The target therapy of ovarian clear cell carcinoma

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Abstract: Clear cell adenocarcinoma (CCC) of the ovary accounts for 10% of epithelial ovarian cancer and is a distinct entity from other epithelial ovarian carcinomas. It arises from the endometriosis. CCC has specific biological and clinical behavior. Compared with other histological types, CCC shows a chemoresistant phenotype, which leads to poorer prognosis. Thus, development of new target-based therapies remains an unmet need for these patients. Mutations in the gene ARID1A have been found to occur in high frequency in CCC. The majority of these mutations lead to a loss of expression of the ARID1A protein, which is a subunit of the SWI/SNF chromatin remodeling complex and considered as a bona fide tumor suppressor. Upregulation of the PIK3/AKT/mTOR pathway, particularly through mutations of PIK3CA and inactivation of PTEN, is involved in tumorigenesis of CCC. Targeting angiogenesis, the Met protooncogene pathway, and HER2 are also discussed in this review.

Keywords: ARID1A, PIK3/AKT/mTOR pathway, angiogenesis, Met protooncogene pathway, HER2

Introduction
Epithelial ovarian cancer (EOC) is the most lethal gynecologic malignancy. A majority of cases (61%) are diagnosed at an advanced stage, with a corresponding 5-year survival rate of only 27%. Clear cell adenocarcinoma (CCC) accounts for 10% of EOC and is a distinct entity from other epithelial ovarian carcinomas. Unlike serious EOC, which is thought to originate in the distal fallopian tube, the CCCs and endometrioid carcinoma were thought to arise from the endometriosis. A large epidemiological pooled analysis of 13 case-control studies, including 13,226 controls and 7,911 women with invasive ovarian cancer, investigated the frequency of self-reported endometriosis and found significantly increased risks for ovarian cancer in women with a history of endometriosis, with an odds ratio (OR) 3.05, 95% CI 2.43–3.84, \( P < 0.0001 \). Importantly, a significant association of the histological subtypes of clear cell (odds ratio [OR] 3.05, 95% CI 2.43–3.84, \( P < 0.0001 \)), endometrioid (OR 2.04, 95% CI 1.67–2.48, \( P < 0.0001 \)), and low-grade serous ovarian carcinomas (OR 2.11, 95% CI 1.39–3.20, \( P < 0.0001 \)) was found with endometriosis in this study. In contrast, there was no association of endometriosis with mucinous and high-grade serous cancer as well as borderline tumors.

CCC has specific biological and clinical behavior. Compared with other histological types, CCC has been recognized to show a chemoresistant phenotype, leading to poorer prognosis. The response rate by paclitaxel plus carboplatin was lower than the other histologic type, ranging from 22% to 56%. Thus, development of new target-based...
therapies remains an unmet need for these patients. This review discusses molecular characteristics of CCC, frequent mutations and pathways involved, and potential implications of these factors on choice of therapy.

**Targeting ARID1A**

ARID1A gene mutations are the most frequent mutations in CCC. ARID1A somatic mutations were seen in 46%–57% of ovarian clear cell carcinomas, 30% endometrioid carcinomas, and none of the high-grade serous ovarian carcinomas. ARID1A encodes BAF250a, a key component of the SWItch/Sucrose NonFermentable (SWI/SNF) chromatin remodeling complex. Loss of the BAF250a protein correlated strongly with the ovarian clear cell carcinoma and endometrioid carcinoma subtypes. ARID1A mutations and loss of BAF250a expression were also evident in the tumor and contiguous ovarian CCC patients. Loss of ARID1A expression was identified in nine (15%) of 60 samples. Loss of ARID1A in ovarian clear cell carcinoma is a negative prognostic factor with p53, and mutations in the ARID1A and TP53 genes were mutually exclusive in the tumor specimens examined. ARID1A is a bona fide tumor suppressor that collaborates with p53 to regulate CDKN1A and SMAD3 transcription and tumor growth in CCC and other endometriosis-related ovarian cancers.

It can be speculated that once the mechanisms of the cellular dysfunctions caused by ARID1A mutation in CCCs are accurately demonstrated, they can be explored for the development of novel targeted therapies.

**Targeting the PI3K/AKT/mTOR signaling pathway**

The PI3K/Akt/mTOR pathway is a central regulator in both normal cell physiology and in cancer proliferation, tumorigenesis, and metastasis. The pathway is comprised of three main driving molecules: PI3 kinase (PI3K), AKT, and mammalian target of rapamycin (mTOR). The pathway is frequently abnormal in CCC, including mutations in PIK3CA, deletion in PTEN, amplification of AKT1, AKT2, and AKT3, which all lead to an aberrant functioning PI3K/AKT/mTOR pathway.

