RESEARCH ARTICLE

Relationships of Ex-Vivo Drug Resistance Assay and Cytokine Production with Clinicopathological Features in the Primary Cell Culture of Thai Ovarian and Fallopian Tube Cancer Patients

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Abstract

Objective: Our goal was to determine the ex-vivo drug resistance assay, as well as the cytokine production, in response to platinum-based chemotherapy treatment in primary culture cells established from the tumor tissue of ovarian or fallopian tube carcinoma patients, and to predict the clinical responses to chemotherapy. **Methods:** Sensitivity to the platinum-based drug was analyzed in two ovarian cancer cell lines and 19 tumor samples using the primary cell culture obtained from 19 patients having ovarian or fallopian tube cancer that had undergone surgery from 2014 to 2017. **Results:** Our findings in the ovarian cancer cell lines showed that SKOV3 cells displayed 10-fold greater resistance to cisplatin and 5.8 times more resistance to carboplatin than A2780 cells. SKOV3 cells displayed platinum-induced IL-6 and IL-8 overproduction whereas wild type A2780 displayed no detectable cytokine production. Regarding the primary cell culture obtained from patients, ex-vivo drug resistance assay results revealed that although extreme drug resistance was correlated with late stage ovarian cancer (P= 0.031), it could not independently predict or alter the outcomes of patients with ovarian or fallopian tube cancer. No relationship was found between basal cytokine secretion and the clinical parameters. However, carboplatin-induced IL-6 and IL-8 production had a significant association with the clinical response to chemotherapy (P=0.016 and P=0.038 respectively). Carboplatin-induced IL-8 overproduction was correlated with FIGO staging III-IV (P=0.026), but no correlation between carboplatin-induced IL-6 and FIGO staging (P= 0.061) was noted. **Conclusion:** These results suggest that cytokine production in response to platinum-based chemotherapy in primary culture cells may be useful as a predictive marker for the therapeutic outcomes among ovarian or fallopian tube cancer patients.

Keywords: Ovarian cancer- primary culture- drug resistance assay- Cytokines-IL6-IL8

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Introduction

The term 'ovarian cancer' is often used to describe cancers that begin in the cells in the ovary, fallopian tube or peritoneum. Ovarian cancer is one of the leading causes of death resulting from gynecologic malignancies worldwide including in Thailand. According to global estimates 225,000 new cases were detected each year and more than 60% mortality rate within five years (Ferlay et al., 2015). Regardless of improvements in the detection and management of this form of cancer, the prognosis in patients with ovarian cancer remain poor and only a minority of patients survive diagnosis over a 5-year period. The gold standard of treatment for ovarian cancer would be surgery followed by chemotherapy. Currently, the chemotherapy used for the treatment of ovarian cancer involves a combination of carboplatin and the drug paclitaxel (Cristea et al., 2010). This is also the drug of choice for ovarian cancer patients in Maharaj Nakorn Hospital. Although platinum-based chemotherapy regimens are effective forms of treatment for the majority of ovarian cancer patients, recurrence is common and often leads to death. Hence, sensitivity to anticancer drugs is thought to be an important prognostic factor among patients with ovarian cancer.

Although immortal cancer cell lines present an easily usable set of biological models to investigate cancer biology and explore the potential efficacy of anticancer

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drugs, the derivation and short-term culture of the primary cells obtained from solid tumors has significant importance in personalized cancer therapy treatments. Ex-vivo drug sensitivity and the resistance testing of individual patient samples might provide important functional information for therapeutic outcomes.

Interestingly, cytokines also play a functional role in the natural history of various malignant diseases. Some studies have revealed that the autocrine production of IL-6 confers to cisplatin and paclitaxel resistance in ovarian cancer cells and has been linked to poor outcomes in ovarian carcinoma (Wang et al., 2010). IL-6 is a pleiotropic cytokine, which is upregulated by the JAK/STAT pathway (Ji et al., 2013), and plays a major role in the inflammatory processes. In relation to ovarian cancer biology, there is emerging evidence that IL-6 mediates the events related to tumor growth, angiogenesis, invasion and chemo-resistance that lead to poor prognoses (Pinciroli et al., 2013). Moreover, another proangiogenic cytokine, IL-8, also plays an important role in carcinoma and its increased expression is associated with most human cancers, including ovarian carcinoma (Wang et al., 2012). In addition, IL-8 gene silencing of liposome-encapsulated small interfering RNA decreases tumor growth and increases sensitivity to clinically useful chemotherapeutic agents in solid tumors (Merritt et al., 2008). Research into the biology of ovarian cancer and the role of inflammatory cytokines in cancer progression, suggests that antagonists to certain cytokines may complement standard chemotherapy and prolong disease-free survival rates among cancer patients.

