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GYNECOLOGICAL CANCER (H KATABUCHI AND H TASHIRO, SECTION EDITORS)

Ovarian Cancer and Drug Resistance

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Abstract Epithelial ovarian cancer (EOC) is the most difficult cancer to cure in gynecological malignancy. Over 70 % of patients respond to chemotherapy initially, but the majority will relapse. Despite the emergence of a variety of cytotoxic anti-cancer agents and targeted therapy such as bevacizumab, control over the progression of EOC remains inadequate. Chemoresistance limits the survival of advanced cancer patients receiving chemotherapy. Many drugs have been developed so far; however, the improvement in the prognosis of ovarian cancer patients is insufficient. Recent evidence suggests that epigenetic change of DNA and multiple cellular pathways contribute to acquired drug resistance to chemotherapy. Identification of the molecular mechanisms associated with chemoresistance is a crucial step toward improving patient survival. A new treatment paradigm for overcoming the resistance of ovarian carcinoma is urgently needed. This review describes the recent advances in the molecular mechanisms of chemoresistance in EOC and strategies for overcoming them.

Keywords Epithelial ovarian cancer · Chemoresistance · Stem cells · Tumor heterogeneity · Epithelial mesenchymal transition · Microenvironment · Drug resistance gene · Transcription · Epigenetics · DNA methylation · Histone modification · microRNA · Cell signaling

Introduction

Among gynecological malignancies, epithelial ovarian cancer (EOC) is the most difficult to cure. Maximum debulking surgery followed by chemotherapy consisting of taxane and platinum is the standard treatment for advanced EOC [1]. Over 70 % of patients respond to chemotherapy initially, but the majority will relapse. Recurrent tumors can acquire resistance to chemotherapy during treatment, and chemotherapy becomes ineffective in the end (Fig. 1). Despite the emergence of a variety of cytotoxic anti-cancer agents and targeted therapy such as bevacizumab, the progression of EOC has been difficult to control [2, 3].

Drug resistance, namely chemoresistance, is a major cause of therapeutic failure. Many mechanisms of drug resistance have been proposed. More than 20 years ago, the expression of multidrug resistance genes in cancer cells was considered a main cause of chemoresistance. Stem cell theory, tumor heterogeneity, and epithelial-mesenchymal transition have recently been proposed to explain chemoresistance. The importance of the tumor microenvironment has also been suggested. Recent advances in molecular genetic techniques have revealed that genetic and epigenetic factors are involved in the chemoresistance of EOC cells. However, overcoming anticancer drug resistance of EOC has not been achieved because the mechanism is complicated.

Identification of the molecular mechanisms associated with chemoresistance is a crucial step towards improving patient survival. In this review, we will describe recent advances in the understanding of the molecular mechanisms of chemoresistance in EOC and strategies for overcoming them.

Tumor and Microenvironment

Stem Cells

Cancer stem cells (CSCs) are a subgroup of tumor cells in many malignant tumors that possess characteristics of normal stem cells, with the ability to self-renew and differentiate [8]. CSCs are considered to be the cause of metastasis, recurrence and resistance. A CSC-based model of drug-resistant cells with chemotherapy has been put forward to explain chemoresistance relapse in EOC [4]. In other words, CSCs are the putative mediators of chemoresistance [5, 6]. It is thought that CSCs are able to survive conventional

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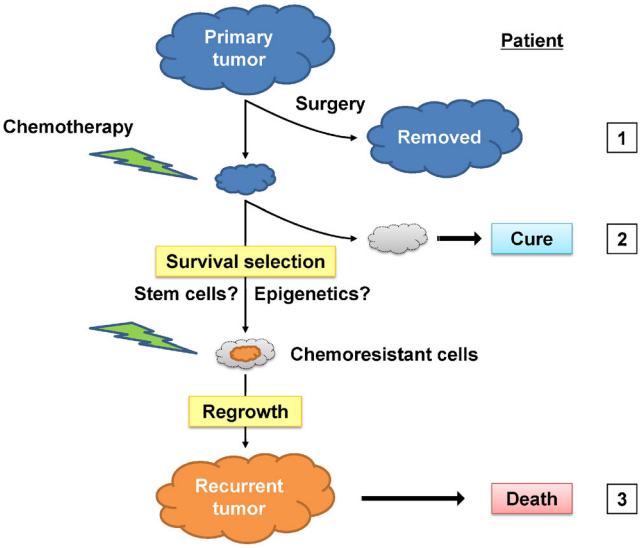

Fig. 1 Overview of acquired drug resistance Chemoresistance is acquired during treatment of epithelial ovarian cancer as follows. 1. The primary tumor is incompletely removed at the cellular level in debulking surgery. 2. If complete eradication of tumor cells is achieved by chemotherapy, the patient is cured. 3. Cancer cells that have acquired stemness, with tumor re-growth after developing chemotherapy resistance, ultimately result in death of the host

chemotherapeutic treatment [7]. It has been shown that primary treatment with chemotherapeutic agents results in increased drug-resistant CSCs, which leads to recurrence [8]. For example, the percentage of side population cells is increased in the ascites of patients with an initial recurrence after platinum-based chemotherapy compared with that of chemo-naive patients [9]. Despite being a critical issue, there is no specific marker for ovarian CSCs. Some proteins that have been identified in other malignancies, such as CD44, CD133, CD117, ALDH1A1 and EpCAM (CD326), are used as markers of "stemness" for EOC [10–15]. Therefore, whether CSCs are present remains controversial. However, therapeutic strategies that target stem cell-like properties of tumors are meaningful for overcoming the resistance of EOC.

