Cancer Therapy: Clinical

IL6-STAT3-HIF Signaling and Therapeutic Response to the Angiogenesis Inhibitor Sunitinib in Ovarian Clear Cell Cancer

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Abstract

**Purpose:** Ovarian clear cell adenocarcinoma (OCCA) is an uncommon histotype that is generally refractory to platinum-based chemotherapy. We analyze here the most comprehensive gene expression and copy number data sets, to date, to identify potential therapeutic targets of OCCA.

**Experimental Design:** Gene expression and DNA copy number were carried out using primary human OCCA tumor samples, and findings were confirmed by immunohistochemistry on tissue microarrays. Circulating interleukin (IL) 6 levels were measured in serum from patients with OCCA or high-grade serous cancers and related to progression-free and overall survival. Two patients were treated with sunitinib, and their therapeutic responses were measured clinically and by positron emission tomography.

**Results:** We find specific overexpression of the IL6-STAT3-HIF (interleukin 6-signal transducer and activator of transcription 3-hypoxia induced factor) pathway in OCCA tumors compared with high-grade serous cancers. Expression of PTHLH and high levels of circulating IL6 in OCCA patients may explain the frequent occurrence of hypercalcemia of malignancy and thromboembolic events in OCCA. We describe amplification of several receptor tyrosine kinases, most notably MET, suggesting other potential therapeutic targets. We report sustained clinical and functional imaging responses in two OCCA patients with chemotherapy-resistant disease who were treated with sunitinib, thus showing significant parallels with renal clear cell cancer.

**Conclusions:** Our findings highlight important therapeutic targets in OCCA, suggest that more extensive clinical trials with sunitinib in OCCA are warranted, and provide significant impetus to the growing realization that OCCA is molecularly and clinically distinct to other forms of ovarian cancer. Clin Cancer Res; 17(8); 2538–48. ©2011 AACR.

Introduction

Ovarian clear cell adenocarcinoma (OCCA) is a histologic subtype of ovarian cancer that is characterized by a particularly poor response rate to current chemotherapy regimens. The occurrence of OCCA is associated with coexistent endometriosis (1). OCCAs are generally refractory to platinum-based therapy, with a response rate of only 11% to 15% (2). Given the poor response rates, there is a need to develop novel clinical approaches to OCCA and this rests on first gaining insight into the biology of the disease. Investigation of OCCA has been neglected in favor of the more common high-grade serous carcinoma (HGSC), and most studies include only a small number of OCCA samples as part of a larger series of ovarian tumors (3–6).

Relatively little is known about the signaling pathways that drive OCCA. Hepatocyte nuclear factor 1 beta (HNF1B) was discovered as a biomarker of OCCA (7). It seems to be a lineage-specific marker that is expressed in preneoplastic lesions and may play a role in apoptotic escape. A gene expression pattern typical of OCCA has recently been derived from OCCA cell lines and involves genes associated with oxidative stress, glyconeogenesis, and mitogen activated protein kinase and cytokine activation (8). Independent karyotypic and array-based genome-wide measures of DNA copy number have identified...
gain at 17q23-25 (9–11) as being associated with overexpression of PPM1D, a protein phosphatase that regulates the stress-induced extracellular signal-regulated kinase p38.

To date, no published genomic studies have involved characterization of a large number of tumors by using high-resolution contemporary gene expression and DNA copy number platforms. Here we explore the genomic features of a cohort of 59 OCCAs, showing amplification and overexpression of multiple cytokine and growth factor receptors and signaling components.

Methods

Patient cohorts

OCCA samples were drawn from the AOCS, a population-based cohort of more than 1,800 women with ovarian cancer recruited between 2002 and 2006 (12). Patients for this project were ascertained from AOCS initially by using abstracted pathology reports, followed by review of diagnostic hematoxylin and eosin–stained sections from tissue collected at primary surgery by a panel of anatomic pathologists to confirm an OCCA diagnosis. Samples from a cohort of primary OCCA patients were subjected to partially overlapping genomic, IHC, and serum IL6 measurements. Summary clinical characteristics of this OCCA cohort and a control set of HGSC samples for IHC are provided in Table 1. Details of the analyses carried out with individual AOCS samples are provided in Supplementary Table S1. For validation studies, single nucleotide polymorphism (SNP) microarray data were obtained from 18 Japanese OCCA samples (Supplementary Table 1).