In this study, we determined the ex-vivo drug resistance assay and the cytokine production in response to platinum-based chemotherapy treatments in the primary culture cells established from the tumor tissues of ovarian/fallopian tube carcinoma patients and to predict the clinical responses to chemotherapy. We hope that our approach serves as the basis for a deeper investigation into drug responses and the establishment of personalized cancer therapy for ovarian cancer patients.

Materials and Methods

Patients and Samples

This study was approved by Chiang Mai University Ethics Committee for Human Research (Ethic number 194/2556) and written informed consent was obtained from each patient. A total of 19 tumor samples were obtained from patients who underwent surgery for ovarian carcinoma at the Department of Gynecologic Oncology, Division of Surgery, Maharaj Nakorn Chiang Mai Hospital between the years of 2014 and 2017. Clinical details were recorded and specimens were registered and handled in accordance with the Human Tissue Act. Patients were comprehensively staged as per the International Federation of Obstetricians and Gynecologists (FIGO) staging system for ovarian cancer. Patients were further stratified by FIGO stage into two groups as follows: early stage (FIGO I and II) and advanced stage (FIGO III and IV). Out of 19 patients, 17 patients were found to be chemo naïve and two patients had undergone reoperations after receiving chemotherapy. For serous, mucinous and endometroid types, the patients were treated with surgery followed by first-line adjuvant chemotherapy consisting of 6 cycles of paclitaxel and carboplatin. For granulosa cell carcinoma patients, the BEP regime (bleomycin, etoposide, cisplatin) was used. The effect of chemotherapy was evaluated after six cycles of first-line chemotherapy. Platinum resistance was defined as patients who experienced a progression of cancer during the first-line platinum chemotherapy treatment or who had a relapse within 6 months after treatment. Complete response (CR) and partial response (PR) were defined as responsive. Stable disease (SD) and progressive disease (PD) were defined as non-responsive.

Primary culture obtained from solid ovarian tumor tissue

Solid tumor tissues were collected after a number of surgical procedures and were confirmed by a pathologist from the Department of Gynecologic Pathology before being transferred to the cell culture laboratory. Once in the laboratory, the solid tumors were dissected into 2 mm^3 pieces using a sterile scalpel and were then placed in a Petridish containing 5 ml of DMEM with 20% streptomycin/penicillin. For enzymatic digestion, the tissue samples were added into an enzyme mixture (Collagenase A 0.15 U/ml and Dispase II 2.4 U/ml diluted in DMEM) and then were incubated at 37°C for 30 to 120 minutes. In the meantime, for the purposes of mechanical digestion, the test tubes containing the tissue and enzyme mixture were vortexed at 10-minute intervals. After that, the cell filtrate was centrifuged at 1200 rpm for 7 minutes. The culture flasks were pre-coated with type-1 collagen coating matrix (Invitrogen, CA) to enhance the cell attachment to the flasks. After resuspension was achieved in DMEM containing 20% FBS, samples were evaluated for cell viability. The medium was changed 24 hours after initial plating and every three days for the following two weeks. When 80% confluency was achieved, subculturing was done using 0.25% trypsin. The experiments were performed between passages 4 and 9.

Ovarian cancer cell lines

Cisplatin resistance (SKOV3) ovarian carcinoma cell lines were purchased from the American Type Culture Collection (Manassas, VA). SKOV3 cells were originally obtained from the ascites of an ovarian adenocarcinoma patient with intrinsic resistance to cisplatin. Cisplatin sensitive (A2780) ovarian carcinoma cell lines were purchased from Health Protection Agency Culture Collections (Salisbury, UK). This cell line was established from an untreated patient with ovarian endometroid adenocarcinoma. These cell lines were cultured in DMEM with 10% fetal bovine serum (FBS), 5 mM L-glutamine, 50 mg/mL penicillin and 50 mg/mL streptomycin. These cell lines were maintained in a humidified incubator with an atmosphere comprised of 95% air and 5% CO2 at 37°C. When the cells reached 70–80% confluence, they were harvested and plated for subsequent investigations.