Tumor Heterogeneity

Recent evidence showed that tumor heterogeneity leads to chemoresistance. The presence of multiple tumor clones within a patient provides an opportunity for survival selection during chemotherapy. The problem of gene mutation needs to be solved for tumor eradication, as tumor heterogeneity results in major difficulties in implementing targeted therapy in EOC. The clonal evolution model has been proposed to explain tumor heterogeneity. Surviving cells that were naturally selected by treatment are responsible for tumor progression. The proliferating cells that have acquired additional mutations will have new characteristics [16]. Therefore, in the clonal evolution model, any cancer cell has the potential to become resistant to chemotherapy.

Khaique et al. [17] analyzed the genetic alterations of primary EOC tumors and suggested that monoclonal proliferating cells are selected from a genetically distinct mixed population [18]. Another study of recurrent serous carcinoma confirmed that chemotherapy-resistant clones arise from minor clones that are present in primary tumors. Castellarin et al. [19] investigated the change of p53 mutations of cells that were collected from the ascites of high-grade serous carcinoma (HGSC) patients receiving chemotherapy, using whole exome sequencing. The vast majority of somatic variants found in recurrent tumors were present in primary tumors. These results demonstrated that cancer cells could be resistant to the effects of chemotherapy. Elucidation of the molecular events that control tumor heterogeneity may lead to the development of strategies for overcoming chemoresistance.

Epithelial-Mesenchymal Transition

Epithelial-mesenchymal transition (EMT) converts epithelial cancer cells into mesenchymal cancer cells with migratory capability and the capacity to invade and metastasize. EMT is characterized by the loss of epithelial polarity and differentiation markers such as E-cadherin and β-catenin, and the gain of mesenchymal markers such as N-cadherin and vimentin [20, 21]. Some signaling pathways involved in EMT include Wnt/β-catenin, TGF-β, Notch and Hedgehog [22]. Among them, Wnt/β-catenin pathway is one of the major signaling pathways thought to be involved in EMT. Wnt/β-catenin signaling plays an important role in the transcription of multidrug resistance genes such as ABCB1/MDR-1 [23]. Furthermore, this pathway is an important pathway in cell survival and has been implicated in the mechanism of chemoresistance of ovarian CSCs [24]. Thus, Wnt/β-catenin pathway may be a potential target for chemosensitization in EOC.

Recently, chemoresistance has been reported to be associated with EMT in EOC cells [25–27]. Clinical studies have implicated a link between EMT-related gene expression and relapse after platinum-based treatment [28], as well as a link between EMT and innate resistance to platinum-based chemotherapy [29] in EOC. Through platinum-based treatment, EOC cells acquire not only resistance to platinum, but also to mesenchymal phenotypes [30–32]. A latest study confirmed that metastasizing EOC cells taken from patients have a different molecular structure from primary tumor cells and display genetic signatures consistent with EMT [33]. Miow et al. [34] assessed the cellular responses to cisplatin using expression microarray analyses of EOC cell lines. Their results show that epithelial-like and mesenchymal-like EOC cells exhibit distinct responses following cisplatin administration. This distinction may suggest a need for differential therapeutic

regimens in the treatment of EOCs based on the EMT status of cancer cells.

Microenvironment

Recently, substantial evidence supports the key role of a heterogeneous tumor microenvironment in carcinogenesis and cancer progression, including various stromal compartments such as fibroblasts, endothelial cells, leukocytes, pericytes and extracellular matrix [35, 36]. There is also growing evidence that highlights the importance of tumor microenvironment-mediated chemoresistance mechanisms in EOC [37]. Accumulating evidence of tumor microenvironment-mediated chemoresistance in EOC suggests that targeting relevant molecules and signaling pathways is critical for overcoming chemoresistance in EOC [38]. Direct cell-to-cell contact, trogocytosis and efflux pumps of drugs are known mechanisms of chemoresistance that involve cancer-associated mesenchymal stem cells (CA-MSCs). In addition, a recent report demonstrated that CA-MSCs protected an EOC cell line from carboplatin-induced apoptosis through inhibiting the inactivation of X-linked inhibitor of apoptosis protein (XIAP) [39]. Lim et al. [40] found that tumor-associated macrophages (TAMs) in the tumor microenvironment produced high levels of VEGF-C and transduced signals to cancer cells through VEGF-C interactions with VEGFR-3, a primary receptor of VEGF-C on tumor cells. Upregulation of the ATP-binding cassette genes by insulin-like growth factor (IGF)-I via the PI3K, MEK and JAK2/STAT3 signaling pathways was another mechanism of chemoresistance. Benabbou et al. [41] demonstrated that the OVCAR-3 cell line showed significant drug resistance to paclitaxel or carboplatin when co-cultured with Hospicells. Triulzi et al. [42] showed that the overexpression of maspin, a member of the serpin protease inhibitor family, led to doxorubicin resistance through the maspin-induced, collagen-enriched microenvironment.

