Effect of culture conditions on the chemosensitivity of ovarian cancer cell lines

Augusta Fernando, Sharon Glaysher, Mark Conroy, Marcin Pekalski, Jason Smith, Louise A. Knight, Federica Di Nicolantonio and Ian A. Cree

The aim of this study was to define the chemosensitivity profile of a series of human ovarian cancer cell lines representing the human primary ovarian tumours under altered culture conditions and to compare the results with those from tumour-derived cells. In this study, we used a standardized ATP-based tumour chemosensitivity assay to measure the activity of cytotoxics in the seven ovarian carcinoma cell lines and ovarian tumour-derived cells. The use of adherence-free polypropylene plates and a serum-free medium slowed down cell proliferation in all cell lines tested, mimicking the slow growth rate of solid tumours in this type of plastic. The seeding density was optimized for each cell line and was in the range of 2000–4000 cells/well. Heterogenous sensitivity to different cytotoxics was observed in the seven ovarian cancer cell lines tested in the ATP-based tumour chemosensitivity assay. The human ovarian carcinoma cell line, OVCA433, was found to be the most resistant cell line and 75% of the drugs showed an $Index_{SUM}$ above 300. Our results suggest that the use of appropriate culture conditions i.e. a serum-free culture environment, adherence-free growth and optimum seeding density can induce cell lines to behave more like tumour-derived cells in response to cytotoxic agents. On the basis of the comparison of chemosensitivity profiles of tumour-derived cells and cell lines derived from the corresponding tumour, a panel of cell lines can be selected. Such a panel could be used to screen and develop anticancer drugs. *Anti-Cancer Drugs* **17:913–919**

Anti-Cancer Drugs 2006, **17**:913–919

Keywords: chemosensitivity, ovarian cancer cell lines, serum-free culture conditions

Translational Oncology Research Centre, Queen Alexandra Hospital, Portsmouth, UK.

Correspondence to I.A. Cree, Translational Oncology Research Centre, Queen Alexandra Hospital, Portsmouth PO9 6AH, UK.
Tel: +44 23 92 286378; fax: +44 23 92 286379; e-mail: ian.cree@porthosp.nhs.uk.

Sponsorship: This work was supported by the Lord Dowding Fund.

Received 13 February 2006 **Accepted** 10 May 2006

Introduction

Cancer research organizations and pharmaceutical companies rely on panels of cell lines and xenograft models to determine the efficacy of new anticancer agents [1,2]. These screening programmes work on the premise that in-vitro drug sensitivity will correlate with in-vivo performance. Drug discovery is an important element in transforming preliminary biological research into making available treatment for cancer patients in the clinics. Human tumour cell lines have played an important role in understanding cancer, and have been extensively used in the discovery and characterization of new chemotherapeutic drugs. A potential weakness of such cell lines is that they may have lost important properties that the tumour originally possessed *in vivo*, including potential targets for therapy [3].

The use of cultured cell lines has several advantages: they are relatively inexpensive to maintain and provide a limitless supply of cells for experimental use. The current in-vitro drug screening panels used by the National Cancer Institute [4] and by most pharmaceutical companies are composed of human tumour cell lines derived from multiple sequential in-vitro subcultures of human tumour explants, then passaged repeatedly over many generations. Cells with a higher growth capacity in serum-containing media rapidly predominate, giving rise to a homogenous cell population [5]. In the National Cancer Institute screening programme, the candidate drugs are tested for efficacy on three cell lines from a panel of 60 lines, representing all the major human cancers. Active compounds progress to be tested against a panel of human tumour xenografts in nude mice. If successful, the drugs enter phase I and phase II clinical trials.

In contrast to cell lines, the tumour neoplastic cell phenotype is influenced by the cytokine environment within the tumour and often by direct contact with nonmalignant cells. This leads to differences between cell lines and tumour-derived cells that are reflected by differences in sensitivity to cytotoxic drugs [6]. For instance, colorectal adenocarcinoma rarely responds to anthracyclines such as doxorubicin as these tumours show constitutive expression of MDR1. Many colorectal cell lines, however, show sensitivity to these agents due to a loss of MDR1 expression under prolonged cell culture or in heterotopic sites owing to loss of modulation by cytokines from non-neoplastic cells [7,8].