Drug resistance assay

The differential cytotoxic potential of platinum-based drugs on the ovarian cancer cells was determined by MTT assay. Briefly, ovarian cancer cell lines and primary culture

cells (4 x10^3cells/well) obtained from patients were seeded in 96-well plates and incubated at 37 °C, 5% CO_2 overnight in DMEM containing 10% FBS. The cells were treated with various concentrations of carboplatin for 72 hours. Then, cell culture supernatants were removed and MTT dye was added and the supernatants were incubated for an additional 4 h. MTT-formazan was dissolved in DMSO and the absorbance was measured using a microplate reader at 540 nm with a reference wavelength of 630 nm. IC50 values (i.e., the drug concentration causing 50% inhibition of cancer cell survival) were obtained from the dose-response curves. Chemotherapy resistance is defined as follows: extreme drug resistance, 1 SD above the median chemotherapy resistance; intermediate drug resistance, between the median and extreme drug resistances; and low drug resistance, less than the median growth. Ex-vivo drug resistance assay, with the percent cell inhibition of more than 1 SD above the median, was termed as displaying extreme drug resistance (EDR). EDR indicates that tumor cell growth was virtually unaffected by the high chemotherapeutic agent exposure. The intermediate drug resistance (IDR) is a result of displaying greater resistance than the median but less resistance than EDR. IDR indicates moderate tumor survival. Low drug resistance (LDR) indicates that tumor cell proliferation was inhibited by the tested agent and consequently, tumor cells demonstrated less median growth. The LDR is a result of being less resistant than the median.

ELISA assay

The amount of cytokines present in response to carboplatin in ovarian cancer cells was detected by sandwich ELISA assay. Briefly, ovarian cancer cells were seeded, incubated overnight and then treated with various concentrations of carboplatin for 72 hours. The supernatant was then collected to determine the production of cytokines by employing the sandwich ELISA assay (BioLegend, San Diego, CA), according to the manufacturer's instructions. This assay procedure was based on the capture of human IL-6 and IL-8 cytokines by specific IL-6 and IL-8 monoclonal antibody (first antibody) immobilized on a 96-well microtiter plate. After unbound materials had been washed, biotinylated anti-human IL-6 and IL-8 detection antibody (second antibody) was added producing an antibody-antigen-antibody 'sandwich'. Avidin-horseradish peroxidase was subsequently added, followed by tetramethylbenzidine substrate solution producing a blue color in proportion to the concentration of IL-6 and IL-8 present in the sample. Finally, the color development was stopped with the addition of a stop solution and the absorbance of the calibrators and specimens were measured at 450 nm with a microplate reader.

Statistical analysis

All statistical analyses were performed using SPSS 17.0 software (SPSS, Chicago, USA). Descriptive analysis was used to describe the patient demographics. Data are presented as mean ± standard deviation. Cytokine levels of both the untreated and treated groups were tested by Student's t-test. Association between ex-vivo chemosensitivity, cytokine levels and clinicopathological parameters were assessed by Chi Square test and Fisher's exact test. Correlation of IC50 to carboplatin and clinical response was analyzed by nonparametric Spearman's test. Correlations between cytokines (IL-6 and IL-8) concentration and IC50 to carboplatin in primary cell culture were analyzed by the Pearson's test. $P < 0.05$ was considered statistically significant.

Results

Sensitivity to platinum drugs and platinum-induced IL-6 secretion in human ovarian cancer cell lines

We first examined the sensitivity to platinum drugs in two human ovarian cancer cell lines, such as cisplatin sensitive A2780 ovarian cancer cells and intrinsic cisplatin resistance SKOV3 cells. The concentration of cisplatin that inhibited cell survival by 50% was found to be 1 ± 7.050 μM for A2780 parental cell lines and 10 ± 2.985 μM for SKOV3 cell lines. The IC50 of carboplatin in A2780 and SKOV3 were found to be 17 ± 6.010 μM, and 100 ± 4.375 μM, respectively (Figure 1). SKOV3 cells displayed 10-fold greater resistance to cisplatin and 5.8 times more resistance to carboplatin than A2780 cells. We next determined the levels of IL-6 and IL-8 in ovarian cancer cells after treatment with platinum drugs. Both IL-6 and IL-8 levels were approximately 3-fold higher in a dose dependent manner after cisplatin and carboplatin drug

Table 1. Characteristics of Included Patients

Clinicopathological Parameters	Number (N=19)	Percentage (%)
Age		
Medium age (years)	52.8	
Age range	18 – 77	
< 60	12	63.1
≥ 60	7	36.8
Histopathology		
i. Serous	13	68.4
ii. Mucinous	2	10.5
iii. Endometroid	3	15.8
iv. Granulosa cell carcinoma	1	5.3
FIGO staging		
Stage I	4	21.1
Stage II	4	21
Stage III	7	36.8
Stage IV	4	21.1
Clinical response to chemotherapy		
CR or PR	7	36.8
SD or PD	3	15.8
Under treatment	6	31.6
No follow up	2	10.5
Death without chemotherapy	1	5.3

CR, Complete response; PR, Partial response; SD, Stable disease; PD, Progressive disease; Poor prognosis (recurrent, progression, drug resistance)