Table 1. Sample cohort used for genomic and IHC analyses

<table>
<thead>
<tr>
<th></th>
<th>OCCA</th>
<th>HGSC</th>
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<tr>
<td></td>
<td>Overall cohort</td>
<td>SNP 6.0</td>
</tr>
<tr>
<td>Grade³</td>
<td>(n = 59)</td>
<td>(n = 39)</td>
</tr>
<tr>
<td>1</td>
<td>26</td>
<td>19</td>
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<td>31–78</td>
</tr>
<tr>
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<td>39</td>
<td>24</td>
</tr>
<tr>
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<td>4</td>
</tr>
<tr>
<td>Plt only</td>
<td>12</td>
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Abbreviations: Plt/Tx, platinum-taxane; Plt, platinum.

³All OCCA tumors are considered “high-grade” by convention.
Validation of IHC data was done using 246 ovarian tumor samples (29 OCCAs, 217 HGSCs) obtained from the British Columbia Cancer Agency (Supplementary Table S2). Two patients with platinum-resistant OCCA were treated with the small molecule inhibitor sunitinib, which was obtained from Pfizer. Patient consent, sample collection, and project design were approved by Institutional Review Boards at the contributing institutions.

Sample processing
DNA and RNA were isolated from serial sections (9–12 mm sections that were dewaxed, rehydrated, and stained using a Dako autostainer with Envision+ amplification (Dako). Staining was visualized with diaminobenzidine. Antibodies were HIF1A (Novus; NB100-479 at 1:1,500), pSTAT3 (Cell Signaling Technologies; D3A7 at 1:50), LYN (Cell Signaling Technologies; C13F9 at 1:200), and IL6. Two sample sets were used to generate TMAs for IHC analysis. One set of 40 OCCA specimens was obtained from AOCS, of which 22 specimens were used for gene expression analysis and 29 specimens were used for DNA copy number analysis (Supplementary Table S1). A second set of samples was obtained from the OvCaRe group at the British Columbia Cancer Agency and was completely independent of the genomic analysis (Supplementary Table S2). Results for both TMAs were scored by an anatomic pathologist (C.B.G). Statistical comparisons of protein expression in OCCA and serous cancers were made using the AOCS and Canadian samples independently. In both cases, scoring systems were binarized into high and low groups during statistical analysis (see figure legends).

Cell culture
Ovarian cell lines were obtained from public repositories (American Type Culture Collection: TOV112D, TOV21G, and ES2; National Cancer Institute: SKOV3 and IGROV1; Human Science Research Resource Bank: RMG1; RIKEN: JHOC5, JHOC7, and JHOC9). HAC2 was kindly provided by Dr. Aikou Okamoto (Japan) and OVHS1 from Dr. Ian Campbell (Australia). All cell lines were maintained in RPMI 1640 with 10% FBS in a humidified incubator at 37°C with 5% CO2. Cell culture supernatants were removed after 48 hours of culture, and cytokine concentrations were measured using Quantiikine ELISA kits following manufacturer's instructions (R&D Systems). OCCA and HGSC serum samples were obtained from the AOCS cohort, relying only on patients wherever serum samples were collected immediately presurgically. Clotted blood samples were collected in plain tubes, allowed to clot, serum collected by centrifugation at 2,500 rpm or 1,300 × g for 10 minutes, and stored in 1 mL aliquots at −80°C until processed. Serum IL6 concentrations were estimated using electrochemiluminescence detection with Meso Scale Discovery (MSD) assays and done according to the manufacturer’s protocol. After defrosting at 4°C, samples were centrifuged briefly at 2,000 × g for 1 to 2 minutes at 4°C. The calibrator standards and samples from patients and normal healthy controls were incubated on MSD single-plex IL6 microplates (MSD cat no. K151AKB-2). Plates were washed and read using SECTOR Imager 2400 software (MSD). Statistical significance of difference between OCCA and HGSC serum samples was calculated using a Wilcoxon rank-sum test. A Kaplan–Meier survival curve was plotted, and log-rank statistic was computed to test the significance of serum IL6 levels versus patient outcome.