Genetic Factors

Drug Resistance Genes

Adenosine 5'-triphosphate (ATP)-binding cassette (ABC) and solute carrier (SLC) transporters are known to lower intracellular drug concentrations and are important multidrug resistance factors. The most important ABC transporters in the context of EOC drug resistance are ABCB1 (also known as P glycoprotein [P-gp]), ABCC2 (multidrug resistance-associated protein-2 [MRP2]), and breast cancer resistance protein (BCRP), which are encoded by the *ABCB1* (multidrug resistance protein 1 [MDR1]), *ABCC2* and *ABCG2* genes, respectively [43, 44]. P-gp is able to actively expel nearly 20 cytostatics, including paclitaxel and doxorubicin. A recent in vitro study confirmed that the main mechanisms of drug resistance were due to P-gp expression in the doxorubicin-resistant, vincristine-resistant and paclitaxel-resistant cell lines, and BCRP expression in the topotecan-resistant cell line [45]. Clinically, Naniwa et al. [46] demonstrated that the expression of the *MDR-1* gene in EOC tumors was significantly higher in nonresponders of chemotherapy, which consisted of paclitaxel and carboplatin.

Currently, microarray-based multigene assays are available. Comprehensive analyses of genetic alterations of drug resistance genes in ovarian cancer cells are being carried out, but the detailed mechanism of drug resistance remains unknown [47–49]. These approaches still require extensive validation before they can be considered as putative biomarkers [50]. Furthermore, inhibitors of drug transporters have been evaluated. The results of most of the trials of these agents were disappointing, as the inhibitors lacked specificity and exhibited high systemic toxicity [51].

Transcriptions

Multiple studies have reported the involvement of transcription factors in gynecological cancer progression. The tumor suppressor gene TP53 encodes a DNA-binding transcription factor that induces cell growth arrest, senescence and cell death by apoptosis upon cellular stress [52]. Once activated by DNA damage detection or UV radiation, p53 induces the expression of many well-known apoptosis inducers and other tumor suppressors such as p21, BAX, PTEN and TSP-1. Mutation of TP53, the gene encoding p53, is very common in EOC [53]. At least 50 % of all ovarian tumors have p53 mutations. Serous carcinoma is classified as high-grade or low-grade (HGSC or LGSC). HGCS accounts for 67 % of all EOCs and often leads to chemoresistance. The majority of HGCS have an inactive p53 because of genetic mutation [54].

P53 protein aggregation is associated with p53 inactivation and platinum resistance. A recent study showed that overexpression p14ARF, a p53-positive regulator, inhibited MDM2-mediated p53 degradation. The authors of the study also demonstrated that inhibition of p14ARF could suppress p53 aggregation and sensitize cancer cells to platinum treatment in vitro and in vivo. Furthermore, they discovered that the aggregated p53 might function by interacting with proteins that are critical for cancer cell survival and tumor progression by using two-dimensional gel electrophoresis and mass spectrometry. These findings suggested that p53 aggregation is a new marker for chemoresistance of HGCS. In addition, this indicated that inhibition of p53 aggregation can reactivate the pro-apoptotic function of p53 [55•]. Therefore, p53

aggregation is a promising therapeutic target for overcoming resistance to chemotherapy.

TWIST1 has been shown to be important in the regulation of programmed cell death and inflammation [56, 57]. Recently, it was found that TWIST1 is involved in the process of tumor metastasis via modulation of epithelial-mesenchymal transition (EMT). Some studies have reported that TWIST1 is overexpressed in EOC [58, 59]. Emerging evidence suggests that TWIST1 plays an important role in the chemoresistance of cancer cells. Kajiyama et al. [60] found that TWIST1 expression predicts poor clinical outcomes in patients with clear cell carcinoma (CCC) of the ovary. They also found that positive TWIST1 was an independent prognostic factor for survival of EOC patients [61].

TWIST2 is a novel zinc finger transcription factor that has been shown to be an important inducer of EMT. A recent study demonstrated that TWIST2 also plays a crucial role in the chemoresistance of EOC. Downregulation of TWIST2 expression facilitated apoptosis and previously chemoresistant EOC regained sensitivity through the AKT/GSK-3β pathway [62].

Many other transcription factors are being studied as potential targets in general cancer treatment, such as STATs, NF-κB and Notch1 [63–65]. However, clinical treatments that target transcription factors have not been realized.

Epigenetic Factors

Epigenetics is a phenotypical change in gene expression without any alteration of the DNA sequence. The epigenetic change in tumors generates the diverse gene expression involved in drug resistance. This allows cancer cells to acquire chemoresistance. Furthermore, this phenomenon may complicate the selection of chemotherapy that is based on mutation biomarkers.