0959-4973

Cell lines consist of a homogenous population of rapidly proliferating cells and thus there is a degree of uniformity in the cell population. In contrast, tumour cells taken directly from the patient generally contain a mixed population of malignant and nonmalignant cells. This may lead to a change in the character of cell lines from their originating tumour, due to changes from the tumour to a culture environment [7,8]. In addition, some cells may proliferate *in vitro* that would not normally proliferate *in vivo*. The relevance of drug efficacy results obtained in cell lines thus becomes difficult to compare with drug efficacy results obtained from patients.

The differential sensitivity of cell lines and tumour-derived cells to anticancer drugs may be explained by the homogeneity of cell lines and by genetic drift during the many passages required to obtain homogeneous cell lines, but the differences in the percentage of growing cells may also be important. It is usually necessary to set up tumour-derived cells at 10 times the plating density of cell lines. Solid tumour growth rates are low: breast cancers have around 10% of cells in S phase [9], whereas cell lines have much greater proliferation fractions than tumour-derived cells [10]. As most anticancer agents target growing cells, this means that cell lines are usually much more sensitive than tumour-derived cells, as we have shown in previous studies [6,10,11]. The result is that new anticancer agents developed using cell line screens tend to target proliferation, which is just one of the hallmarks of cancer [12].

It is clearly difficult to change the genetics of cells lines back to something closer to their originating tumours. Nor is it possible or desirable to alter the homogeneity and ease of growth of large numbers of cells for experiments. It is, however, possible to alter the environment in which cell lines are tested to develop anticancer drugs. We hypothesized that by altering the environment in which cells are cultured, it would be possible to induce cell lines to behave more like tumour-derived cells and to develop a panel of cell lines whose chemosensitivity is representative of their corresponding tumour type under defined conditions. We chose to look first at ovarian cancer as we have considerable experience of testing tumour-derived ovarian cells in the ATP-based tumour chemosensitivity assay (ATP-TCA) and a large data set for comparison with cell line data, some of which we have published previously [13,14].

Methods

Cell lines

The ovarian cancer cell lines 1847, 1847ad, OVCAR-3, OVCA433, jama2 and SKOV-3, ovarian cell lines (obtained from Cancer Research UK, Sutton, UK) and OAW42 (obtained from ECACC, Salisbury, UK) were cultured in Dulbecco's modified Eagle's medium (Sigma, Poole, UK; Cat. No. D6171) supplemented with 10% heat-inactivated fetal calf serum (FCS) (Biowest, Ringmer, UK; Cat. No. S185H), 2 mmol/l L-glutamine (Sigma; Cat. No. G7513) and 50 μg/ml penicillin/streptomycin (Sigma; Cat. No. P0781), in 75 cm^2 polystyrene flasks (Corning Life Sciences, High Wycombe, UK; Cat. No. 430641) and maintained at 37°C in a humidified atmosphere with 5% CO_2. Growth and morphology were monitored and cells were passaged when they had reached 90% confluence. All cell lines were screened regularly for mycoplasma using the PCR Mycoplasma Detection set (TaKaRa Bio Healthcare, Shiga, Japan; Cat. No. TAK-6601) and found to be mycoplasma-free.

Tumour-derived cells

Cells were obtained from surgical resection material or ascitic fluid over the last 5 years, many as part of the series previously reported [13,14]. The majority of tumours represented recurrent ovarian cancer following previous platinum therapy. All tissue samples were obtained with written informed consent, and the study was approved by the Multi-Centre Research Ethics Committee. Only tumour surplus to diagnostic requirements was used in these assays to avoid compromising the histopathology or cytological diagnosis of malignancy. Specimens were transported to the laboratory in sterile 25-ml universal containers or 250-ml bottles containing a basic transport medium (Dulbecco's modified Eagle's medium, Sigma-Aldrich, Poole, Dorset UK) with penicillin, streptomycin and gentamicin as additives. Ascites bottles also contained 5000 IU heparin sulphate. The samples were immediately packed into chilled polystyrene containers and transported by courier to arrive in the laboratory (Queen Alexandra Hospital, Portsmouth, UK) within 24 h. All specimens were confirmed for malignancy by histology or cytology. The number of samples tested for each drug or combination varied depending upon the number of cells available for testing, and their clinical priority for testing. No tumours were therefore tested with methotrexate or vincristine, and small numbers with 5-fluorouracil (5-FU) and 4-hyroxycyclophosphamide.