Results
Clear cell ovarian cancer accounts for less than 10% of invasive ovarian cancer diagnoses in Western countries and it was therefore necessary to access a large patient cohort to obtain substantial numbers of samples. Patients were obtained from the Australian Ovarian Cancer Study (AOCS), a population-based cohort of more than 1,800 women accrued from 2002 to 2006 (12). Details of the clinical characteristics of the AOCS OCCA patient cohort
and genomic analyses done are provided in Table 1 and Supplementary Tables S1 and S2.

Overexpression and amplification of cytokine and receptor tyrosine kinase signaling

Affymetrix U133 2.0 GeneChip arrays were used to obtain comprehensive gene expression signatures for 31 OCCA samples, which were compared with data generated previously by our group on more than 200 HGSC and endometrioid cancers (12) and human ovarian surface epithelium (HOSE; ref. 15). Unsupervised clustering of the top ~9,500 most variant genes easily segregated OCCA from other ovarian cancer histotypes (Fig. 1A). A large coregulated cluster of genes was highly expressed in OCCA that included the immunohistochemical (IHC) biomarker of OCCA \( \text{HNF1}\beta \) (TCF2; ref. 9), the transcriptional targets of \( \text{HNF1}\beta \), \( \text{PKHD1} \) (16) and P450 xenobiotic metabolism genes \( \text{CYP2C9}, \text{CYP2C19}, \text{and CYP2C18} \) (17), the prolactin receptor (\( \text{PRLR} \)), and UDP glycosyl transferases involved in fatty acid and steroid metabolism (Fig. 1B). IHC studies have shown that immune infiltration is less prevalent in OCCA than in HGSC (18), and this was reflected in the low-level expression of immune markers (Fig. 1B).

We noted highly specific upregulation of \( \text{PTHLH} \) (\( \text{PTHrP} \); Fig. 1C; Supplementary Fig. S1), a gene whose expression is commonly associated with hypercalcemia of malignancy (19), and stanniocalcin-1 (\( \text{STC1} \); Fig. 1C), a protein hormone that regulates calcium/phosphate homeostasis and is upregulated by interleukin (IL) 6 in response to hypoxia (20). Strong and specific overexpression of \( \text{IL6} \) was observed \( (P < 0.001; \) Fig. 1C and Supplementary Fig. S1). \( \text{IL6} \) is known to regulate \( \text{PTHLH} \) expression (21) and to be strongly proangiogenic in ovarian cancer (22). \( \text{IL6} \) expression was tightly correlated with both

Figure 1. Gene expression of OCCA. A, dendrogram of agglomerative unsupervised clustering including OCCA samples, HOSE, serous and endometrioid ovarian carcinomas (“Tothill molecular subtypes” of HGSC; ref. 12), low-grade/borderline serous carcinomas, and low-grade endometrioid carcinomas. OCCA samples are distinctly separated from the other groups of ovarian tumors. B, an expression heatmap of HOSE, molecular subtypes of serous and endometrioid carcinomas, and OCCA. Samples are ordered by subtype class, showing discrete clusters of coregulated genes. A coregulated gene cluster highly specific to OCCA samples including specific biomarkers of OCCA such as \( \text{HNF1}\beta \) and a number of its transcriptional targets is clearly visible. Molecular subtypes of serous and endometrioid carcinomas defined by Tothill and colleagues (NCBI GEO accession GSE12172) are labeled and used for comparison in (A) and (B), as per color legend, as are HOSE samples from Bonome and colleagues (data donated by Dr. Michael Birrer; ref. 15). C, zoomed image of selected genes from the larger heatmap whose expression is tightly correlated with the OCCA subtype. Serous and clear cell histologic types are shown.
PTHLH \( (PTHRP; P < 0.0001) \) and EPAS1 (HIF2A; \( P < 0.0001 \); Supplementary Fig. S1). Ontology showed an enrichment of genes associated with coagulation, inflammation-mediated cytokine signaling, and hypoxia and oxidative stress (Supplementary Table S3). Expression of proliferative markers and cell-cycle–associated genes was reduced in OCCA compared with HGSC. The gene expression profile was concordant with a previously described OCCA cell line–derived signature (ref. 8; Supplementary Fig. S2), indicating that the epithelial fraction of OCCA tumors rather than stromal infiltration dominates the gene expression profile.