DNA Methylation

DNA methylation is the most frequent epigenetic phenomenon. In cancer cells, DNA hypermethylation is associated with gene silencing, while DNA hypomethylation is associated with gene expression. DNA methyltransferase (DNMT), an enzyme that catalyzes the transfer of a methyl group to DNA, is essential for this process [66]. DNA methylation has attracted attention as a target for overcoming chemoresistance. The efficacy of some demethylating agents in treating EOC has been examined in clinical trials [67, 68]. Human mutL homolog 1 (hMLH1) is a promising target. Hypermethylation of hMLH1 inhibits the apoptotic response to platinum chemotherapy. This is considered a major molecular cause of acquired resistance to platinum chemotherapy in EOC [69]. In addition, the presence of methylated hMLH1 DNA in plasma after chemotherapy predicts poor survival for EOC patients [70]. In EOC cells, histone deacetylation at the RGS10-1 promoter correlates with suppression of RGS10 and chemoresistance [71]. This data suggest the possibility of using histone biomarkers to determine the appropriate selection of therapy in cases of EOC chemoresistance [71, 72]. Recent preclinical research on resensitizing platinum-resistant cells using a novel DNMT inhibitor SGI-110 is noteworthy. This study demonstrated that $ALDH^+$ EOC cells possessing stem cell characteristics are enriched in platinum-resistant EOC cell lines, human tumors and residual xenografts after platinum therapy. SGI-110 inhibited $ALDH^+$ cell viability, sphere formation and tumor-initiating capacity; repressed stem cell-associated gene transcription; and resensitized platinum-resistant EOC cells to platinum [73•].

Histone Modification

Histone modifications also play important roles in epigenetic regulation. Histones are dynamic proteins that can become methylated or acetylated at specific amino acid residues, which correlate with active or repressive transcription [74, 75]. By tightly winding and condensing chromatin or loosening up the structure of chromatin, transcription factors and other proteins are denied or permitted access to DNA for transcription. Histone deacetylases (HDAC) cause repression of gene expression by regulating the condensation of chromatin. Aberrant expression of HDACs in gynecological cancers is associated with chemoresistance. The use of HDAC inhibitors such as valproic acid, belinostat and vorinostat in combination therapy for overcoming chemoresistance has been proposed. The results of clinical trials of combination therapy with belinostat and carboplatin have been reported for platinum-resistant EOC [76, 77]. Clinical trials of EOC epigenetic therapeutics are ongoing.

MicroRNAs

MicroRNAs (miRNAs) are small, non-coding RNAs that negatively regulate gene expression at the post-transcriptional level. MiRNAs play an important role in carcinogenesis and cancer progression. Certain miRNAs have also recently emerged as important epigenetic modulators of autophagy in cancer cells [78]. Two major approaches in targeting transcription factors are post-transcriptional silencing by siRNAs or miRNAs, or blocking the binding of transcription factors to DNA. Multiple studies have focused on the roles of miRNAs in overcoming resistance to chemotherapy for EOC.

It has been reported that many miRNAs are expressed differentially between chemosensitive and chemoresistant ovarian cell lines such as miR-30c, miR-130a and miR-335 [79]. MiR-214 induced cell survival and cisplatin resistance through direct targeting of PTEN and inactivation of the AKT pathway [80]. Vecchione et al. [81] analyzed miR signatures associated with chemoresistance in 198 samples of serous ovarian cancer. They demonstrated that the presence miR-217, miR-484 and miR-617 could predict chemoresistance. MiR-199a-5p was shown to increase chemoresistance by simultaneously promoting autophagy and suppressing apoptosis. By downregulating Beclin-1 expression, miR-30a and miR-376b downregulate not only autophagy but also apoptosis, since the level of free antiapoptotic BCL-2 protein decreased in the cell. MiR-30a was found to be deregulated in stage I ovarian cancer patients, together with other miRNAs. In particular, it was downregulated in samples from patients with relapse [82, 83]. MiR-27a increases MDR1/P-glycoprotein expression in EOC cells by targeting HIPK2 [84]. Similarly, miR-451 and miR-21 facilitate MDR1/P-glycoprotein overexpression, leading to paclitaxel resistance in EOC cells [85, 86]. Let-7a is a potential biomarker for the selection of chemotherapy in EOC. Patients with low let-7 showed a good response to platinum–paclitaxel combination therapy [87]. The down-regulation of let-7i is associated with resistance of EOC cells to cisplatin [88]. Let-7 g downregulates the multiple drug resistance 1 (MDR1) gene, one of the major factors causing paclitaxel resistance in EOC [89]. Lower expression of miR-31 and higher expression of MET significantly correlated with PTX resistance and poor prognosis in ovarian cancer patients [90]. Epigenetic silencing of miR-199b-5p is associated with chemoresistance in EOC through the activation of JAG1/Notch1 signaling [91].

As noted above, many of the miRNAs are involved in chemoresistance. Further research is needed before miRNAs can be used in clinical treatment.

Related Signaling Pathways

Ubiquitin–Proteasome Pathway

The ubiquitin–proteasome pathway is responsible for maintaining cellular homeostasis by regulating the degradation of proteins. Disruption of this pathway can result in cell cycle arrest and apoptosis as a result of incompatible regulatory protein accumulation within the cell [92]. Cancer cells generally have higher levels of proteasome activity and are more sensitive to the proapoptotic effects of proteasome inhibition than normal cells [93]. Bortezomib is a reversible proteasome inhibitor that targets the chymotrypsin-like and caspase-like active sites of the proteasome complex [94]. By inhibiting the proteasome, bortezomib acts through several mechanisms to suppress tumor survival pathways and to arrest tumor growth, metastasis and angiogenesis. These mechanisms of action underlie the rationale for the combined use of bortezomib with other chemotherapeutic and targeted agents, some of which have been evaluated in EOC clinical trials [95–97]. A recent study demonstrated that proteasome inhibition affects microtubule stabilization in a manner similar to taxanes, and increases sensitivity to paclitaxel [98].

