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Short communication

Reduced accumulation of platinum drugs is not observed in drug-resistant ovarian cancer cell lines derived from cisplatin-treated patients

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ABSTRACT

The resistance of ovarian cancer towards front-line chemotherapy, usually cisplatin or carboplatin in combination with paclitaxel or docetaxel, remains a major clinical challenge. Resistance to these agents has been largely studied using cell lines selected for resistance to agents *in vitro*. We examined a series of paired cell lines derived from patients with ovarian cancer prior to chemotherapy (PEO1, PEO4, PEO14 and PEA1), and following the acquisition of resistance to a platinum-based chemotherapy regimen (PEO6, PEO23 and PEA2, respectively). All resistant patient lines showed resistance to cisplatin (2-5-fold), but this did not correspond with lowered accumulation. No general cross-resistance was observed for oxaliplatin, paclitaxel or docetaxel, and paclitaxel accumulation was not affected. PEO1 cells carrying BRCA2 mutations were hypersensitive to the PARP inhibitors olaparib and velaparib, but all other cell lines expressing functional forms of BRCA2 were less sensitive. While reduced drug accumulation was not observed, we believe these pairs of lines are of use to researchers studying Pt drug resistance and experimental therapeutics against drug-resistant ovarian cancer.

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Introduction

The resistance of cancer cells towards chemotherapeutics is an enduring clinical challenge. For example, following surgical debulking, a front-line therapeutic regimen for treating ovarian cancer is a taxane (paclitaxel or docetaxel, day 1) in combination with a 'platinum' (cisplatin or carboplatin, day 2) repeated every 21 days [1]. Despite initial efficacy, patients usually relapse and those with platinum-resistant disease are often cross-resistant to other drugs [2]. In the laboratory, resistance to agents such as cisplatin or paclitaxel is examined individually by selecting cell lines *in vitro* for resistance to an agent [3], though a small number of cell lines have been generated for resistance to both a platinum and a taxane [4]. As such, our understanding of cisplatin resistance has been largely derived from studies on cell lines where the resistance has been generated *in vitro* [5], and these mechanisms have informed development of new platinum-based analogs that circumvent cisplatin resistance [6].

One important feature of both platinum [7] and taxane resistance *in vitro* is decreased accumulation [8]. This is mediated by the efflux transporter P-glycoprotein (*ABCB1*) for taxanes, and by a pleiotropic mechanism that involves decreased uptake and altered endocytic processes for platinum compounds. For both drug classes there are multiple other pathways involved in resistance acquisition, and it is not clear whether diminished accumulation is clinically relevant [5]. One study of Pt levels in frozen samples of surgically resected non-small cell lung cancer samples demonstrated a correlation between Pt levels and survival for 44 patients [9]. While extremely informative, a limitation of this study is that patients had received platinum chemotherapy on average 37 days prior to surgery, yet only a single data point exists for each patient. As such, the impact of resistance acquisition on accumulation cannot be assessed—that is, there is no way to determine whether diminished accumulation was intrinsic or acquired.

Our interest in accumulation changes in ovarian cancer led us to examine three sets of ovarian cancer cell lines derived from patients prior to and following chemotherapy (Fig. 1) [10]. PEO1 cells were collected from a patient 22 months after cisplatin, 5-fluorouracil and chlorambucil treatment and the patient continued to show response to cisplatin therapy. PEO4 cells were taken from the same patient after relapse occurred 10 months later, and PEO6 cells were collected from that patient 3 months later, following further cisplatin treatment that had produced no benefit [11]. PEA1 cells were collected from a different patient prior to treatment, and PEA2 cells from the same patient