DNA copy number data were generated using Affymetrix SNP6.0 arrays (Supplementary Table S1). Regions of copy number change were both smaller and less frequent in OCCA than in HGSC (Fisher exact test; \( P < 0.05 \)). Although some amplifications and deletions are shared between HGSC and OCCA (e.g., 8q24 involving MYC), a distinct pattern of chromosomal aberration was observed in OCCA samples (Fig. 2A). In contrast, the pattern of copy number

Figure 2. Patterns of DNA copy number changes in OCCA. Comparison of DNA copy number change in (A) OCCA versus HGSC or (B) OCCA derived from the AOCS cohort versus an independent set of Japanese OCCA tumors. Differential regions of gain (red) or loss (blue) were assessed at each cytoband using Fisher’s exact test and the \( P \)-values were plotted after correction for multiple testing. OCCA and HGSC show distinctly different patterns of chromosomal change compared with OCCA derived from independent data sets. C, frequency (0%–100%) of copy number changes across the genome and annotated with genes of interest associated with peak regions of gain (red) or loss (blue). D, frequency plot of chromosome 7 associated with the MET gene (red line) for HGSC and OCCA tumors. E, histogram depicting the distribution of amplified regions of different lengths [megabases] associated with the MET locus for individual HGSC and OCCA tumors. The frequency plot and histogram show that MET gain is associated with focal regions of gain in OCCA compared with being more commonly included in a broad region of copy number gain in HGSC.
change was highly consistent with that obtained from an independent set of 18 Japanese OCCA samples (Fig. 2B), indicating that the variation observed between HGSC and OCCA cancers represented a meaningful difference between the biology of the 2 tumor types. A frequency plot of copy number change shows numerous regions of gain and loss associated with genes involved in oncogenic signaling (Fig. 2C), including gains associated with several receptor tyrosine kinases (RTK) including ERBB2 (13/39 samples), ERBB3 (8/39), and MET (5/39). Gain of MET is of particular interest, as it was focal and corresponded to the peak of amplification (Fig. 2D and E). Approximately 50% of OCCAs overexpressed MET mRNA by more than 8-fold in OCCA over HGSC (P < 0.0001; Fig. 1C and Supplementary Fig. S1), suggesting amplification-dependent and -independent pathways of MET expression in OCCA. Specific overexpression of the MET ligand HGF was observed in OCCA compared with HGSC tumors (P < 0.004; Supplementary Fig. S1). Changes were also found in intracellular signaling components, including gain of STAT3 (14/39), IL6R and SHC1 (13/39), src-family kinase LYN (29/39), PPM1D (12/39), and heterozygous loss of the STAT3 phosphatase PTPRD. Minimal regions of chromosomal change involving at least 4 samples identified 76 regions of gain and 30 regions of loss (Fig. 2C, bottom panel; Supplementary Table S4). A gene ontology analysis of genes associated with minimal regions showed enrichment of genes related to cytokine- and stress-related pathways (Supplementary Table S5) including JAK (Janus activated kinase)-STAT signaling (P < 0.001).

**IL6 and downstream signaling proteins are strongly overexpressed in OCCA**

Figure 1C shows that IL6 overexpression is a consistent feature of OCCA. IL6 signals via STAT3 (23) to hypoxia induced factor (HIF; ref. 24), a key hypoxia-induced transcription factor controlling angiogenesis and VEGF-A expression. Consistent with our array data, IHC analysis of primary tumors showed high-level nuclear expression of phospho-STAT3 (pSTAT3; P = 0.0008; Fisher exact) and

![Image](https://example.com/image.png)