Toll-like Receptor 4 Signaling

Myeloid differentiation factor 88 (MyD88) is an adaptor protein that is required for Toll-like receptor 4 (TLR4) signaling. The activation of the TLR4/MyD88 signal pathway can induce the activation of the Akt survival pathway and enhance the expression of the antiapoptotic protein XIAP. Furthermore, knockout of Myd88 reduces MRP1, which is a pump involved in chemoresistance. For the above reasons, MyD88 is involved in resistance to chemotherapy. In particular, EOC cells expressing MyD88 induces resistance to cisplatin and paclitaxel [99, 100]. MyD88 is expected to be a new therapeutic target.

Conclusions

Chemoresistance limits the survival of advanced cancer patients receiving chemotherapy. Many drugs have been developed so far, however, the improvement of the prognosis of EOC patients is insufficient. Chemoresistance occurs as a result of a complex interplay of the various factors described above. We can only confirm the status of the tumor clinically. Thus, it is important to identify a simple clinical biomarker that indicates the mechanisms of chemotherapy resistance of a recurrent tumor. There is a need for detailed validation studies of tumor and plasma at the time of relapse and clinically acquired resistance, for optimizing tailored therapy. A new treatment paradigm for overcoming the resistance of ovarian carcinoma is urgently needed.

Compliance with Ethics Guidelines

Conflict of Interest Seiya Sato and Hiroaki Itamochi declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:
- Of importance

