Clear cell carcinoma (CCC) accounts for 4% to 12% of epithelial ovarian cancer in Western countries and, for some unknown reasons, it comprises more than 20% of such cancers in Japan. CCC shows unique clinical features such as a high incidence of stage I disease, a large pelvic mass, an increased incidence of vascular thromboembolic complications, and hypercalcemia. It is frequently associated with endometriosis. Compared to serous adenocarcinoma (SAC), CCC is relatively resistant to conventional platinum, or taxane-based chemotherapy which is associated with its poor prognosis. However, the mechanisms underlying CCC’s resistance to chemotherapy have not been understood. Although several mechanisms involved in drug resistance exist in CCC, including decreased drug accumulation, increased drug detoxification, and an increased DNA repair activity; however, no particular chemoresistance system has been identified. On the other hand, an in vitro study revealed that low cell proliferation may cause the insensitivity of CCC to cisplatin. The Ki-67 labeling index in CCC tumors was significantly lower than SAC. The Ki-67 labeling index for responders was significantly higher than that for non-responders in both tumor types. A multivariable analysis revealed that Ki-67 labeling index and residual tumor size were independent prognostic factors in CCC. Therefore, lower proliferation of the tumor cells may contribute to their resistance to chemotherapy. However, further investigation into the molecular biology and genetics of CCC is warranted. This review discusses the current state of knowledge of the chemoresistance mechanism in CCC and novel treatment strategies for CCC. (Cancer Sci 2008; 99: 653–658)

Clinicopathological features

CCC tumors show distinctly different clinical behavior from that of other epithelial ovarian cancers. When compared with serous adenocarcinoma (SAC), CCC is often associated with a large pelvic mass and rarely occur bilaterally. An increased incidence of vascular thromboembolic complications and hypercalcemia is also seen in patients with CCC. Histopathologically, the tumors were diagnosed to be CCC if 90% or more of all histological specimens show large cuboidal, hobnailled or flattened epithelial cells containing abundant clear cytoplasm lining tubules and cysts, and growing in solid/tubular or glandular patterns. CCC is often associated with endometriosis (22–70%), whereas the hobnail cells bear a very strong morphological resemblance to endometrial Arias-Stella cells. It has been reported that endometriosis frequently shows a sequential change to epithelial ovarian cancer, including CCC; therefore, atypical endometriosis is considered to be a precancerous change.

Several reports have shown that CCC frequently appears during an early stage, especially stage Ic. The percentage of patients with stage I was higher in patients with CCC than in patients with SAC, and among those patients stage Ic was observed at 32.2% to 38.8% in CCC and at 7.1% to 9.4% in SAC. On the contrary, the patients with advanced stage (III and IV) were lower in CCC than in SAC (Table 1). These findings suggest that the proliferation and progression of CCC are different from those of SAC.

Several authors have discussed the prognosis of patients with CCC in comparison to that of patients with SAC. In early stage (I and II), the prognosis of patients with CCC was not different significantly to that of SAC, while the recurrence rate of CCC was higher than that of SAC. However, a high recurrence rate in patients with stage Ic CCC and lower survival rate of these patients was seen in comparison to those of SAC. It has been thought that CCC in its advanced stages (III and IV) showed lower survival rate due to resistance to platinum- or taxane-based chemotherapy relative to SAC. Although neither the estimated 3-year nor 5-year survival rates in patients with no gross tumor were significantly different between CCC and SAC, both survival rates in the patients with <2 cm residual disease and those with ≥2 cm residual disease were significantly lower in CCC than in SAC. In addition, the overall response rate to platinum-based chemotherapy for CCC was significantly lower than that for SAC (11.1% vs 72.5%). Another author demonstrated that platinum-based chemotherapy did not appear to improve the survival of patients with CCC, in comparison to the preferred treatment for CCC is a complete resection of the tumor, but this is difficult to accomplish when the disease is advanced. This review discusses the mechanisms of chemoresistance in CCC and some potential novel treatment strategies for advanced CCC.
Mechanisms of drug resistance