**Figure 3.** IHC staining supports activation of a central IL6-STAT3-HIF1A pathway in OCCA, with potential involvement of the src-family kinase LYN. Typical high-level IHC positivity is shown in OCCA samples for (A) nuclear HIF1A, (B) the src-family kinase LYN, and (C) pSTAT3 (Y705). Bottom, mosaic plots summarizing IHC results of OCCA and HGSC samples (D, E, and F); both the vertical (score) and horizontal (cohort) dimensions are proportional to the number of samples in each class. A thin column at the right of each mosaic plot represents the score proportions for all samples. High- and low-staining groups within serous and clear cell tumors were binarized for analysis (high staining was >2 for HIF1A and pSTAT3, >0 for LYN). For all 3 markers tested, OCCA samples showed a higher proportion of high-level positive staining (P < 0.001; see also Supplementary Methods).
HIF1A (P < 0.0001; Fisher exact) in the epithelial fraction of the tumor (Fig. 3). Specific staining of the src-family kinase LYN was also found in OCCA samples (P < 0.0001; Fig. 3). Staining was further validated in an independent cohort of OCCA and HGSC samples from Gynaecologic Tissue Bank at Vancouver General Hospital (Supplementary Methods). In the Vancouver cohort, HIF1A staining in OCCA was also higher than in HGSC (P < 0.0001) and pSTAT3 was of borderline significance (P = 0.0678). Strong IL6 protein expression was seen in the epithelial fraction of OCCA tumors (Fig. 4A). However, in contrast to the mRNA data, differential IHC staining of IL6 in OCCA compared with HGSC was not readily apparent, likely due to the secretion of IL6 from cells. High levels of secreted IL6 were detected in the media of OCCA cultures (Fig. 4B). Significantly higher levels of circulating IL6 were found in sera collected from OCCA patients than from HGSC patients (P < 0.01; Fig. 4C). Although the level of IL6 in HGSC was associated with increasing tumor stage, IL6 concentrations in OCCA patients were independent of extent of disease (Fig. 4D). Increased serum IL6 levels were associated with reduced progression-free and overall survival in OCCA patients in a univariate analysis (Supplementary Fig. S5). Although there were too few samples to fit into a multivariate model, we note that IL6 levels were not associated with FIGO (International Federation of Gynecology and Obstetrics) stage in OCCA tumors but were so in HGSC patients (Fig. 4D), suggesting that the prognostic significance of IL6 in OCCA may be independent of stage.

Recent clinical data suggest that low-stage OCCA has a better outcome than equivalently staged HGSC (25). We therefore carried out a supervised analysis of gene expression and DNA copy number against progression-free survival, overall survival, and tumor stage (Supplementary Methods). Although a number of genes and regions were obtained, no genes or regions of chromosomal aberration were statistically significantly associated with these clinical parameters after correcting for multiple testing (Supplementary Tables S6 and S7, respectively). Mutation in ARID1A has been reported in ~46% of OCCA samples (26). ARID1A mutation status was determined for 24 AOCS OCCA samples in the study by Wiegand and colleagues (26). Consistent with the notion that wild-type tumors are disrupted in the SWI/SNF pathway by mechanisms independent of ARID1A mutation (26), we found no difference in gene expression between tumors with or without mutation (Supplementary Table S8).

Sunitinib response in chemotherapy-resistant OCCA patients

The profound upregulation of HIF1A and EPAS1 (HIF2A) we observed in OCCA and the molecular parallels with renal clear cell cancer (RCCC) suggest that patients might benefit from treatment with sunitinib, a potent inhibitor of several tyrosine kinases including VEGFR, PDGFR, and KIT, that has significant activity in RCCC (27) and is approved for clinical use. This is supported by our experience in 2 patients who were treated with sunitinib and response assessed by

Figure 4. IL6 is strongly expressed in OCCA. A, IHC staining of IL6 in a representative OCCA sample, with strong cytoplasmic expression apparent in the epithelial fraction of the tumor. B, OCCA cell lines show high levels of secreted IL6 compared with other ovarian cancer lines. C, serum samples collected presurgically show that OCCA patients have higher levels of circulating IL6 compared with HGSC patients. D, serum IL6 levels are associated with increasing tumor stage in HGSC patients but not in OCCA patients.
monitoring of cancer antigen 125 (CA125) levels and with serial F-18 fluoro-deoxyglucose (18-FDG) positron emission tomography (PET). Both patients had progressed within 6 months of initial platinum and paclitaxel chemotherapy and had not responded to liposomal doxorubicin. Patient 1 had widespread painful, enlarged lymph nodes and subcutaneous tumors and was initially treated with 50 mg daily of sunitinib for 4 weeks, followed by a 2-week break. She showed a rapid response as judged by decreasing CA125 levels (Fig. 5A) and decreased uptake of 18-FDG (Fig. 5B), as well as a decrease in size of measurable tumors. However, her CA125 levels rose and nodal disease worsened during the off-treatment phase. After 2 further cycles, she was switched to continuous sunitinib 37.5 mg daily. There was an ongoing decrease in CA125 levels, the size and number of involved lymph nodes and subcutaneous tumors, and an overall clinical improvement. She maintained a clinical response for 20 months, at which time several nodal deposits progressed and sunitinib treatment was ceased. Patient 2 had an ovarian carcinosarcoma with predominantly clear cell differentiation with a large pelvic mass, multiple bulky liver metastases, and peritoneal nodules. She was also treated with 50 mg daily of sunitinib, using the 4 weeks on and 2 weeks off schedule. She had significant improvement in symptoms including reduced pain and fatigue, decreased CA125 levels, and improvement on serial PET-CT (computed tomographic) scans (Supplementary Fig. S4). The sunitinib dose was reduced to 37.5 mg from cycle 3 because of diarrhea, nausea, and hand–foot syndrome. Clinically and radiologically, her disease remained stable for the next 3 cycles. However, CA125 levels began to fluctuate with an increase during the 2-week off-treatment phase, followed by a decline in the 4-week on-treatment phase. She developed a Mallory–Weiss tear during the sixth cycle following vomiting that was probably related to sunitinib, and treatment was withheld for 3 weeks. Despite reintroduction of the sunitinib, disease progression occurred and she died 11 months after commencing sunitinib treatment.