Table 2. The IC_{50} Values of Carboplatin in the Primary Cell Cultures of Ovarian/Fallopian Tube Cancer Patients and Their Response to Chemotherapy

Patients	IC_{50} to carboplatin	Clinical response to chemotherapy	Patients	IC_{50} to carboplatin	Clinical response to chemotherapy
Patient 1	275 ± 7.071 µM	Nonresponsive	Patient 11	125 ± 4.630 µM	Responsive
Patient 2	500 ± 3.536 µM	Responsive	Patient 12	132 ± 4.950 µM	Responsive
Patient 3	295 ± 4.681 µM	No follow up data	Patient 13	250 ± 2.828 µM	No follow up data
Patient 4	320 ± 5.090 µM	Nonresponsive	Patient 14	500 ± 3.36 uM	During treatment
Patient 5	345 ± 7.012 µM	Expired before chemotherapy	Patient 15	>500 µM	Nonresponsive
Patient 6	480 ± 5.341 µM	Responsive	Patient 16	168 ± 4.243 µM	During treatment
Patient 7	180 ± 5.657 µM	No follow up data	Patient 17	340 ± 5.657 µM	During treatment
Patient 8	175 ± 8.55 µM	During BEP regimen treatment	Patient 18	132 ± 7.638 µM	Responsive
Patient 9	500 ± 0.013 µM	Responsive	Patient 19	250 ± 4.43 µM	During treatment
Patient 10	500 ± 6.633 µM	Responsive			

The values are presented in the mean ± SD of three independent experiments. 50% inhibitory concentration (IC_{50}) was calculated from dose-response curves determined by the MTT assay. The correlation of IC_{50} to carboplatin and clinical response was analyzed by the Spearman's test and the result showed that there was no significant correlation (P=0.829).

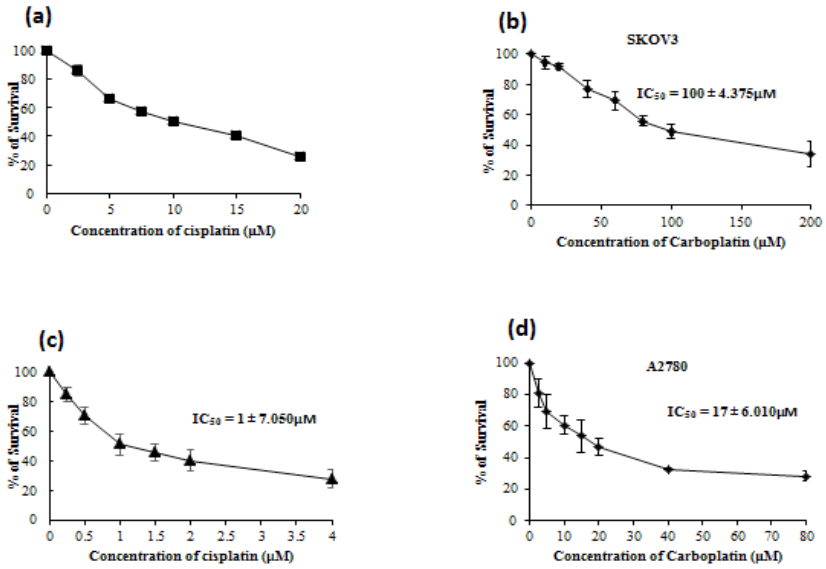

Figure 1. Sensitivity to Platinum Drugs in Two Human Ovarian Cancer Cell Lines. Natural drug resistance SKOV3 cells (a, b) and drug sensitive A2780 (c, d) were treated with different concentrations of cisplatin or carboplatin as indicated and incubated for 72 h, and then cell viability was assessed by MTT assay. Data are shown as the mean ± SD of three independent experiments.

Table 3. The Relationships of *ex-vivo* Drug Resistance Assay with Clinicopathological Features and the Clinical Responses to Chemotherapy among Ovarian/Fallopian Cancer Patients

		Ex-vivo drug resistance assay			
Clinicopathological parameters		EDR (n=6)	IDR (n=3)	LDR (n=10)	*P* value
Age (y) (n=19)	< 60 (n=12)	5	1	6	0.326
	≥ 60 (n=7)	1	2	4	
FIGO staging (n=19)	Early (n=8)	1	0	7	0.031*
	Late (n=11)	5	3	3	
Histological types (n=19)	Serous (n=13)	5	3	5	0.168
	Mucinous (n=2)	1	0	1	
	Endometroid(n=3)	0	0	3	
	Granulosa (n=1)	0	0	1	
Clinical response to chemotherapy (n=10)	Responsive (n=7)	4	0	3	0.27
	Non-responsive (n=3)	1	1	1	

EDR, extreme drug resistance; IDR, intermediate drug resistance; LDR, low drug resistance.