1. Sato S, Itamochi H. Neoadjuvant chemotherapy in advanced ovarian cancer: latest results and place in therapy. Ther Adv Med Oncol. 2014;6(6):293–304.
2. Garcia AA et al. Phase II clinical trial of bevacizumab and low-dose metronomic oral cyclophosphamide in recurrent ovarian cancer: a trial of the California, Chicago, and Princess Margaret Hospital phase II consortia. J Clin Oncol. 2008;26(1):76–82.
3. Penson RT et al. Phase II study of carboplatin, paclitaxel, and bevacizumab with maintenance bevacizumab as first-line chemotherapy for advanced mullerian tumors. J Clin Oncol. 2010;28(1):154–9.
4. Agarwal R, Kaye SB. Ovarian cancer: strategies for overcoming resistance to chemotherapy. Nat Rev Cancer. 2003;3(7):502–16.
5. Dean M, Fojo T, Bates S. Tumour stem cells and drug resistance. Nat Rev Cancer. 2005;5(4):275–84.
6. Huang EH et al. Cancer stem cells: a new paradigm for understanding tumor progression and therapeutic resistance. Surgery. 2007;141(4):415–9.
7. Rosen JM, Jordan CT. The increasing complexity of the cancer stem cell paradigm. Science. 2009;324(5935):1670–3.
8. Valent P et al. Cancer stem cell definitions and terminology: the devil is in the details. Nat Rev Cancer. 2012;12(11):767–75.
9. Rizzo S et al. Ovarian cancer stem cell-like side populations are enriched following chemotherapy and overexpress EZH2. Mol Cancer Ther. 2011;10(2):325–35.
10. Zhang S et al. Identification and characterization of ovarian cancer-initiating cells from primary human tumors. Cancer Res. 2008;68(11):4311–20.
11. Chau WK et al. c-Kit mediates chemoresistance and tumor-initiating capacity of ovarian cancer cells through activation of Wnt/beta-catenin-ATP-binding cassette G2 signaling. Oncogene. 2013;32(22):2767–81.
12. Alvero AB et al. Molecular phenotyping of human ovarian cancer stem cells unravels the mechanisms for repair and chemoresistance. Cell Cycle. 2009;8(1):158–66.
13. Steg AD et al. Stem cell pathways contribute to clinical chemoresistance in ovarian cancer. Clin Cancer Res. 2012;18(3):869–81.
14. van der Gun BT et al. EpCAM in carcinogenesis: the good, the bad or the ugly. Carcinogenesis. 2010;31(11):1913–21.
15. Ferrandina G et al. Expression of CD133-1 and CD133-2 in ovarian cancer. Int J Gynecol Cancer. 2008;18(3):506–14.
16. Campbell LL, Polyak K. Breast tumor heterogeneity: cancer stem cells or clonal evolution? Cell Cycle. 2007;6(19):2332–8.
17. Cooke SL et al. Genomic analysis of genetic heterogeneity and evolution in high-grade serous ovarian carcinoma. Oncogene. 2010;29(35):4905–13.
18. Khalique L et al. Genetic intra-tumour heterogeneity in epithelial ovarian cancer and its implications for molecular diagnosis of tumours. J Pathol. 2007;211(3):286–95.
19. Castellarin M et al. Clonal evolution of high-grade serous ovarian carcinoma from primary to recurrent disease. J Pathol. 2013;229(4):515–24.
20. Tarin D, Thompson EW, Newgreen DF. The fallacy of epithelial mesenchymal transition in neoplasia. Cancer Res. 2005;65(14):5996–6000. *discussion -1*.
21. Thompson EW, Newgreen DF, Tarin D. Carcinoma invasion and metastasis: a role for epithelial-mesenchymal transition? Cancer Res. 2005;65(14):5991–5. *discussion 5*.
22. Talbot LJ, Bhattacharya SD, Kuo PC. Epithelial-mesenchymal transition, the tumor microenvironment, and metastatic behavior of epithelial malignancies. Int J Biochem Mol Biol. 2012;3(2):117–36.
23. Takahashi-Yanaga F, Kahn M. Targeting Wnt signaling: can we safely eradicate cancer stem cells? Clin Cancer Res. 2010;16(12):3153–62.
24. Takebe N et al. Targeting cancer stem cells by inhibiting Wnt, Notch, and Hedgehog pathways. Nat Rev Clin Oncol. 2011;8(2):97–106.
25. Abubaker K et al. Short-term single treatment of chemotherapy results in the enrichment of ovarian cancer stem cell-like cells leading to an increased tumor burden. Mol Cancer. 2013;12:24.
26. Latifi A et al. Cisplatin treatment of primary and metastatic epithelial ovarian carcinomas generates residual cells with mesenchymal stem cell-like profile. J Cell Biochem. 2011;112(10):2850–64.
27. Ricci F et al. Ovarian carcinoma tumor-initiating cells have a mesenchymal phenotype. Cell Cycle. 2012;11(10):1966–76.
28. Marchini S et al. Resistance to platinum-based chemotherapy is associated with epithelial to mesenchymal transition in epithelial ovarian cancer. Eur J Cancer. 2013;49(2):520–30.
29. Haslehurst AM et al. EMT transcription factors snail and slug directly contribute to cisplatin resistance in ovarian cancer. BMC Cancer. 2012;12:91.
30. Yue P et al. Hyperactive EGF receptor, Jaks and Stat3 signaling promote enhanced colony-forming ability, motility and migration of cisplatin-resistant ovarian cancer cells. Oncogene. 2012;31(18):2309–22.
31. Rosano L et al. Acquisition of chemoresistance and EMT phenotype is linked with activation of the endothelin A receptor pathway in ovarian carcinoma cells. Clin Cancer Res. 2011;17(8):2350–60.
32. Wintzell M et al. Repeated cisplatin treatment can lead to a multiresistant tumor cell population with stem cell features and sensitivity to 3-bromopyruvate. Cancer Biol Ther. 2012;13(14):1454–62.
33. Lili LN et al. Molecular profiling supports the role of epithelial-to-mesenchymal transition (EMT) in ovarian cancer metastasis. J Ovarian Res. 2013;6(1):49.
34. Miow QH et al. Epithelial-mesenchymal status renders differential responses to cisplatin in ovarian cancer. Oncogene. 2014.
35. Pietras K, Ostman A. Hallmarks of cancer: interactions with the tumor stroma. Exp Cell Res. 2010;316(8):1324–31.
36. Naora H. Heterotypic cellular interactions in the ovarian tumor microenvironment: biological significance and therapeutic implications. Front Oncol. 2014;4:18.
37. Meads MB, Gatenby RA, Dalton WS. Environment-mediated drug resistance: a major contributor to minimal residual disease. Nat Rev Cancer. 2009;9(9):665–74.
38. Suh DH et al. Metabolic orchestration between cancer cells and tumor microenvironment as a co-evolutionary source of chemoresistance in ovarian cancer: A therapeutic implication. Biochem Pharmacol. 2014.
39. Castells M et al. Microenvironment mesenchymal cells protect ovarian cancer cell lines from apoptosis by inhibiting XIAP inactivation. Cell Death Dis. 2013;4:e887.
40. Lim JJ et al. VEGFR3 inhibition chemosensitizes ovarian cancer stemlike cells through down-regulation of BRCA1 and BRCA2. Neoplasia. 2014;16(4):343–53 e1-2.
41. Benabbou N et al. Hospicells promote upregulation of the ATP-binding cassette genes by insulin-like growth factor-I via the JAK2/STAT3 signaling pathway in an ovarian cancer cell line. Int J Oncol. 2013;43(3):685–94.