Discussion

OCCA is an uncommon ovarian cancer histotype and, consequently, with the exception of a recent study involving a large series of cell lines (8), individual high-resolution genomic studies have involved small numbers of samples of typically less than 10 primary tumors (3–6). By drawing on a very large population cohort (AOCs) and independent sample sets in Japan and Canada for validation, we have provided the first large, high-resolution gene expression and DNA copy number data sets for OCCA.

The contrast between the genomic features of OCCA and HGSC was striking, with OCCA tumors showing a distinct pattern of gene expression and a more limited and largely different pattern of chromosomal change. HGSCs most likely arise from secretory cells in the distal fallopian tube (28) and are characterized by near ubiquitous TP53 mutations (29) and frequent mutation in BRCA1 or BRCA2 pathway (30). These molecular events are much less frequent in OCCA, which has a different pattern of mutations involving, for example, activating mutations in PIK3CA and loss of PTEN (31). The progenitor cell for OCCA is unknown; however, the well-established association of OCCA with endometriosis (1, 32–34) and recent observations linking molecular changes in atypical endometriosis to those found in coexisting clear cell carcinoma (35), including ARID1A mutations present in OCCA and adjacent atypical endometriotic lesions (26), strongly implicate metaplastic endometrial cells as a source of these tumors. It should be noted that endometriosis is not always reported in OCCA patients, and specific molecular differences between OCCA cases presenting with (atypical) endometriotic lesions or without evidence of endometriosis have not been thoroughly investigated. In any case, apparent...
differences in progenitor cells and distinct molecular features of OCCA and HGSC underscore the conclusion that these diseases are largely unrelated and require different approaches to therapeutic intervention.

In breast cancer, some tumors are characterized by loss of homologous recombination and chromosomal instability whereas others are more karyotypically normal and are characterized by features of oncogene addiction (36). HGSC and OCCA provide a parallel with breast cancer, in which loss of HR repair is a key feature of HGSC and deregulation of cytokine signaling pathways seem central to OCCA. Gain was observed in several RTKs, most notably MET, which is a validated therapeutic target in a range of solid cancers (37). MET gain seemed to be highly focal in OCCA, and this together with specific overexpression of MET in OCCA samples suggests that MET may be an important therapeutic target in a subset of OCCA samples. The overexpression of its ligand, HGF, supports this notion. A number of genes and chromosomal regions were associated with time-to-relapse and overall survival; however, none were significant after correcting for multiple testing. Given the relatively small number of samples evaluated against a large number of genes and regions, the failure to maintain an association with outcome is not surprising and these loci deserve further attention in larger studies. Previously, gain at 17q23-25 encompassing PPM1D has been associated with outcome and was observed in 12 of 39 cases. In our series of cases, the peak region of amplification as assessed by GISTIC (Genomic Identification of Significant Targets in Cancer) analysis (38) mapped adjacent to PPM1D (Supplementary Fig. S3), suggesting that it may not be the driver of amplification at this locus in our series of patients.