Table 4. The Relationships of Basal Cytokine Levels with Clinicopathological Features and the Clinical Responses to Chemotherapy among Ovarian/Fallopian Tube Cancer Patients

Clinicopathological parameters		Basal IL-6 level				Basal IL-8 level			
		Low (n=1)	Medium (n=13)	High (n=5)	P value	Low (n=2)	Medium (n=12)	High (n=5)	P value
Age (y) (n=19)	< 60 (n=12)	0	8	4	0.311	2	7	3	0.52
	≥ 60 (n=7)	1	5	1		0	5	2	
FIGO staging (n=19)	Early (n=8)	0	6	2	0.662	0	6	2	0.413
	Late (n=11)	1	7	3		2	6	3	
Histological types (n=19)	Serous (n=13)	1	8	4	0.168	2	6	5	0.077
	Mucinous (n=2)	0	2	0		0	2	0	
	Endometroid(n=3)	0	2	1		0	3	0	
	Granulosa (n=1)	0	1	0		0	1	0	
Clinical response (n=10)	Responsive (n=7)	0	6	1	0.49	1	4	2	0.788
	Non-responsive (n=3)	0	2	1		0	2	1	
Ex-vivo drug sensitivity	EDR (n=6)	0	5	1	0.172	2	3	1	0.298
(n=19)	IDR (n=3)	1	1	1		0	2	1	
	LDR (n=10)	0	7	3		0	7	3	

EDR, extreme drug resistance; IDR, intermediate drug resistance; LDR, low drug resistance.

treatment in SKOV3 cells (Figure 2a-d). However, there was no detectable cytokine production in drug sensitive A2780 cells (Figure 2e-h).

Patients

The characteristics of the patients are summarized in Table 1. The median age of the enrolled patients was 52.8 (18-77) years. Among these 19 patients, 12 (63.1%) were under 60 years old, 11 (57.9%) were at advanced stage III and IV and 13 (68.4%) were diagnosed as having serous type adenocarcinoma. After performing platinum-based chemotherapy on ten ovarian cancer patients, seven (70%) patients displayed a complete or partial response to chemotherapy, and three (30%) patients showed a level of progression and resistance to platinum-based chemotherapy. Six patients were still receiving chemotherapy treatment and had not yet finished the course. There was insufficient follow-up information on two patients and one patient expired before chemotherapy could be initiated, and these patients were excluded from analysis. Of the 19 patients who underwent platinum-based chemotherapy after surgery, ten cases met the criteria for statistical evaluation.

Table 5. The Relationships of Cytokine Enhancement in Response to Carboplatin with Clinicopathological Features and the Clinical Responses to Chemotherapy among Ovarian/Fallopian Tube Cancer Patients.

Clinicopathological parameters		IL-6 level after carboplatin treatment (72 h) in primary cell culture			IL-8 level after carboplatin treatment (72 h) in primary cell culture		
		No or very little change (n=12)	Increased (>2 fold) (n=7)	P value	No or very little change (n=11)	Increased (> 2 fold) (n=8)	P value
Age (y) (n=19)	< 60 (n=12)	7	5	0.568	7	5	0.96
	≥ 60 (n=7)	5	2		4	3	
FIGO staging	Early (n=8)	7	1	0.061	7	1	0.026*
(n=19)	Late (n=11)	5	6		4	7	
Histological types (n=19)	Serous (n=13)	7	6	0.216	6	7	0.127
	Mucinous (n=2)	2	0		2	0	
	Endometroid (n=3)	3	0		2	1	
	Granulosa (n=1)	0	1		1	0	
Clinical response to chemotherapy (n=10)	Responsive (n=7)	7	0	0.016*	5	2	0.038*
	Non-responsive (n=3)	1	2		0	3	
Ex-vivo drug sensitivity (n=19)	EDR (n=6)	4	2	0.502	3	3	0.473
	IDR (n=3)	1	2		1	2	
	LDR (n=10)	7	3		7	3	

EDR, extreme drug resistance; IDR, intermediate drug resistance; LDR, low drug resistance