ATP-based tumour chemosensitivity assay

The ATP-TCA was performed as previously published [13,15,16]. Cell line cultures were grown to confluence, cells were harvested using trypsin ethylene diaminetetraacetic acid (Sigma; Cat. No. T4174) and then checked for viability before ATP-TCA.

A single-cell suspension obtained from cell lines were then plated in 96-well polypropylene plates (Corning; Cat. No. 3790) at an optimum seeding density established for each cell line in a serum-free complete assay medium (CAM; DCS Innovative Diagnostik Systeme, Hamburg, Germany) by comparing the ATP results from cells seeded at 4000, 2000, 1000 or 500 cells per well.

Drugs were added to triplicate wells at serial dilutions corresponding to 6.25–200% of a test drug concentration (TDC) estimated from pharmacokinetic data, including the degree of protein binding. All TDCs were within clinically achievable levels. Two controls were included in each plate: a no drug control consisting of media only (MO), and a maximum inhibitor (MI) control which killed all cells present. The plates were incubated for 6 days at 37°C with 5% CO_2. At the end of the incubation period, remaining cells were lysed by addition of cell extraction reagent (DCS Innovative Diagnostik Systeme). An aliquot of the lysate from each well was added to the corresponding wells of a white 96-well microplate (Thermo Life Sciences, Basingstoke, UK), followed by addition of luciferin–luciferase reagent (DCS Innovative Diagnostik Systeme). The light output corresponding to the level of ATP present was measured in a luminometer (MPLX, Berthold Detection Systems, Hamburg, Germany). All cell line experiments were performed at least three times.

Drugs

The panel of drugs tested was chosen to include most classes of cytotoxic drug and those used commonly with in previous ATP-TCA experiments with tumour-derived cells. Alkylating agents included 4-hydroxycyclophosphamide, tested as the active metabolite of cyclophosphamide and ifosfamide, and treosulphan (Medac, Hamburg, Germany), an alkylating agent with a different mechanism of action [17]. The antimetabolites 5-FU (Faulding Pharm, Royal Leamington Spa, UK), methotrexate (Pharmacia, Walton-on-the-Hill, UK) and gemcitabine (Eli Lilly, Basingstoke, UK) were tested, as were the spindle active agents, paclitaxel (Bristol-Myers Squibb, Uxbridge, UK), vincristine (Eli Lilly), and vinorelbine (Pierre Fabre, Winchester, UK). Topotecan (Merck Pharmaceuticals, West Drayton, UK) was included as a representative topoisomerase inhibitor, and epirubicin (Pharmacia) as a representative topoisomerase IIα inhibitor. The list included one combination: cisplatin (Approved Prescription Services, Eastbourne, UK) and gemcitabine, as gemcitabine has been shown to reverse resistance to cisplatin, probably mediated by increased DNA repair [18].

All drugs or combinations were tested in triplicate at six dilutions corresponding to 200, 100, 50, 25, 12.5 and 6.25% of the TDC estimated from pharmacokinetic data to be clinically achievable in patients [13,16]. TDCs for each drug were as follows: 4-hydroxycyclophosphamide – 10.2 μmol/l, treosulfan – 72 μmol/l, cisplatin – 10 μmol/l, gemcitabine – 40 μmol/l, 5-FU – 346 μmol/l, methotrexate – 6.1 μmol/l, vinorelbine – 15 μmol/l, paclitaxel – 16 μmol/l, epirubicin – 0.86 μmol/l and topotecan – 1.64 μmol/l. Combinations were tested by simultaneous addition. All drugs were obtained from the Queen Alexandra Hospital pharmacy (Portsmouth, UK).