We noted strong and specific upregulation of IL6 in OCCA compared with HGSC and an association of serum IL6 levels with progression-free and overall survival. IL6 has tumor-promoting actions on both malignant and stromal cells in a range of experimental cancer models (23, 39). It is also expressed by myeloid cells in colitis-associated cancer (39), wherein it stimulates the proliferation of premalignant epithelial cells and protects them from cell death. We observed strong overexpression of IL6 in OCCA in the epithelial fraction of the tumor and IL6 mRNA and protein were strongly and specifically overexpressed in OCCA compared with HGSC cell lines. IL6 expression in solid cancers is also associated with immune cell infiltration; however, OCCA has lower levels of immune cell infiltration, including macrophages, than HGSC (18). What may be the role of IL6 in OCCA? IL6 has been shown to be a downstream effector of oncogenic ras (40) and has been implicated in several human cancers including multiple myeloma (reviewed in ref. 41). It exists in a cytokine network with TNF, CXCL12, and IL8, which are important for the growth of HGSC (42). IL6 levels are elevated in women with endometriosis (43, 44), a precursor of OCCA, and are overexpressed in endometriotic samples compared with eutopic endometrium (45). Therefore deregulation of IL6 expression seems to be an early event in the development of OCCA. IL6 signals via STAT3 and activates expression of downstream genes including PTHLH and HIF1A (24, 41, 46). We observed strong expression of activated pSTAT3 in tumors and cell lines and nuclear HIF1A in TMA, consistent with autocrine activation of the pathway. Whether PTHLH and HIF1A are direct targets of the IL6 pathway in OCCA as in other cell types remains to be tested. We note that the expression of IL6 and EPAS1 (HIF2A) is particularly closely correlated, supporting a causal association. HIF2A is a proangiogenic transcription factor that seems to be more important than HIF1A for promoting VEGF expression in RCCC (47), a solid tumor type that is molecularly related to OCCA (6). The regulation of HIF2A is poorly defined; however, it is a transcriptional target of Oncostatin M, a member of the IL6 family (48).

The prominent expression of IL6, PTHLH, and HIF proteins may explain important clinicopathologic features of OCCA. Intense PTHLH expression was a consistent feature of OCCA. Overexpression of PTHLH induces hypercalcemia of malignancy, a condition that is more commonly associated with OCCA than any other ovarian histotype (49). Thromboembolic events are also more common in OCCA than other types of ovarian cancer (50, 51), and IL6 overexpression is associated with increased risk of thrombosis (52). Anti-IL6 antibodies are in clinical development and a phase II trial in ovarian cancer has been recently completed (F. Balkwill personal communication). The pronounced expression of IL6 in OCCA suggests that this histotype may deserve special attention in the development of anti-IL6 therapy. In addition, the high levels of circulating IL6 observed in many patients may have implications for dosing schedules if effective treatment of OCCA is to be attempted.

Consistent with strong upregulation of HIF1A and HIF2A in OCCA, and the previously shown molecular similarity of OCCAs and RCCCs (6), we observed a favorable clinical response in 2 patients treated with sunitinib, which is approved for the treatment of RCCC. Before commencing sunitinib, both patients had progressive disease that was refractory to conventional chemotherapy. Sunitinib targets multiple RTKs (27) expressed in tumor and stromal cells, although it has been suggested that collapse of the vascular network at concentrations lower than required to directly kill the epithelial fraction may be most important (53). How sunitinib acts in OCCA is unclear, but given the pronounced expression of HIF1A and HIF2A, it is likely that inhibition of VEGF signaling is important. Previous completed trials of antiangiogenic agents in ovarian cancer have been conducted in populations with predominantly HGSC (54–57). Sunitinib is currently being tested in a phase II clinical trial of OCCA expected to complete accrual in late 2012. The responses observed in the 2 patients treated, together with data obtained from our genomic studies, indicate that a molecularly targeted approach to OCCA is likely to provide an improvement over the current limited response rates and poor prognosis observed with the standard platinum-taxane chemotherapy.
Disclosure of Potential Conflicts of Interest

The authors declare no conflicts of interest.

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# Clinical Cancer Research

**IL6-STAT3-HIF Signaling and Therapeutic Response to the Angiogenesis Inhibitor Sunitinib in Ovarian Clear Cell Cancer**

Michael S. Anglesio, Joshy George, Hagen Kulbe, et al.

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