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Review

Novel mechanisms and approaches to overcome multidrug resistance in the treatment of ovarian cancer

Fang Ren [a,b], Jacson Shen [b], Huirong Shi [a], Francis J. Hornicek [b], Quancheng Kan [a,*], Zhenfeng Duan [a,b,*]

[a] Department of Gynecologic Oncology, The First Affiliated Hospital of Zhengzhou University, 1 Jianshe East Road, Zhengzhou 450052, People's Republic of China
[b] Sarcoma Biology Laboratory, Department of Orthopaedic Surgery, Massachusetts General Hospital and Harvard Medical School, 55 Fruit Street, Jackson 1115, Boston, MA 02114, United States

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ABSTRACT

Ovarian cancer remains the leading cause of gynecological cancer-related mortality despite the advances in surgical techniques and chemotherapy drugs over the past three decades. Multidrug resistance (MDR) to chemotherapy is the major cause of treatment failure. Previous research has focused mainly on strategies to reverse MDR by targeting the MDR1 gene encoded P-glycoprotein (Pgp) with small molecular compound inhibitors. However, prior Pgp inhibitors have shown very limited clinical success because these agents have relatively low potency and high toxicity. Therefore, identification of more specific and potent new inhibitors would be useful. In addition, emerging evidence suggests that cancer stem cells (CSCs), deregulated non-coding RNA (ncRNA), autophagy, and tumor heterogeneity also contribute significantly to drug sensitivity/resistance in ovarian cancer. This review summarizes these novel mechanisms of MDR and evaluates several new concepts to overcome MDR in the treatment of ovarian cancer. These new strategies include overcoming MDR with more potent and specific Pgp inhibitors, targeting CSCs and ncRNA, modulating autophagy signaling pathway, and targeting tumor heterogeneity.

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1. Introduction

Ovarian cancer is the most lethal gynecological malignancy and the second leading cause of cancer-related deaths among women throughout the world [1]. Due to the absence of specific symptoms at early stages and lack of early detection, the vast majority of the ovarian cancer patients are often diagnosed at an advanced stage with 60–70% of patients having stage III–IV disease at diagnosis. The current standard care for advanced ovarian cancer includes surgical debulking followed by platinum and taxane-based chemotherapy. Despite the development of various new treatment strategies (targeted therapy, immune therapy, *etc.*), the overall 5-year survival rate of ovarian cancer patients has improved slightly from 33.7% in 1975 to 45.6% in 2011 [2]. Intrinsic or acquired multidrug resistance (MDR) to chemotherapy is still a major

* Corresponding authors at: Department of Gynecologic Oncology, The First Affiliated Hospital of Zhengzhou University, 1 Jianshe East Road, Zhengzhou 450052, People's Republic of China.
E-mail addresses: qckan19632012@163.com (Q. Kan), zduan@mgh.harvard.edu (Z. Duan).

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challenge to ovarian cancer treatments. Almost 90% of patient deaths with advanced disease (stages III–IV) are caused by the development of MDR [2,3]. No biomarkers for the prediction of response to chemotherapy are currently in clinical use. Combining a prognostic model, named "Classification of Ovarian Cancer" (CLOVAR), with clinically related features to predict outcome to therapy may provide a rationale for optimal combination of patient and treatment regimens [4]. To date, however, overcoming MDR is still a high priority of clinical and investigational oncology, but remains an elusive outcome. This review summarizes the recent literature on novel mechanisms of MDR and evaluates several novel MDR reversal strategies, including more potent and specific P-glycoprotein (Pgp) inhibitors, cancer stem cell (CSC)-based targeting agents, applications of non-coding RNA (ncRNA), autophagy modulation, and targeting tumor heterogeneity in chemoresistance in ovarian cancer.

2. Novel Pgp inhibitors and compounds for overcoming MDR in ovarian cancer

Many studies have confirmed that overexpression of ATP-binding cassette, sub-family B member 1/Multidrug resistance gene 1 (ABCB1/MDR1) is the major factor in limiting the efficacy of chemotherapy drugs *in vitro* [5,6]. MDR1 gene encoded Pgp is the most extensively studied ABC transporter. Pgp can utilize the energy of ATP hydrolysis to transport various structurally and functionally unrelated drugs out of the cells [7]. In addition to their physiologic expression in normal tissues, overexpression of Pgp results in the development of MDR in human tumors, including in ovarian cancers [6]. Thereby, overcoming Pgp-based MDR has been extensively studied for more than three decades. Several distinct strategies have been used to target MDR1-Pgp, such as small interfering RNAs (siRNAs) and small molecular Pgp inhibitors.

Pgp inhibitors can be divided into four generations according to their potency, selectivity, and drug-drug interaction potential [8]. Among these agents, clinical toxicities associated with their use at the required concentrations to inhibit Pgp function have prohibited their widespread utility. First-generation Pgp inhibitors, such as verapamil, have lower affinity to Pgp than to other target proteins (*e.g.*, calcium channel protein) [9]. When administered at sufficiently high doses, verapamil can cause cardiac toxicity. Second-generation Pgp inhibitors significantly inhibit the metabolism and excretion of conventional chemotherapy drugs, thus leading to unacceptable toxicity that has necessitated dose reductions of chemotherapy drugs in clinical trials [10]. Among the third-generation Pgp inhibitors, XR9576 (tariquidar) [11], LY335979 (zosuquidar) [8], and R101933 (laniquidar) [8] are being studied for mitigating drug resistance. Although the third-generation Pgp inhibitors are more potent than the first and second-generation, the phase III clinical trials using the third-generation Pgp inhibitors have been disappointing due to increased toxicity and interaction with chemotherapy drugs [12]. In general, the results from previous Pgp inhibitor clinical trials in ovarian cancer have shown almost no survival benefits, consistent with Pgp inhibitors studied in other types of human cancers up to now. The development of more potent and selective Pgp inhibitors is an important unrealized necessity for cancer patients.