Data analysis

Data from the luminometer were transferred automatically to an Excel spreadsheet where the results were expressed as a percentage inhibition at each of the six TDCs tested. Inhibition was calculated using the equation; $1-(\text{test}-\text{MI})/(\text{MO}-\text{MI}) \times 100$. IC_{50} and IC_{90} were determined, and area under the concentration-inhibition curve ($Index_{AUC}$) values were calculated from the data using the trapezoidal rule. Previous ATP-TCA studies have found that a natural logarithmic sum index ($Index_{SUM}$) calculated by direct addition of the percentage survival at each concentration tested ($Index = 600 - \sum\%Inhibition_{6.25\ldots200}$) provides a better indication of sensitivity or resistance to different drugs in cell lines [13,16]. Total inhibition of growth results in an index of 0 and no inhibition of growth at any concentration produces an index of 600. All experiments were performed three times and judged acceptable if the results showed a coefficient of variation below 25%. The results of each experiment were entered into an access database for further analysis and compared with existing data [14] for tumour-derived cells using descriptive statistics. Further statistical tests (Analyse-It Software, Leeds, UK) were performed when direct comparisons were necessary: the Wilcoxon rank-sum test was used to compare paired series.

Results

Effect of serum and adherence on cell line proliferation

Preliminary experiments were performed to determine the effect of serum concentration and polystyrene or polypropylene on cell proliferation. The use of adherence-free polypropylene plates slows down cell proliferation in all cell lines tested compared with polystyrene (Wilcoxon, $P < 0.0001$). As shown in Fig. 1, serum addition (1%) significantly increased ATP levels in polystyrene plates (Wilcoxon, $P < 0.02$), whereas there was a strong statistical trend for the seven cell lines studied in polypropylene (Wilcoxon, $P < 0.07$, NS). All the cell lines chosen for study were able to survive and grow to a limited extent in polypropylene plates in serum-free CAM. The optimum seeding density, usually higher than would normally be required in serum-containing cell culture, was established individually for each cell line. Optimal ATP values were obtained for 1847ad, OVCAR433 and OVCAR3 at 2000 cells per well, and at 4000 cells per well for the remaining lines.

Effect of serum on chemosensitivity

The effect of the increased growth rate on chemosensitivity was examined in each of the ovarian cancer cell lines tested, all of which showed greater sensitivity to the agents tested in the presence of 10% FCS. As an example, the results for OVCA433 are shown in Fig. 2. In serum, this cell line was found to be more sensitive to all of the drugs tested, particularly paclitaxel, which had an IC_{90} of

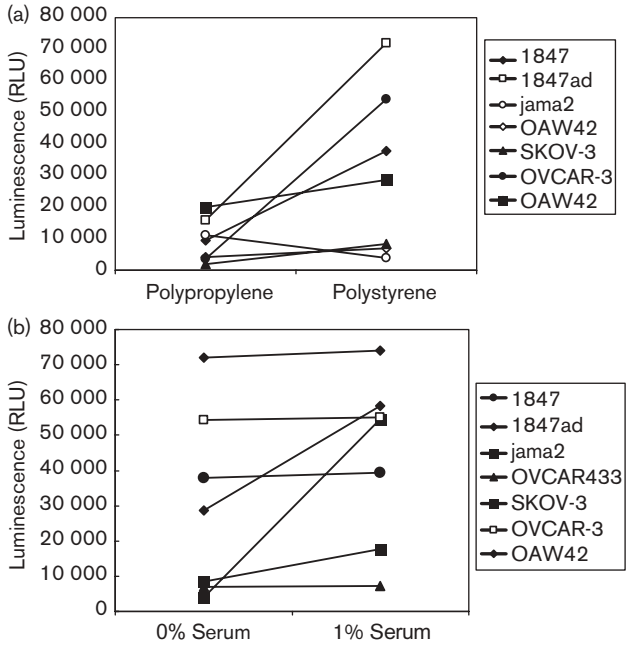

Fig. 1

(a) The effect of polypropylene versus polystyrene growth of all seven cell lines grown in standard growth medium [(RPMI + 10% fetal calf serum (FCS), Wilcoxon, $P < 0.0001$]. (b) The effect of addition of just 1% serum on the growth of all seven cell lines in standard polystyrene plates with standard growth medium (RPMI + 10% FCS). There is a large effect on two of the cell lines, with a relatively small effect on the remainder, although all seven show a rise in cell growth in this low serum concentration (Wilcoxon, $P < 0.02$). RLU, relative light units.