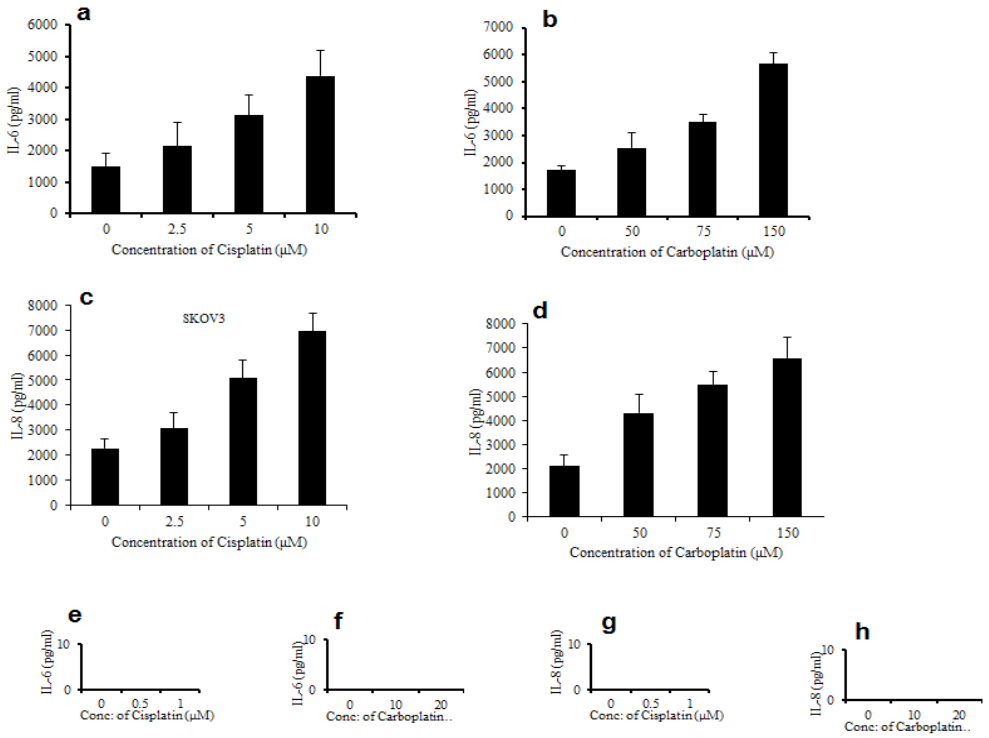

Figure 2. Platinum-Induced Cytokine (IL-6 and IL-8) Secretion in Ovarian Cancer Cell Lines. The SKOV3 (a-d) and A2780 (e-h) ovarian cancer cells were treated with different concentrations of cisplatin or carboplatin for 48 h, and then the supernatant was collected to determine drug induced cytokine production by ELISA. Data are presented as the mean ± SD of three independent experiments. *p<0.05, **p<0.01 compared with the untreated control.

Ex-vivo drug resistance assay versus clinical responses

We studied the sensitivity to the platinum-based chemotherapeutic drug, carboplatin, in the primary cell cultures obtained from ovarian cancer patients, as no effects had yet been observed in individual patients. IC50 of carboplatin in ovarian/fallopian cancers from patients were shown in Table 2. There was no significant relationship between IC50 of carboplatin in the primary culture cells and clinical response (P=0.829). We then grouped the degree of drug resistance into EDR, IDR and LDR based on IC50 values and compared the impact of ex-vivo drug resistance assay on the clinical response to

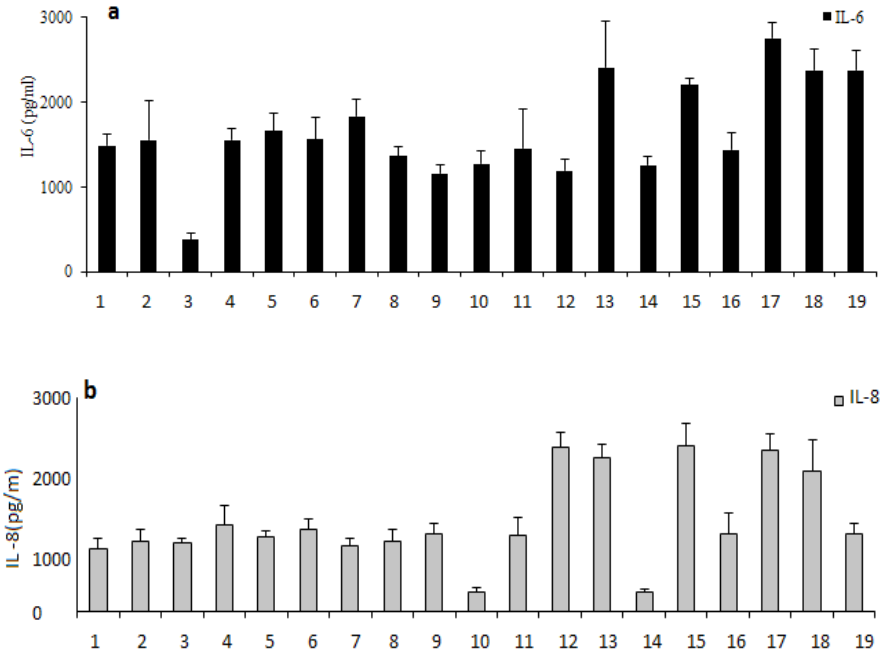

Figure 3. Basal level of cytokine (IL-6 and IL-8) production in primary cell culture from ovarian/fallopian tube cancer patients. The supernatant of the primary culture cells were collected to determine the basal level of cytokines production by ELISA. Data are presented as the mean ± SD of three independent experiments.

chemotherapy in ovarian cancer patients (Table 2 and 3). Our findings on the ex-vivo drug resistance assay results reveled that extreme drug resistance was not associated with age, histological types and clinical responses, but it was significantly associated with late stage ovarian cancer (P=0.031).