Several novel MDR1 inhibitors have been identified in recent years (Table 1). NSC77037, also known as Tetrandrine or CBT-1®, is a bisbenzylisoquinoline alkaloid compound, which was originally isolated from the dried root of *Stephania tetrandra* [13]. NSC77037 is able to reverse MDR in Pgp-overexpressed ovarian cancer cell lines by directly stimulating Pgp ATPase activity and inhibiting the function of Pgp. The inhibitory effect of NSC77037 is not altered after co-incubation with the Pgp inhibitor verapamil, suggesting that NSC77037 itself is not a substrate of Pgp [14]. A phase I clinical trial with NSC77037 and doxorubicin was conducted in patients with advanced cancer, including ovarian cancer [15]. The antitumor activity of NSC77037 was encouraging in that, out of 25 evaluable patients, there were nine patients with stable disease (containing two ovarian cancer patients) and five with tumor shrinkage. Compared to other Pgp inhibitors, NSC77037 did not affect the pharmacokinetics of doxorubicin and the side effects were mild [15]. In combination with paclitaxel, the effect of NSC77037 on Pgp-mediated efflux of rhodamine 123 from normal liver and peripheral blood mononuclear cells was evaluated in a phase II clinical trial. The results showed that NSC77037, with lack of toxicity, could inhibit Pgp-mediated efflux to a degree, which was achieved by using other Pgp inhibitors [16]. NSC73306 is a thiosemicarbazone compound that was initially identified from National Cancer Institute (NCI) Developmental Therapeutics Program (DTP) [17]. It displays greater toxicity against cells expressing functional Pgp than against other cells. Furthermore, pre-treatment of Pgp positive cells with NSC73306 decreases Pgp expression and resensitizes MDR cancer cells to the chemotherapy drugs that previously induced resistance [17]. The precise mechanism of NSC73306 that inhibits Pgp-mediated transport remains unclear. It neither stimulated nor inhibited the ATPase, suggesting that NSC73306 does not interact with Pgp directly. NSC23925, another novel and long-acting methoxyphenyl piperidinyl compound, was identified from screening the NCI Diversity Set library. NSC23925 can reverse MDR by inhibiting the function of Pgp and therefore increase intracellular accumulation of chemotherapy drugs. NSC23925 itself is also not a substrate of Pgp [18]. The mechanism of NSC23925 that reverses MDR is similar to that of NSC77037, which directly interacts with Pgp and stimulates transporter ATPase activity. Compared with chemotherapy drugs alone, the use of a combination of chemotherapy drugs (paclitaxel/doxorubicin) with NSC23925 significantly induced cell death and apoptosis. In follow-up studies, the combination of NSC23925 with paclitaxel prevented the onset of Pgp expression or anti-apoptotic meditated paclitaxel resistance in ovarian cancer cell lines both *in vitro* and *in vivo* [19,20]. More recently, a study showed that NSC23925b exhibits positive preclinical pharmacokinetic characteristics and limited toxicity *in vivo* [21]. In addition, compounds such as procyanidin (GSP), extracted from grape seed, have also been shown to exhibit MDR reversal activities [22]. In chemoresistant ovarian cancer cells, GSP significantly increases the efficacy of paclitaxel and adriamycin by inhibiting the function and expression of Pgp [22]. However, the contribution of MDR1/Pgp to clinical ovarian cancer resistance remains controversial.

3. Ovarian CSCs in MDR

Experimental evidence has implicated that other mechanisms, including deregulation of ovarian CSCs, may also contribute to chemotherapy drug resistance.

CSCs, or tumor initiating cells, are cancer stem cells that possess characteristics associated with normal stem cells. CSCs have the ability to transform into all types of cells comprising the tumor mass, and possess the characteristics of self-replication and inducing resistance to chemotherapy. These features are responsible for the progression and recurrence of malignant tumors [23–26]. The number of ovarian CSCs might be used to predict progression of disease in early stages of epithelial ovarian cancer [27].

CSCs are defined by several markers, which can be used as therapeutic targets in ovarian cancer, such as CD133, CD44, CD117 (c-kit), epithelial cell adhesion molecule (EpCAM), and aldehyde dehydrogenase isoform 1 (ALDH1) [28–33] (Table 2). Different ovarian CSCs defined by distinct marker profiles may in fact reveal the chemoresistant mechanism of CSCs in ovarian cancer. The cell surface transmembrane glycoprotein CD44 is associated with poor prognosis and resistance to chemotherapy of ovarian cancer [34]. In a study that evaluated the expression of CSCs surface markers in biopsies taken from the initial laparoscopies and interval surgeries, an enriched CD44 tumor cell population was identified on biopsies after neoadjuvant chemotherapy. Thus, changes in the expression of CD44 may be used as a predictor of chemoresistance in epithelial ovarian cancer [35]. EpCAM, also referred to as EGP-40 or CD326, is involved in resistance to platinum-based