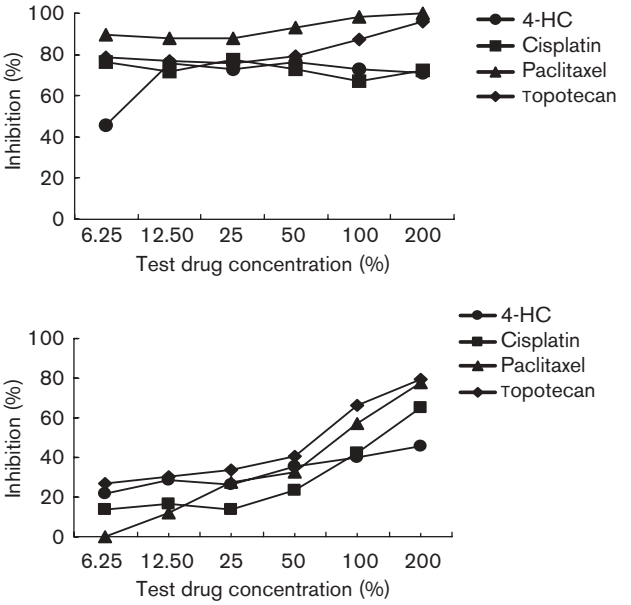

Fig. 2

(a) The ATP-based tumour chemosensitivity assay (ATP-TCA) inhibition curves for human ovarian cancer cell line, OVCA433 exposed to 4-hydroxycyclophosphamide (4-HC), cisplatin, paclitaxel and topotecan performed in the presence of 10% fetal calf serum. (b) The ATP-TCA inhibition curves for human ovarian cancer cell line, OVCA433 exposed to 4-HC, cisplatin, paclitaxel and topotecan in serum-free complete assay medium (CAM). Comparisons for each drug in RPMI + 10% serum versus CAM are statistically significant (Wilcoxon, $P < 0.04$).

5.5 μmol/l, in comparison in serum-free medium OVCA433 had an IC_{90} of 38.1 μmol/l. OVCA433 cells, treated with topotecan, had IC_{90} values of 2.1 and 3.7 μmol/l in serum-containing medium and serum-free medium, respectively (Wilcoxon, $P < 0.04$).

ATP-based tumour chemosensitivity assay results

The seven ovarian cancer cell lines tested in the same serum-free medium used with the cell lines showed heterogenous sensitivity to the different cytotoxic drugs tested in the ATP-TCA (Table 1). Even with the changed culture conditions, most cell lines still showed increased sensitivity to the cytotoxics tested when compared with the 95% confidence intervals of the tumour-derived cell data for each drug (Table 1). OVCA433 was found to be the most resistant cell line and only 25% of the drugs tested showed an $Index_{SUM}$ over 300.

Considerable heterogeneity of sensitivity exists between different cell lines to the different agents tested, as shown in Table 1. Cisplatin, methotrexate, vincristine, paclitaxel, epirubicin and topotecan exhibit greater activity with IC_{50} values below 1 μmol/l in the ovarian cancer cell line 1847, and methotrexate, vinorelbine, vincristine, epirubicin and topotecan show greater sensitivity with IC_{50} values below 1 μmol/l in the ovarian cancer cell line jama2. In the ovarian cancer cell line OVCA433, only vincristine and epirubicin show IC_{50} values below 1 μmol/l. The cell lines 1847, SKOV-3, OVCAR3 and OAW42 are particularly sensitive to treosulfan. OAW42 showed greatest chemoresistance to vinca alkaloids, paclitaxel and epirubicin.