It is generally accepted that CCC’s insensitivity to conventional platinum-based chemotherapy tends to be followed by a poor prognosis. Drug resistance is an important factor in the poor prognosis of patients with CCC. Several mechanisms involved in drug resistance have been proposed, including decreased drug accumulation, increased drug detoxification, and increased DNA repair activity (Fig. 1).20–25

Drug efflux. Adenosine 5′-triphosphate (ATP)-binding cassette (ABC) transporters, such as ABCC1 (also known as P-glycoprotein), ABCC1 (also known as multidrug resistance associated protein-1) and lung resistance protein, are known to affect intracellular drug concentrations and are important multidrug resistance factors.26 ABCB1 and ABC1 function as an ATP-dependent efflux pump for cytotoxic drugs, such as paclitaxel (PTX) and platinum agents.22,23 Indeed, the expression of ABCB1 in CCC cell lines was associated with cisplatin (CDDP), etoposide (VP-16), and mitomycin-C (MMC) resistance.28

An immunohistochemical study of ABCB1 and ABCC1 expression in 41 patients with CCC and 90 patients with SAC who had measurable disease after initial surgery was conducted.7 All patients underwent cytoreductive surgery followed by platinum-based chemotherapy and response rate to chemotherapy was 14.6% for CCC and 72.2% for SAC. The overall positive rates of ABCB1 and ABCC1 were 41.5% (17/41) and 19.5% (8/41) for CCC, and 46.7% (42/90) and 27.8% (25/90) for SAC, respectively. The expression of ABCB1 and ABCC1 did not differ between CCC and SAC. In addition, no significant differences were observed in the expression of ABCB1 and ABCC1 between responders and non-responders in both tumors. Therefore, these results suggest that these multidrug resistance proteins are not contributing factors to chemoresistance in CCC.

Ohishi et al.29 examined the mRNA expression of ABC superfamily members, ABCC3, ABC1, ABC2, and ABC3, in tumors of CCC and SAC and found that only ABCC3 gene expression in CCC was significantly higher than that in SAC. ABC3 is known to transport various substrates, including anticancer drugs, and contributes to drug resistance against platinum agents.26 Therefore, they concluded that increased expression of ABCC3 may be associated with, at least in part, the chemoresistant phenotype of CCC.

Tsuda et al.30 found a significant increased in DNA and mRNA copy number of 12 genes in CCC tumors in comparison to those in SAC by genomic and gene expression array analyzes and focused on the ABC transporter F2 (ABCF2) gene on 7q35–36. The validation studies using real-time quantitative polymerase chain reaction (PCR) and immunohistochemical staining showed significantly higher ABCF2 DNA and mRNA copy number and protein levels in CCC. Furthermore, ABCF2 cytoplasmic staining was significantly higher in non-responders to platinum-based chemotherapy than that in the responders for patients with CCC. These data suggests that ABCF2 protein could be a useful prognostic marker for CCC. However, the function of ABCF2 is unknown.

Drug inactivation. Several drug detoxification systems can also diminish the amount of intracellular drug activity. Cellular detoxification via the glutathione system is known to be involved in the metabolism of various cytotoxic agents, including the platinum agents, VP-16, and MMC.22,23,27,31 Indeed, the glutathione concentrations of CCC cell lines was significantly increased after exposure to CDDP or MMC in comparison to before treatment.28

A gene expression study showed glutathione peroxidase 3 (GPx3), glutaredoxin (GLRX), and superoxide dismutase (SOD2) were highly expressed in CCC tumors and high levels of these and perhaps other antioxidant proteins may render these tumors more resistant to chemotherapy.32

DNA repair. Nucleotide excision repair is a multienzyme DNA repair pathway in eukaryotes that has been implicated in drug resistance in human tumor cells.33 Reed et al.34 examined the mRNA expression levels of two key genes, excision repair
kinase and phosphatidylinositol 3-kinases and can activate both the mitogen-activated protein (EGFR) and v-erb-b2 erythroblastic leukemia viral oncogene (HER2), which are expressed in CCC in 50 to 70% of cases of advanced serous adenocarcinoma (48,49). In ovarian cancer, HER2 protein is overexpressed as a consequence of HER2 gene amplification in 20 to 25% of cases and is a marker of poor prognosis (43,46).

