K signaling.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: S.J. Davis, K.E. Sheppard, J. Pfisterer, F. Hilpert, K.J. Simpson, I.G. Campbell, K.L. Gorringe

Table 3. Cell line sensitivity data to PF-04691502

<table>
<thead>
<tr>
<th>Cell line</th>
<th>GI50 (nM)</th>
<th>PF502 (nM)</th>
<th>Sensitive (S)</th>
<th>Resistant (R)</th>
<th>GAB2 mRNA</th>
<th>PI3K pathway</th>
<th>MAPK/ERK pathway</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGROV1</td>
<td>16</td>
<td></td>
<td>S</td>
<td>R</td>
<td>High</td>
<td>PTEN</td>
<td></td>
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<tr>
<td>OAW28*</td>
<td>29</td>
<td></td>
<td>S</td>
<td>R</td>
<td>High</td>
<td>PTEN</td>
<td></td>
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<tr>
<td>RMGII</td>
<td>32</td>
<td></td>
<td>S</td>
<td>R</td>
<td>High</td>
<td>PTEN</td>
<td></td>
</tr>
<tr>
<td>59M*</td>
<td>77</td>
<td></td>
<td>S</td>
<td>R</td>
<td>High</td>
<td>PTEN</td>
<td></td>
</tr>
<tr>
<td>OAW42</td>
<td>81</td>
<td></td>
<td>High</td>
<td></td>
<td></td>
<td>PIK3CA</td>
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<tr>
<td>JHOC7</td>
<td>90</td>
<td></td>
<td>S</td>
<td>R</td>
<td></td>
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</tr>
<tr>
<td>OVCAR3*</td>
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<td></td>
<td>High</td>
<td></td>
<td></td>
<td>PIK3CA</td>
<td></td>
</tr>
<tr>
<td>FUOVI*</td>
<td>108</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>JHOC9</td>
<td>117</td>
<td></td>
<td>S</td>
<td>R</td>
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<td></td>
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<tr>
<td>CHI</td>
<td>200</td>
<td></td>
<td>S</td>
<td>R</td>
<td></td>
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<td>CADOV3*</td>
<td>216</td>
<td></td>
<td>R</td>
<td>High</td>
<td></td>
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<tr>
<td>2008</td>
<td>296</td>
<td></td>
<td>R</td>
<td>S</td>
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<tr>
<td>RMGI</td>
<td>311</td>
<td></td>
<td>R</td>
<td>S</td>
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<td></td>
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<td>R</td>
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<tr>
<td>MCAS</td>
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<td>OVCAR8*</td>
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<td>OVCAR432</td>
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<tr>
<td>OV90*</td>
<td>461</td>
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<td>R</td>
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<td>EFO27</td>
<td>464</td>
<td></td>
<td>R</td>
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<tr>
<td>SKOV3</td>
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<td>S</td>
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<tr>
<td>TOV21G*</td>
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<td></td>
<td>R</td>
<td>S</td>
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<tr>
<td>ES2</td>
<td>576</td>
<td></td>
<td>R</td>
<td>S</td>
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<tr>
<td>JHOC5</td>
<td>641</td>
<td></td>
<td>R</td>
<td>S</td>
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<td></td>
</tr>
</tbody>
</table>

Note: The mean GI50 was used to define sensitive and resistant cell lines. High GAB2 expression corresponds to greater than the mean of all cell lines.

*High-grade serous cell lines according to Domcke and colleagues (25) or Anglesio and colleagues (10). Note that not all cell lines used here were assessed by these studies.
Development of methodology: S.J. Davis, K.E. Sheppard, S. Kommoss, K.J. Simpson


Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): S.J. Davis, J. George, S. Fereday, J. Pisters, R.B. Pearson, K.J. Simpson, K.L. Gorringe

Writing, review, and/or revision of the manuscript: S.J. Davis, K.E. Sheppard, S. Kommoss, K.J. Simpson

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): S.J. Davis, S. Fereday, M.P. Intermaggio, S. Kommoss, S.J. Ramus, D.G. Huntsman

Study supervision: K.J. Simpson, I.G. Campbell, K.L. Gorringe

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Correction: Enhanced GAB2 Expression Is Associated with Improved Survival in High-grade Serous Ovarian Cancer and Sensitivity to PI3K Inhibition

In this article (Mol Cancer Ther 2015;14:1495–1503), which appeared in the June 2015 issue of Molecular Cancer Therapeutics (1), an error in extracting the survival data led to a number of errors in the published data and the authors request the opportunity to correct the record.

The nature of the error is related to survival data from the Arbeitsgemeinschaft Gynäkologische Onkologie (AGO-OVAR3) randomized controlled trial in Germany. The authors were informed, postpublication, that there had been an error in extracting the survival data by the clinical trial data manager. The authors have since received the correctly extracted data with sample key, and have repeated the survival analysis.

The corrected data, which comprises 40% of the dataset for the first survival analysis and 17% of the combined second survival analysis, changes P values for all genes as would be expected, and some genes have altered statistical significance (Table 2; Supplementary Table S3). These differences affect only the first Results section, with the other four sections and the Discussion unchanged. The association of GAB2 expression with survival is reduced in the first subset analysis, but importantly, the association of GAB2 with overall survival by both Akaike Information Criterion analysis and in the large combined expression cohort is still statistically significant. Thus, the overall conclusions of the article remain unchanged.

The corrected results are addressed in the Abstract, the first Results section (including Fig. 1A), Table 2, Supplementary Figs. S1, S3, and S4, Supplementary Data S1, and Supplementary Table S3. In these changes, some genes were newly statistically significant.

The online version of the article has been updated to reflect the correction. The authors regret the error.

Reference


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Molecular Cancer Therapeutics

Enhanced GAB2 Expression Is Associated with Improved Survival in High-Grade Serous Ovarian Cancer and Sensitivity to PI3K Inhibition

Sally J. Davis, Karen E. Sheppard, Michael S. Anglesio, et al.

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