42. Triulzi T et al. Maspin influences response to doxorubicin by changing the tumor microenvironment organization. Int J Cancer. 2014;134(12):2789–97.
43. Leonard GD, Fojo T, Bates SE. The role of ABC transporters in clinical practice. Oncologist. 2003;8(5):411–24.
44. Choi CH. ABC transporters as multidrug resistance mechanisms and the development of chemosensitizers for their reversal. Cancer Cell Int. 2005;5:30.
45. Januchowski R et al. MDR gene expression analysis of six drug-resistant ovarian cancer cell lines. Biomed Res Int. 2013;2013: 241763.
46. Naniwa J et al. Genetic diagnosis for chemosensitivity with drug-resistance genes in epithelial ovarian cancer. Int J Gynecol Cancer. 2007;17(1):76–82.
47. Gyorffy B et al. Gene expression profiling of 30 cancer cell lines predicts resistance towards 11 anticancer drugs at clinically achieved concentrations. Int J Cancer. 2006;118(7):1699–712.
48. Whiteford CC et al. Credentialing preclinical pediatric xenograft models using gene expression and tissue microarray analysis. Cancer Res. 2007;67(1):32–40.
49. Januchowski R et al. Drug transporter expression profiling in chemoresistant variants of the A2780 ovarian cancer cell line. Biomed Pharmacother. 2014;68(4):447–53.
50. Unger FT, Witte I, David KA. Prediction of individual response to anticancer therapy: historical and future perspectives. Cell Mol Life Sci. 2014.
51. Yu M, Ocana A, Tannock IF. Reversal of ATP-binding cassette drug transporter activity to modulate chemoresistance: why has it failed to provide clinical benefit? Cancer Metastasis Rev. 2013;32(1–2):211–27.
52. Vousden KH, Prives C. Blinded by the Light: The Growing Complexity of p53. Cell. 2009;137(3):413–31.
53. Marks JR et al. Overexpression and mutation of p53 in epithelial ovarian cancer. Cancer Res. 1991;51(11):2979–84.
54. Integrated genomic analyses of ovarian carcinoma. Nature. 2011;474(7353):609-15.
55.• Yang-Hartwich Y et al. p53 protein aggregation promotes platinum resistance in ovarian cancer. Oncogene. 2014. *This study demonstrated that p53 protein aggregation is associated with p53 inactivation and platinum resistance in a population of high-grade serous ovarian carcinoma cancer cells with cancer stem cell properties. In addition, results indicated that inhibition of p14ARF with overexpression of a p53-positive regulator could suppress p53 aggregation and sensitize cancer cells to platinum treatment, suggesting that inhibiting p53 aggregation can reactivate p53 pro-apoptotic function.*
56. Cheng GZ et al. Twist is transcriptionally induced by activation of STAT3 and mediates STAT3 oncogenic function. J Biol Chem. 2008;283(21):14665–73.
57. Cheng GZ, Zhang W, Wang LH. Regulation of cancer cell survival, migration, and invasion by Twist: AKT2 comes to interplay. Cancer Res. 2008;68(4):957–60.
58. Yin G et al. TWISTing stemness, inflammation and proliferation of epithelial ovarian cancer cells through MIR199A2/214. Oncogene. 2010;29(24):3545–53.
59. Chen R et al. Regulation of IKKbeta by miR-199a affects NF-kappaB activity in ovarian cancer cells. Oncogene. 2008;27(34): 4712–23.
60. Kajiyama H et al. Twist expression predicts poor clinical outcome of patients with clear cell carcinoma of the ovary. Oncology. 2006;71(5–6):394–401.
61. Hosono S et al. Expression of Twist increases the risk for recurrence and for poor survival in epithelial ovarian carcinoma patients. Br J Cancer. 2007;96(2):314–20.
62. Wang T et al. Twist2 contributes to cisplatin-resistance of ovarian cancer through the AKT/GSK-3beta signaling pathway. Oncol Lett. 2014;7(4):1102–8.
63. Sen M et al. First-in-human trial of a STAT3 decoy oligonucleotide in head and neck tumors: implications for cancer therapy. Cancer Discov. 2012;2(8):694–705.
64. Raskatov JA et al. Modulation of NF-kappaB-dependent gene transcription using programmable DNA minor groove binders. Proc Natl Acad Sci U S A. 2012;109(4):1023–8.
65. Moellering RE et al. Direct inhibition of the NOTCH transcription factor complex. Nature. 2009;462(7270):182–8.
66. Robertson KD, Jones PA. DNA methylation: past, present and future directions. Carcinogenesis. 2000;21(3):461–7.
67. Matei D et al. Epigenetic resensitization to platinum in ovarian cancer. Cancer Res. 2012;72(9):2197–205.
68. Fu S et al. Phase 1b-2a study to reverse platinum resistance through use of a hypomethylating agent, azacitidine, in patients with platinum-resistant or platinum-refractory epithelial ovarian cancer. Cancer. 2011;117(8):1661–9.
69. Watanabe Y et al. A change in promoter methylation of hMLH1 is a cause of acquired resistance to platinum-based chemotherapy in epithelial ovarian cancer. Anticancer Res. 2007;27(3B):1449–52.
70. Gifford G et al. The acquisition of hMLH1 methylation in plasma DNA after chemotherapy predicts poor survival for ovarian cancer patients. Clin Cancer Res. 2004;10(13):4420–6.
71. Ali MW et al. Transcriptional suppression, DNA methylation, and histone deacetylation of the regulator of G-protein signaling 10 (RGS10) gene in ovarian cancer cells. PLoS One. 2013;8(3): e60185.
72. Hooks SB et al. Regulators of G-Protein signaling RGS10 and RGS17 regulate chemoresistance in ovarian cancer cells. Mol Cancer. 2010;9:289.
73.• Wang Y et al. Epigenetic targeting of ovarian cancer stem cells. Cancer Res. 2014;74(17):4922–36. *This study tested the hypothesis that novel hypomethylating agents may be able to reset ovarian cancer stem-like cells (OCSC) and drive them toward a differentiated phenotype, by examining the effect of the new DNA methyltransferase inhibitor SGI-110 on OCSC phenotype. The results offered preclinical evidence that epigenome-targeting strategies have the potential to delay tumor progression by reprogramming residual cancer stem-like cells.*
74. Esteller M. Cancer epigenomics: DNA methylomes and histone-modification maps. Nat Rev Genet. 2007;8(4):286–98.
75. Kouzarides T. Chromatin modifications and their function. Cell. 2007;128(4):693–705.
76. Dizon DS et al. A phase II evaluation of belinostat and carboplatin in the treatment of recurrent or persistent platinum-resistant ovarian, fallopian tube, or primary peritoneal carcinoma: a Gynecologic Oncology Group study. Gynecol Oncol. 2012;125(2):367–71.
77. Dizon DS et al. Phase II activity of belinostat (PXD-101), carboplatin, and paclitaxel in women with previously treated ovarian cancer. Int J Gynecol Cancer. 2012;22(6):979–86.
78. Titone R et al. Epigenetic control of autophagy by microRNAs in ovarian cancer. Biomed Res Int. 2014;2014:343542.
79. Sorrentino A et al. Role of microRNAs in drug-resistant ovarian cancer cells. Gynecol Oncol. 2008;111(3):478–86.
80. Yang H et al. MicroRNA expression profiling in human ovarian cancer: miR-214 induces cell survival and cisplatin resistance by targeting PTEN. Cancer Res. 2008;68(2):425–33.
81. Vecchione A et al. A microRNA signature defines chemoresistance in ovarian cancer through modulation of angiogenesis. Proc Natl Acad Sci U S A. 2013;110(24):9845–50.
82. Lee H et al. MicroRNA expression in ovarian carcinoma and its correlation with clinicopathological features. World J Surg Oncol. 2012;10:174.