Cell-cycle control. Cell proliferation depends on the ability of the cell to successfully pass through the G1, S, G2, and M phases of the cell cycle (Fig. 2). Such cell progression is controlled by cyclin-dependent kinases (CDKs), which themselves are regulated by cyclin binding, phosphorylation, and CDK inhibitors. The quiescence state is called G0 phase. There are two known CDK inhibitor families, p21<sup>WAF1</sup>/p27<sup>KIP1</sup> and p27<sup>KIP1</sup>, which inhibit CDK2 and p16<sup>INK4a</sup> inhibits CDK4 and CDK6.

DNA mismatch repair systems (MMR), which correct errors occurring during DNA replication, also play a critical role in the sensitivity of DNA damaging agents. In experimental systems, MMR deficient cells are highly tolerant to the methylating chemotherapeutic drugs streptozocin and temozolomide and, to a lesser extent, to CDDP and doxorubicin (35). Loss of MMR may be caused either by a germline mutation of two major MMR genes, hMLH1 or hMSH2, or by somatic MMR gene inactivation through epigenetic silencing via methylation of the hMLH1 promoter. Cai et al. (36) reported that high expression of hMLH1 and hMSH2 protein are involved in the development of a subset of CCC, and there is a strong correlation between alterations in the expression of hMLH1 and hMSH2 and the presence of MSI in CCC tumors.

Growth-factor signaling. Epidermal growth-factor receptor (EGFR) and v-erb-b2 erythroblastic leukemia viral oncogene homolog 2 (ERBB2; HER2) are cell-surface-receptor tyrosine kinases and can activate both the mitogen-activated protein kinase and phosphatidylinositol 3-kinase (PI3K) – Akt signaling pathways (37). Activating these pathways leads to phosphorylated Bcl-2 antagonist of cell death (BAD) and B-cell leukemia/lymphoma (Bcl)-2, thereby inhibiting chemotherapy-induced apoptosis (38). Amplified PI3K and activated Akt have been found in 30–40% of ovarian carcinomas and they could thus represent a mechanism of drug resistance in patients with ovarian cancer (39).

An overexpression of EGFR has been associated with chemoresistance and poor prognosis in ovarian cancer. An immunohistochemical study showed that EGFR was detected in 61% of CCC tumors (40). Molecular analyzes of various types of ovarian tumors showed HER2 to be overexpressed in CCC relative to other major histological types of epithelial ovarian cancer (41). The HER2 proto-oncogene encodes a 185-kDa transmembrane growth factor receptor tyrosine kinase (42). Several types of tumors that overexpress HER2 have shown poor sensitivity to conventional anticancer agents and poor prognosis (43,44). In ovarian cancer, HER2 protein is overexpressed as a consequence of HER2 gene amplification in 20 to 25% of cases and is a marker of poor prognosis (43,46).

Cytotoxic drugs are primarily effective against proliferating cells; therefore, quiescent cells show a degree of drug resistance relative to cycling cells (35). Dimanche-Boitrel et al. (32) reported that intracellular drug accumulation decreases in resting cells. The proliferation activity and CDDP sensitivity of 11 CCC and 5 SAC cell lines were examined, and this showed that the doubling time for CCC cells was significantly longer than that for SAC cells (61.4 vs. 29.8 h). (53). There was a significant reverse correlation observed between the S-phase fraction and the response to CDDP (Fig. 3). These findings may relate to the high incidence of stage I patients with CCC (Table 1), and also suggests that the resistance of CCC to CDDP may be caused by low cell proliferation. In addition, Ki-67, a nuclear antigen, which is expressed in all states of the cell cycle except in resting cells in G0, has a significantly lower labeling index in CCC than in SAC (Fig. 4). (7) Furthermore, a significantly higher Ki-67 labeling index is observed in responders than in non-responders in both CCC and SAC tumors. A multivariable analysis revealed that the Ki-67 labeling index and residual tumor size were independent prognostic factors. Other authors also reported that the immunohistochemical staining of CCC revealed a low expression of Ki-67, p53, and cyclin E, which are other histological subtypes (54). These results suggest that CCC had low tumor proliferation activity and that this low proliferation activity in CCC could be associated with chemoresistance.