Cytokine production in primary culture cells versus clinical responses

Since cytokines, especially IL-6 and IL-8, play a role in the drug resistance of various malignant diseases including ovarian carcinoma, we determined the level of basal cytokine production in the primary cell cultures of individual patients. The primary culture cells obtained from the patients can produce IL-6 and IL-8 cytokines at various levels (Figure 3). Patients with considerable amounts of basal IL-6 also revealed a substantial IL-8 level. We determined the correlation coefficient between cytokine concentration and IC50 to carboplatin in primary culture cells by using bivariate Pearson correlation. We found that there was no significant correlation between either IL-6 or IL-8 concentrations and IC50 to carboplatin in primary culture cells (supplemental Figure 1). In our study, a basal cytokine level of more than 1 SD above the median was identified as high basal cytokine production. A basal cytokine level of less than 1 SD below the median was classified as low basal cytokine production. Then, we determined the relationships between basal cytokine secretion and the clinical parameters. Our data indicated that baseline IL-6 and IL-8 levels in the primary cell cultures did not correlate with the age, stages, histological types, ex-vivo drug resistance assay and the clinical responses (Table 3 and 4).

The relationships of cytokine enhancement in response to carboplatin with clinicopathological features in ovarian/fallopian tube cancer patients are shown in Table 4 and 5. It was found that carboplatin-induced IL-6 and IL-8 production had a significant association with a clinical response to chemotherapy (P=0.016 and P=0.038, respectively). There was a statistically significant association between carboplatin-induced IL-8 overproduction and FIGO staging III-IV (P=0.026), but not with carboplatin-induced IL-6 and FIGO staging (P=0.061). The overproduction of cytokines in response to carboplatin was not correlated with age, histological types and ex-vivo drug resistance assay.

Discussion

Preclinical studies involving cancer cell lines have played a significant role in the understanding of tumor biology and the screening for chemotherapeutic drug development. Nonetheless, since immortal cancer cell lines under in vitro conditions have been poorly represented in clinical scenarios (Kirk, 2012), the derivation and short-term culture of primary cells from solid tumors have become of significant importance in personalized cancer therapy treatments (Mitsiades et al., 2011; Schilsky, 2010; Trusheim et al., 2011).

In the present study, we used primary cell culture methods to determine the sensitivity to the platinum-based chemotherapeutic drug, carboplatin, as the effects observed in individual patients had not yet been recorded. This information would be needed in order to evaluate whether or not the anticancer drug efficacy identified in the ex-vivo studies could be inferred to clinical trials and beyond. Since the IC50 values in patients were varied so much individually, the degree of drug resistance probably at least could be grouped into EDR, IDR and LDR based on IC50 values (Parker et al., 2004 and Lyon et al., 2009). Our findings on ex-vivo drug resistance assay results reveled that extreme drug resistance was significantly associated with the advanced stages of ovarian cancer (P = 0.031). However, it does not independently predict or alter the outcomes of patients with epithelial ovarian cancer who had been treated with the current standards of primary cytoreductive surgery followed by platinum-based chemotherapy.

A number of previous studies have compared ex-vivo chemosensitivity and the clinical outcomes in epithelial ovarian cancer patients. Our results and those of some previous studies (Karam et al., 2009; Matsuo et al., 2010) indicated that there was no significant association between the assay results and the clinical outcome, but on the other hand, other studies claimed that significant associations were found between the assay results and various types of short- or long-term clinical endpoints (Gallion et al., 2006; Holloway et al., 2002; Matsuo et al., 2009). In many of these studies, the sample types, staging and study populations were markedly heterogeneous, which might elucidate the lack of a relationship between ex-vivo drug activity and the clinical outcomes in some of the cases. It has been proposed that the different subtypes may characterize different diseases (Soslow, 2008; Kobel et al., 2008). We studied various histological types and stages in studies involving small populations and this has become one of the limitations of our study. Accordingly, ex-vivo assays should be established and evaluated separately for each of these subtypes to improve their performance.