Cisplatin is active as a single agent in four of seven cell lines (Table 1). Of the three cell lines (1847ad, jama2 and OVCA433) resistant to single agent cisplatin (defined as $Index_{SUM} > 300$), the addition of gemcitabine in combination with cisplatin increases their sensitivity as shown by a marked decrease in the $Index_{SUM}$ and IC_{50} or IC_{90} (Table 1), with above 90% inhibition for all concentrations tested. Of the seven cell lines tested in combination for cisplatin and gemcitabine, all show increasing sensitivity to this combination (Wilcoxon, $P < 0.04$).

Table 1 Comparison of the sensitivity of seven ovarian cancer cell lines to all 12 agents tested in comparison with ovarian tumour-derived cells, using the same serum-free media (median IC_{50} μmol/l, IC_{90} μmol/l and $Index_{SUM}$ shown)

Cells	1847	1847ad	jama2	OVCA433	OVCAR3	OAW42	SKOV-3	Ovarian tumour-derived cells (95% CI)
4-HC								
IC_{50}	3.8	7.6	15	25	0.4	0.3	–	14.9 (10.1–39.2)
IC_{90}	17	18.9	30	131	14	18.3	36.7	27.1 (21.1–70.5)
$Index_{SUM}$	317	308	**436**	414	71	137.4	410	493 (428–516) ($n=10$)
Treosulfan								
IC_{50}	3.6	NA	47	70	2.6	3.6	19.8	47.2 (42.3–51.7)
IC_{90}	70	142	139	166	36.4	218	161.8	125.0 (114.3–132.2)
$Index_{SUM}$	107	320	**352**	**374**	64	173	267	361 (343–375) ($n=205$)
Cisplatin								
IC_{50}	0.7	20.8	5.2	13.4	0.4	18.1	2.1	13.7 (11.9–14.6)
IC_{90}	11	37.5	23.8	31	8.9	0.55	18.7	25.8 (24.6–27.2)
$Index_{SUM}$	134	376	371	419	74	206	246	468 (449–482) ($n=225$)
Cisplatin + gemcitabine[a]								
IC_{50}	2	1.6	1.8	2.0	1.6	1.9	2.1	0.6 (0.5–0.6)
IC_{90}	3	2.8	5	5.0	2.9	47.1	16.3	4.5 (3.7–5.3)
$Index_{SUM}$	1	2.0	34	34	6.8	126	61	110 (95–130) ($n=193$)
Gemcitabine								
IC_{50}	1.2	1.3	3.0	2.1	1.3	1.4	1.7	2.8 (2.4–4.8)
IC_{90}	2.0	2.3	27	16.8	2.4	82.0	89	87.7 (82.5–91.7)
$Index_{SUM}$	1.5	1.5	125	80	23.2	69	129	200 (172–224) ($n=220$)
5-FU								
IC_{50}	13.8	65.6	38	325	6.1	21.2	439	148.7 (83.0–221.4)
IC_{90}	69	167.4	448	732	127.1	229.1	912	529.2 (339.0–664.1)
$Index_{SUM}$	54	205	170	321	66	127	476	299 (252–366) ($n=21$)
Methotrexate								
IC_{50}	0.3	0.2	0.2	17	0.2	0.2	0.27	NA
IC_{90}	1.5	0.4	3.5	31	13.4	0.4	17.4	
$Index_{SUM}$	90	34	93	426	92	46	181.1	
Vinorelbine								
IC_{50}	1	NA	0.7	5.6	10.4	13.8	0.8	1.9 (0.9–3.5)
IC_{90}	18	28.8	29	27	0.7	51.2	20.8	20.5 (15.4–24.2)
$Index_{SUM}$	147	191	149	256	93	468	164	199 (182–218) ($n=170$)
Vincristine								
IC_{50}	0.1	0.9	0.08	0.8	0.7	21.1	0.06	NA
IC_{90}	1	1.5	0.09	1.8	0.8	1.6	1.57	
$Index_{SUM}$	242	400	235	470	112	386	229	
Paclitaxel								
IC_{50}	0.7	0.7	1.1	13.1	0.8	20.6	0.7	9.6 (8.1–11.1)
IC_{90}	13	16.6	12.3	38	27.3	31.0	10.3	26.4 (23.9–28.3)
$Index_{SUM}$	100	90	154	428	153	388	93	350 (333–372) ($n=211$)
Epirubicin								
IC_{50}	0.4	3.7	0.1	0.6	0.04	1.6	0.9	NA
IC_{90}	2.0	6.6	1.7	1.3	1.5	2.9	3.1	
$Index_{SUM}$	313	586	211	383	127	415	316	
Topotecan								
IC_{50}	0.1	NA	0	1.1	0.06	0.06	0.4	0.8 (0.7–0.9)
IC_{90}	0.4	1.3	3.3	3.7	0.3	4.0	5.0	3.4 (3.2–3.5)
$Index_{SUM}$	100	167	190	320	60	162	284	324 (308–343) ($n=201$)