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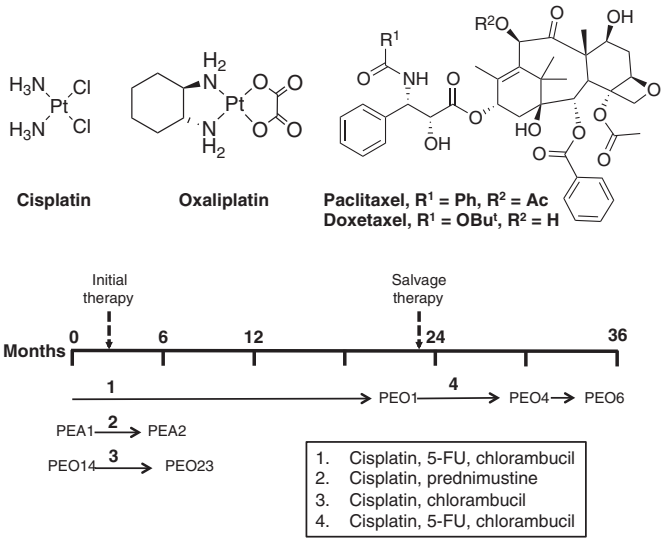

Fig. 1. Top: structures of cisplatin, oxaliplatin, paclitaxel (taxol) and docetaxel. Bottom: scheme demonstrating the timeline for establishment of each cell line studied in this paper, including chemotherapeutic regimens used for each patient. Information incorporated into scheme derived from [10,11].

after cisplatin and prednimustine treatment failed. PEO14 cells were collected from a third patient prior to treatment, and PEO23 cells collected from the same patient after cisplatin and chlorambucil treatment failed. Treatments reflect the chemotherapy regimens utilized prior to the introduction of paclitaxel to the clinic. All cell lines have mutant p53, a hallmark of high-grade serous ovarian carcinoma. PEO1 was shown to carry germ-line BRCA2-inactivating mutations, rendering the cells sensitive to DNA-damaging agents (PEA1 and PEO14 are BRCA2 wild-type) [11]. PEO4 and PEO6 both carry a secondary BRCA2 mutation acquired by the tumor cells in the patient prior to isolation of PEO4 that results in functional restoration of BRCA2 [12]. None of the patients received radiation therapy. Following the initial characterization, it has been shown that PEO1 contained two separate clones with BRCA2 inactivation mutations — one carrying a BRCA2 stop mutation (PEO1-stop) and another carrying a BRCA2 missense mutation (PEO1-mis) [13]. It has been shown that PEO4 and PEO6 cells are resistant to platinum agents and 5-FU compared with PEO1 [14,15].

Our aim was to characterize the patterns of drug sensitivity and resistance of the three sets of cell lines, and to learn whether there was a relationship between sensitivity and drug accumulation in lines obtained from cisplatin-resistant patients, as has been repeatedly observed in previous *in vitro* studies.

We first assessed the cytotoxicity of agents: cisplatin and oxaliplatin as cytotoxic platinums, paclitaxel and docetaxel as taxanes currently used against ovarian cancer, and the PARP inhibitors olapirib and veliparib, as BRCA-mutant cells are deficient in DNA-repair and are known to be hypersensitive to PARP inhibition (and to DNA damaging agents) (see Supplementary methods for details) [16]. It appears that olapirib will soon be granted first-in-class medicine status for treating BRCA-mutant ovarian cancer sufferers [17].