Mutations of PIK3CA (which encodes p110α), resulting in overactivation of PI3K kinase activity, have been identified in 33% of CCC patients and are present in the endometriosis of patients with CCC harboring PIK3CA mutations, indicating that the PI3K pathway has an important role in the pathogenesis of CCC, and PIK3CA mutation is an early event in the development of endometriosis-associated ovarian CCC. In patients with a PIK3CA activating mutation, 40% also had an inactivating mutation in the regulatory genes PIK3R1 or PTEN. Loss of PTEN is a coregulator of PI3K activity and is sufficient in conjunction with a mutant PIK3CA for tumorigenesis to occur.

AKT is the central molecule in the PI3K/AKT/mTOR pathway, activating and modulating numerous
downstream targets. AKT2 amplification has been found in a subgroup of CCCs that are associated with a shorter PFS.14

mTOR is a 289 kDa serine/threonine kinase that belongs to the PI3K-related protein kinase family. An immunohistochemical analysis of phospho-mTOR expression in a tissue microarray of 98 primary ovarian cancers showed that mTOR was more frequently activated in CCC than in serous carcinomas (86.6% versus 50%).20 Another study also confirmed mTORC2 was more frequently activated in CCCs than in serous carcinomas (71.2% versus 45.7%).21

Given that the PI3K/AKT/mTOR signaling pathway is more frequently activated in CCCs, it is reasonable to act as a therapeutic target. Many PI3K/AKT/mTOR pathway inhibitors have been used in preclinical ovarian cancer models. For example, PI3K inhibitor CH513279922 and AKT inhibitor MK220623 have been reported to inhibit cell growth in ovarian cancer cell lines. The mTOR inhibitor everolimus can also markedly inhibit cell growth in both cisplatin-sensitive parental and cisplatin-resistant human CCC cell lines.20 However, the sensitivity of CCC cell lines to BEZ235 or temsirolimus (both are PI3K/mTOR dual inhibitors) was not related to the mutation status, but CCC cells exhibited G1 phase arrest and apoptosis with a higher concentration of BEZ235. BEZ235 significantly inhibited tumor growth in mice bearing OVISE and TUCOC-1 cell tumors.24 Another study revealed that BEZ235 prevents epithelial-mesenchymal transition induced by hypoxia and transforming growth factor-β1.25 Dual PI3K/mTOR inhibitor DS-7423 was recently reported to constitute a promising molecular targeted therapy for CCC, and its antitumor effect might be partly obtained by induction of TP53-dependent apoptosis in TP53 wild-type CCCs.26

The majority of the agents targeting the PI3K pathway are in early-phase clinical trials, and to date, there is no evidence that supports their use in the management of CCCs. An international collaborative study led by the Gynecologic Oncology Group is evaluating the combination of the mTOR inhibitor temsirolimus with carboplatin and paclitaxel for the first-line treatment of patients with advanced CCCs.27

### Targeting angiogenesis

Growth factors have been identified to play key roles in driving angiogenesis and, thus, the formation of new blood vessels that assist in “feeding” cancer. Such molecules include vascular endothelial growth factor (VEGF), platelet derived growth factor (PDGF), fibroblast growth factor, and angiopoietin/Tie2 receptor complex. VEGF has emerged as an important therapeutic target in several solid tumors, including ovarian carcinoma. The monoclonal antibody bevacizumab binds VEGF, thus preventing activation of the VEGF receptor (VEGFR), leading to inhibition of tumor angiogenesis. Four clinical trials including first-line trials of the International Collaborative Ovarian Neoplasm Group trial (ICON7), the Gynecologic Oncology Group trial (GOG218), and the trials in recurrent cases of Ovarian Cancer Study Comparing Efficacy and Safety of Chemotherapy and Anti-Angiogenic Therapy in Platinum-Sensitive Recurrent Disease (OCEANS) and Avastin Use in Platinum-Resistant Epithelial Ovarian Cancer (AURELIA) have shown that the tumor vasculature is a valid target. ICON7 and GOG218 showed improvements in PFS, and in the case of ICON7, increased overall survival in a predefined group of patients at high risk of disease progression. OCEANS and AURELIA also showed improvements in PFS, but response rates in specific histological subgroups were not examined.

Mabuchi et al29 examined the role of VEGF as a therapeutic target in CCC. Immunohistochemical analysis using tissue microarrays of 98 primary ovarian cancers revealed that VEGF was strongly expressed both in early stage and advanced stage CCC. In early stage CCCs, patients who had tumors with high levels of VEGF had significantly higher survival than those with low levels of VEGF. Furthermore, in vitro experiments revealed that VEGF expression was significantly higher in cisplatin-refractory human CCC cells compared with the respective parental cells. In vivo treatment with bevacizumab markedly inhibited the growth of both parental CCC cell-derived and cisplatin-refractory CCC cell-derived tumors. VEGF expression has been shown to be upregulated by factors such as insulin-like growth factor-1 and interleukin (IL)-6.30 Anglesio et al31 found specific overexpression of the IL6-STAT3-HIF (IL-6-signal transducer and activator of transcription 3-hypoxia induced factor) pathway in CCC tumors compared with high-grade serous cancers. The authors also reported sustained clinical and functional imaging responses in two CCC patients with chemotherapy-resistant disease who were treated with sunitinib, a potent inhibitor of several tyrosine kinases including VEGFR, platelet-derived growth factor receptor, and c-Kit, which has significant activity in renal clear cell cancer.32 A study by Sany et al33 also confirmed the use of sunitinib in CCC. They demonstrated that clear cell tumors are exquisitely sensitive to sunitinib compared with serous tumors. There are two international collaborative studies to evaluate efficacy of sunitinib for the treatment of persistent or recurrent CCC.34,35 A case report of chemoresistant CCC confirmed the use of combination therapy with sorafenib, a multikinase inhibitor that targets mitogen-activated protein kinase or...
the Ras/Raf/ERK pathway and also inhibits other kinases (VEGFR, platelet-derived growth factor receptor).\textsuperscript{37}