83. Marchini S et al. Association between miR-200c and the survival of patients with stage I epithelial ovarian cancer: a retrospective study of two independent tumour tissue collections. Lancet Oncol. 2011;12(3):273–85.
84. Li Z et al. MiR-27a modulates MDR1/P-glycoprotein expression by targeting HIPK2 in human ovarian cancer cells. Gynecol Oncol. 2010;119(1):125–30.
85. Zhu H et al. Role of MicroRNA miR-27a and miR-451 in the regulation of MDR1/P-glycoprotein expression in human cancer cells. Biochem Pharmacol. 2008;76(5):582–8.
86. Xie Z, Cao L, Zhang J. miR-21 modulates paclitaxel sensitivity and hypoxia-inducible factor-1alpha expression in human ovarian cancer cells. Oncol Lett. 2013;6(3):795–800.
87. Lu L et al. MicroRNA let-7a: a potential marker for selection of paclitaxel in ovarian cancer management. Gynecol Oncol. 2011;122(2):366–71.
88. Yang N et al. MicroRNA microarray identifies Let-7i as a novel biomarker and therapeutic target in human epithelial ovarian cancer. Cancer Res. 2008;68(24):10307–14.
89. Boyerinas B et al. Let-7 modulates acquired resistance of ovarian cancer to Taxanes via IMP-1-mediated stabilization of multidrug resistance 1. Int J Cancer. 2012;130(8):1787–97.
90. Mitamura T et al. Downregulation of miRNA-31 induces taxane resistance in ovarian cancer cells through increase of receptor tyrosine kinase MET. Oncogenesis. 2013;2:e40.
91. Liu MX et al. Epigenetic silencing of microRNA-199b-5p is associated with acquired chemoresistance via activation of JAG1-Notch1 signaling in ovarian cancer. Oncotarget. 2014;5(4):944–58.
92. Obeng EA et al. Proteasome inhibitors induce a terminal unfolded protein response in multiple myeloma cells. Blood. 2006;107(12):4907–16.
93. Adams J. The proteasome: a suitable antineoplastic target. Nat Rev Cancer. 2004;4(5):349–60.
94. Adams J. Preclinical and clinical evaluation of proteasome inhibitor PS-341 for the treatment of cancer. Curr Opin Chem Biol. 2002;6(4):493–500.
95. Aghajanian C et al. Phase I trial of bortezomib and carboplatin in recurrent ovarian or primary peritoneal cancer. J Clin Oncol. 2005;23(25):5943–9.
96. Ramirez PT et al. Phase I trial of the proteasome inhibitor bortezomib in combination with carboplatin in patients with platinum- and taxane-resistant ovarian cancer. Gynecol Oncol. 2008;108(1):68–71.
97. Aghajanian C et al. A phase II evaluation of bortezomib in the treatment of recurrent platinum-sensitive ovarian or primary peritoneal cancer: a Gynecologic Oncology Group study. Gynecol Oncol. 2009;115(2):215–20.
98. Steg AD et al. Proteasome inhibition reverses hedgehog inhibitor and taxane resistance in ovarian cancer. Oncotarget. 2014;5(16):7065–80.
99. Kelly MG et al. TLR-4 signaling promotes tumor growth and paclitaxel chemoresistance in ovarian cancer. Cancer Res. 2006;66(7):3859–68.
100. Liu G, Du P, Zhang Z. Myeloid Differentiation Factor 88 Promotes Cisplatin Chemoresistance in Ovarian Cancer. Cell Biochem Biophys. 2014.