Figures shown in bold are within the 95% confidence intervals for tumour-derived cells.
NA, data not available; CI, confidence interval; 4-HC, 4-hydroxycyclophosphamide.
[a]Figures for cisplatin + gemcitabine given as μmol/l cisplatin.

In comparison with the median sensitivities shown for tumour-derived cells, the cell lines 1847, jama2 and OVCA433 show the closest profiles. A particular problem exists with drugs like treosulfan, 5-FU, methotrexate, vinorelbine and topotecan, to which most cell lines are more responsive in comparison with tumour-derived cells (Table 1).

Discussion

Cell lines are generated from primary carcinoma cells and adapt to the cell culture environment, thereby developing different characteristics to the primary tumour from which they were obtained. As a result, cell lines are usually much more sensitive to chemotherapeutic agents than tumor-derived cells [19]. The use of a serum-free

CAM and polypropylene plates that do not support cell adherence, however, reduces the growth rate of cell lines causing them to respond in a manner more similar to tumour-derived cells [10]. In this study, seven ovarian cancer cell lines were screened against 11 cytotoxics and one combination using the ATP-TCA, and the results compared with those previously obtained from tumour-derived cells. The drugs tested include representatives of those currently used for ovarian cancer treatment [20,21]. Currently, the standard of care in the UK is single-agent carboplatin, followed by paclitaxel or platinum + paclitaxel on first relapse, with topotecan or liposomal doxorubicin often used in later relapses (National Centre for Clinical Excellence, 2005) [22]. It should be noted that carboplatinum and cisplatin show similar equimolar activity in the ATP-TCA [16], and we have used cisplatin in the ATP-TCA.

The heterogeneity of ovarian cancer chemosensitivity is apparent clinically and in studies with tumour-derived cells, both primary [23] and recurrent [13,14]. Similar heterogeneity becomes apparent between cell lines, particularly cultured under serum-free conditions.

Long-term survival of patients with stage III or IV ovarian cancer is currently unusual and there is a need to develop better drugs for this disease [24–26]. Most of the agents available, and those tested here, are most effective against dividing cells. The identification of cell lines in this study with resistance to the commonly used drugs provides a potentially useful tool to identify active agents for future drug development. Some cell lines, such as OVCAR-3, are very sensitive to existing drugs and therefore of limited value. Other cells lines show greater resistance: Lincet *et al.* [27] studied the effects of cisplatin on cell line growth and apoptosis, and found SKOV-3 cells more resistant to cisplatin than OAW42, just as we have shown here.

Gemcitabine and cisplatin chemotherapy demonstrates synergistic activity against platinum-resistant ovarian carcinoma *in vitro* [18]. It is therefore hardly surprising that the combination of cisplatin and gemcitabine is active in all the cell lines tested, even those showing cisplatin resistance. Others have demonstrated activity with gemcitabine alone or in combination with cisplatin for the treatment of ovarian cancer, including recurrent ovarian cancer. Rose *et al.* [28] and Nagourney *et al.* [29] have used different assays to show that gemcitabine may re-sensitize cancer cells to cisplatin therapy in platinum-resistant patients, and we have shown similar effects in tumour-derived cells in serum-free medium. Cell lines have not previously been studied in serum-free medium with this combination, but our data show that this mimics the effects seen in other systems.