**Targeting the Met protooncogene pathway**

Met protooncogene (also called hepatocyte growth factor receptor or MET) encodes MET kinase, which is composed of three functional domains including the ligand-binding domain, regulatory juxtamembrane domain, and the receptor tyrosine kinase domain. When its ligand, hepatocyte growth factor, binds, the Ras-mitogen-activated protein kinase pathway is activated as well as the phosphatidylinositol 3-kinase-AKT-mTOR signaling pathway and several other pathways.\textsuperscript{38,39} In tumor cells, MET can be activated in a ligand-independent manner through amplification and overexpression of the MET gene. MET amplification was identified in 4\%–10\% of gastric cancers, esophageal cancers, lung cancers, and colorectal cancers.\textsuperscript{40–43} MET overexpression and gene amplification were commonly detected in CCC with their frequencies at 22\% and 24\%, respectively. Such alterations were extremely rare in the other histological subtypes of ovarian carcinoma (ie, serous, endometrioid, and mucinous adenocarcinomas). MET overexpression was associated with a worse prognosis in the CCC patients.\textsuperscript{44} Furthermore, in the case with synchronous endometriosis and with adjacent CCC components, the overall incidence of MET overexpression gradually increased from the precursors of nonatypical form (0\%), through those of atypical form (67\%) and the relatively differentiated carcinoma components (92\%), to the poorly differentiated carcinoma components (100\%). The results indicated that MET alterations occur as an early event in carcinogenesis of the MET amplification-positive CCC, and these alterations might drive the development and progression in a subset of CCC.\textsuperscript{45} Furthermore, Yamashita et al\textsuperscript{46} also revealed MET gene amplification in four out of 13 CCC primary tumors and two out of eight CCC cell lines using an array-based comparative genomic hybridization analysis. A total of 37\% demonstrated MET gene amplifications (more than four copies) were confirmed in 73 CCC cases using real-time quantitative polymerase chain reaction. In the meantime, amplification of the AKT2 gene was also observed in five out of 21 samples by array-based comparative genomic hybridization analysis. MET knockdown in CCC cell lines with MET amplification showed both increased apoptosis and senescence in vitro, which suggested that MET-amplified CCC cells primarily depend on activation of the MET/P13K/AKT pathway for cell proliferation and survival. Targeted therapy inhibiting the MET/P13K/AKT pathway may be promising for CCC treatment.

**HER2 as a therapeutic target**

Tan et al\textsuperscript{19} reported that HER2 gene amplification and protein overexpression were observed in 14\% of CCCs using hierarchical cluster analysis. Using an immunohistochemical method, Fujimura et al reported that HER2 was frequently overexpressed in 42.9\% of CCC patients compared with 20.8\% of ovarian serous adenocarcinoma, 23.1\% of ovarian endometrioid adenocarcinoma, and 30.0\% of mucinous adenocarcinoma specimens. Trastuzumab, a humanized recombinant monoclonal antibody against HER2, significantly and dose-dependently reduced the growth of CCC cell lines in vitro. Trastuzumab also dose-dependently inhibited the growth of xenografted RMG-1 (CCC cell line) tumor. The survival period of trastuzumab-treated mice was longer than that of the control group.\textsuperscript{47} Thus, trastuzumab appears to be a candidate treatment modality for HER2 overexpressing CCC. A clinical trial was conducted to use single-agent trastuzumab to treat HER2-overexpression ovarian and primary peritoneal carcinoma (documented by an immunohistochemical method).\textsuperscript{48} An overall response rate of only 7\% was observed. However, patient selection (41 patients of which seven were CCCs) was based only on HER2 immunohistochemical expression levels, without assessment of HER2 copy number status. Future studies are warranted to assess the predictive value of HER2 amplification and overexpression in CCCs.

The distinct molecular features of CCC and serous ovarian cancer serve to emphasize the need to develop subtype-specific therapeutic approaches in the management of EOC. Patients with CCC have poor prognosis and short survival due to lack of effective therapy. Despite displaying histologically uniform features, CCCs do not constitute a single entity and may be classified into distinct molecular genetic subtypes that also appear to be associated with clinical outcome. Future research is expected to help better understand the pathogenesis of CCC and identify new compounds to block important signaling pathways.

**Disclosure**

The authors report no conflicts of interest in this work.

**References**