Tumor cells synthesize and secrete various cytokines, proteins and growth factors, which can act in a manner associated with autocrine/paracrine in the stimulation of tumor cell proliferation and drug resistance. For example, IL-6 contributes to EOC progression by the inhibition of apoptosis, the stimulation of angiogenesis, increased migration and invasion rates, and the stimulation of cell proliferation (Ding et al., 2016; Guo et al., 2010; Monique et al., 2005) leading to chemotherapy resistance (Duan et al., 1999; Johnson et al., 1993). Furthermore, elevated IL-6 levels measured in the ascites fluid of EOC patients were correlated with shorter progression-free survival rates (Denis et al., 2011; Lo et al., 2011; Samar et al., 2015). Some studies have stated that the increase of the IL-6 serum level appears to be a late-stage phenomenon in cancer patients (Bozena et al., 2013) and serum levels of IL-6, IL-8 and CRP serve as prognostic factors in epithelial ovarian cancer patients (Bodo and Robert, 2016). IL-6, and/or IL-8 produced locally by the tumor may identify a subset of patients who are at the greatest risk for treatment failure in the primary cell culture of endometrial carcinoma (Harriet et al., 2013).

In this study, we determined whether cytokines were

synthesized locally by ovarian tumor cells, and also determined if production rates were correlated with the clinical outcomes. To our knowledge, this is the only study of cytokine production in primary cell cultures derived from fresh ovarian cancer tissues. Baseline IL-6 and IL-8 levels in the primary cell cultures did not correlate with the age, stages, histological types, ex-vivo drug resistance assays and clinical responses. Nonetheless, the positive expression of IL-8 in response to carboplatin was associated with the tumor stage (P=0.026), but was not correlated with age, histological types and ex-vivo drug resistance assays. Early stage cancer cells grown locally do not disseminate, whereas advanced stage cancerous cells have more aggressive features leading to their dissemination to other sites. We believe that cytokines might be one of the precipitating factors for tumor aggressiveness and, therefore, IL-8 overproduction can be associated significantly with the advanced stages of ovarian cancer. When patients who later showed resistance to platinum-based chemotherapy treatments were accordingly compared with those who remained sensitive to the same regimens, IL-6 enhancement was found in two out of three non-responsive patients (P=0.016), and IL-8 enhancement was found in all non-responsive three patients (P=0.038).

In conclusion, based on our findings, the cytokine overproduction in response to carboplatin in the primary cell cultures can serve as a predictive marker for the therapeutic outcomes in ovarian or fallopian tube cancer patients. However, ex-vivo drug resistance assay and basal cytokine secretion cannot serve as predictive markers for the therapeutic outcomes. Large prospective studies with specific histology subtypes are required for further evaluation in order to understand the underlying mechanisms and the role of cytokines in the drug responses among ovarian cancer patients and to find personalized approaches in the treatment of ovarian cancer so as to offer the patient a better quality of life and a longer survival rate.

Statement conflict of Interest

There are no conflicts of interest with respect to the research, authorship, and/or publication of this article.

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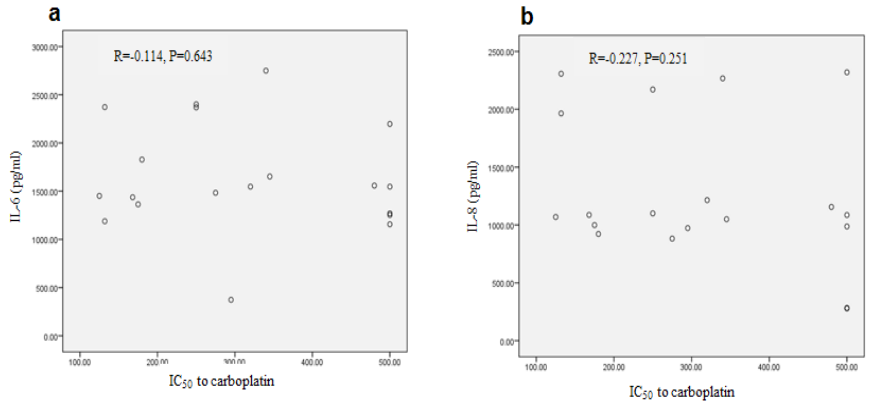

Supplement Data Figure 1. Correlations between Cytokines (IL-6 and IL-8) Concentration and IC50 to Carboplatin in Primary Cell Culture from Ovarian/Fallopian Tube Cancer Patients. The correlation was analyzed by the Pearson's test. The plots show that there was no significant correlation between IL-6 and IC50 to carboplatin (P=0.643) as well as between IL-8 and IC50 to carboplatin (P=0.251) in primary culture of ovarian/fallopian tube cancer patients.