What is an effective agent against clear cell carcinoma of the ovary?

The clinicopathological features of CCC are provocative and suggest a new strategy for chemotherapy in CCC should be treated by a combination of cytotoxic agents.
that codes for two major proteins by alternative splicing of two exons. The two early viral proteins (243 and 289 amino acids in length) can activate or repress transcription of several viral or cellular genes and thereby regulate the cell cycle.\(^{53}\) E1A acts as a tumor suppressor by down-regulating \(HER2\) transcription.\(^{64}\) HER2 protein was detected at various levels by Western blotting in all 10 cell lines used in this study. Trastuzumab did not inhibit the proliferation in any of the four CCC cell lines tested. However, cotreatment with E1A reduced colony formation in all 10 CCC cell lines regardless of the HER2 expression level. Infection of CCC cells with an adenoviral vector encoding E1A led to significant suppression of proliferation and enhancement of cell death. This effect required the stabilization of p53 protein and was associated with the up-regulation of Bax and the cleavage of caspase-9. Treatment with E1A prolonged survival in a CCC xenograft model.\(^{65}\) Therefore, E1A gene therapy is worth exploring as a treatment modality for women with ovarian CCC.

Mitogen-activated protein kinase and phosphatidylinositol 3’-kinase-Akt signaling pathways. Other signaling pathways, such as the mitogen-activated protein kinase (MEK)-extracellular signal-regulated kinase kinase (ERK) signaling cascade and a PI3K-Akt pathway, which are proliferation and cell-survival pathways, are also potential targets for modulating drug resistance. Combining PTX with either MEK inhibitor (PD98059 [PD]) or PI3K inhibitor (LY294002 [LY]) had an additive effect on cell-growth inhibition in five ovarian cancer cell lines.\(^{65}\) In contrast, a synergistic effect was observed when PTX was combined with both PD and LY. Furthermore, treating nude mice with PTX and PD and LY prolonged survival in an ovarian cancer xenograft model, this indicating that the inhibition of both the MEK and PI3K signaling pathways can help to overcome the drug resistance of CCC.

Vascular endothelial growth factor. Neovascularization is essential for tumor growth and it is induced by several growth factors, including vascular endothelial growth factor (VEGF).\(^{66}\) Overexpression of VEGF have been shown to be a poor prognostic factor in ovarian cancer, and VEGF is thought to be one of the most promising targets of therapy.\(^{67}\) Three antiangiogenic drugs, bevacizumab, sunitinib malate, and sorafenib, have been approved by the United States Food and Drug Administration for treatment patients with specific types of cancer. Bevacizumab is a humanized monoclonal antibody that selectively binds and neutralizes all isoforms of VEGF-A. In phase II studies, bevacizumab showed improved survival of patients with ovarian cancer.\(^{68}\) Phase III trials of bevacizumab in combination with first-line chemotherapy in ovarian cancer are ongoing. Both sunitinib and sorafenib are orally administered, small molecule inhibitors of multiple receptor tyrosine kinases, including VEGF receptors and platelet derived growth factor receptor, implicated in tumor growth, angiogenesis, and metastatic progression.\(^{69}\)

A wide range of therapeutic strategies based on molecular biology should be evaluated to improve survival in patients with CCC. These include developing of novel cytotoxic drugs, small molecule inhibitors, monoclonal antibodies, and gene therapy strategies.
molecular biology and genetics of CCC is warranted. Several molecular targeted agents have recently been developed and may modulate resistance to chemotherapy in CCC. Among those targets, growth-factor signaling pathways, such as HER2, EGFR, and VEGF, enhance tumor growth, and inhibition of these pathways is promising to improve survival in patients with CCC.

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