The cell lines derived from the patients after they had had received cisplatin chemotherapy were all more resistant to cisplatin than the initial lines (first column, Table 1, see Fig. 1 as a guide for cell line names and their lineage). The two PEO1 lines (Mis and Stop) were more sensitive to cisplatin than PEA1 and PEO14, consistent with non-functional BRCA2 [18]. The remaining results, examining sensitivity to drugs that the patients had not seen, showed no clear patterns. The PEO1/4/6 and PEO14/23 cells demonstrated cross-resistance to oxaliplatin, but PEA2 cells ($IC_{50} = 124.1 \pm 12.9$ μM) were hypersensitive to oxaliplatin compared with PEA1 ($IC_{50} = 30.2 \pm 9.7$ μM), which is not normally observed in cisplatin-resistant cells [19]. Docetaxel and taxol gave variable data. PEA2 and PEO23 were hypersensitive to the taxanes, consistent with observations from *in vitro* resistant cells and clinical studies [20]. In contrast, the PEO1 cells were less sensitive to docetaxel, PEO4 was sensitized, and PEO6 was strongly resistant. Olaparib and veliparib both demonstrated greater cytotoxicity against the BRCA2 mutant PEO1 cells compared with other lines, consistent with the hypersensitivity to PARP inhibition expected in cells with mutant BRCA2 [21]. Interestingly, the PEO1-Mis line (BRCA2 missense mutation) was more sensitive to both PARP inhibitors than the PEO1-Stop line (BRCA2 stop codon mutation), and cisplatin had the same effect. It may be that the missense mutation is more deleterious than the stop mutation, though little work exists on this topic, but it is known that factors other than BRCA2 status can impact sensitivity to PARP inhibitors [22]. Our interpretation of these results is that the established cell lines retain the cisplatin resistance phenotype of the tumors from which they were derived, but patterns of cross-resistance to other drugs are not predictable.

The accumulation of platinum in all cell lines treated with cisplatin and oxaliplatin was assessed by ICP-MS. Briefly, cells were incubated with drug (20 μM) for 24 h, lysed for Pt measurement, and the measured Pt levels were normalized to protein content (measured by Bradford assay [23]) (Fig. 2). The only statistically significant difference was that PEO4 cells accumulated more Pt than the PEO1 and PEO6 lines. The same was true of oxaliplatin, and PEO23 cells accumulated more Pt than PEO14 — both changes were contrary to the cellular sensitivity patterns for these drugs. The accumulation of [^{3}H]taxol (1 μCi/mL) was assessed after 45 min by tryspinization of cells and scintillography [24], and normalized against cell count (Fig. 3). PEO6 cells accumulated twice the taxol of PEO1/4 cells, and PEO23 cells accumulated almost 10-

Table 1
Cytotoxicity (IC_{50}) of compounds against ovarian cancer cell lines[a].

Cell line	Platinums		Taxane		PARP inhibitors		Status	
	Cisplatin (μM)	Oxaliplatin (μM)	Docetaxel (nM)	Taxol (nM)	Olaparib (μM)	Veliparib (μM)	BRCA2	TP53
PEO1-Mis	3.2 ± 1.4	3.0 ± 0.9	33 ± 13	6.5 ± 0.9	25 ± 3	33 ± 1	Mutated	Mutated
PEO1-STOP	2.0 ± 0.7	3.8 ± 0.7	29 ± 4	13 ± 2	4.3 ± 0.4	22 ± 5	Mutated	Mutated
PEO4	9.1 ± 2.1	13 ± 2	14 ± 1	15 ± 1	46 ± 2	111 ± 6	Functional	Mutated
PEO6	12 ± 1	51 ± 5	137 ± 8	23 ± 8	258 ± 48	92 ± 14	Functional	Mutated
PEA1	9.6 ± 1.5	124 ± 13	261 ± 84	58 ± 20	141 ± 82	41 ± 1	Wild-type	Mutated
PEA2	16 ± 4	30 ± 10	87 ± 13	15 ± 1	245 ± 8	105 ± 2	Wild-type	Mutated
PEO14	4.8 ± 0.1	40 ± 3	262 ± 160	69 ± 2	59 ± 15	111 ± 22	Wild-type	Mutated
PEO23	10 ± 1	71 ± 7	122 ± 90	33 ± 18	51 ± 2	75 ± 18	Wild-type	Mutated

[a] Cells were cultured in RPMI 1640 supplemented with 10% fetal bovine serum, L-glutamine and antibiotics. All compounds were dissolved in DMSO as 20 mM stock solutions, except cisplatin (saline, 3.3 mM) and oxaliplatin (water, 12.6 mM) [30], and cytotoxicity of agents was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [31].