Our results show less correlation with those obtained for paclitaxel, a spindle active agent whose activity is known to be related strongly to proliferation fraction [30]. MacDaid *et al.* [31] reported OAW42 to be sensitive to paclitaxel and suggested that this was dependent on Raf-1, a kinase involved in cell proliferation in response to growth factors present in FCS. In serum-free media this Raf-1 activation may be reduced and our data suggest that OAW42 is an example of a cell line chemoresistant to paclitaxel. Experiments to define gene expression in cell lines rarely take account of these effects and this may lead to discrepancies between the gene signatures in resistant cell lines versus those obtained from tumours in experiments using oligonucleotide arrays [32,33].

We are currently analysing data from a larger set of cell lines representing different tumour types to devise a larger panel of cell lines suitable for drug discovery using serum-free conditions.

Acknowledgements

We wish to thank to all those who submitted ovarian tumour material to Translational Oncology Research Centre (TORC) for testing, particularly those within the NHS Collaborative Research Programme for Predictive Oncology. This project was supported by the Lord Dowding Fund, to whom we are most grateful.

References

1 Cree IA, Kurbacher CM. ATP based tumour chemosensitivity testing: assisting new agent development. *Anticancer Drugs* 1999; **10**:431–435.
2 Sawyers CL, Sausville EA. Overview: the art of cancer drug screening: molecular target versus milieu-based screens. *Curr Opin Investig Drugs* 2002; **3**:478–481.
3 Baguley BC, Marshall ES. *In vitro* modelling of human tumour behaviour in drug discovery programmes. *Eur J Cancer* 2004; **40**:794–801.
4 Monks A, Scudiero D, Skehan P, Shoemaker R, Paull K, Vistica D, *et al.* Feasibility of a high-flux anticancer drug screen using a diverse panel of cultured human tumor cell lines. *J Natl Cancer Inst* 1991; **83**:757–766.
5 Freshney RI. *Culture of animal cells: a manual of basic technique*. New York: Wiley-Liss; 1994.
6 Cree IA, Pazzagli M, Mini E, Mazzei T, Hunter EM, Sutherland LA, *et al.* Methotrexate chemosensitivity by ATP luminescence in human leukemia cell lines and in breast cancer primary cultures: comparison of the TCA-100 assay with a clonogenic assay. *Anticancer Drugs* 1995; **6**:398–404.
7 Cree IA, Petty RD, Sutherland LA, Hunter EMM, Subedi AMC, James EA, *et al.* Elucidation of molecular determinants of tumour chemosensitivity by ATP-based luminescence assay. In: Campbell AC, Stanley PE, Kricka LJ, editors. *Chemluminescence and bioluminescence*. Chichester: Wiley; 1994; 407–410.
8 Fidler IJ, Wilmanns C, Staroselsky A, Radinsky R, Dong Z, Fan D. Modulation of tumor cell response to chemotherapy by the organ environment. *Cancer Metastasis Rev* 1994; **13**:209–222.
9 Van Diest PJ, Baak JP, Brugghe J, Van De Burg ME, Van Oosterom AT, Neijt JP. Quantitative pathologic features as predictors of long-term survival in patients with advanced ovarian cancer treated with cisplatin. *Int J Gynecol Cancer* 1994; **4**:174–179.
10 Andreotti PE, Linder D, Hartmann DM, Cree IA, Pazzagli M, Bruckner HW. TCA-100 tumour chemosensitivity assay: differences in sensitivity between cultured tumour cell lines and clinical studies. *J Biolumin Chemilumin* 1994; **9**:373–378.
11 Cree IA, Andreotti PE. Measurement of cytotoxicity by ATP-based luminescence assay in primary cell cultures and cell lines. *Toxicology In Vitro* 1997; **11**:553–556.
12 Hanahan D, Weinberg RA. The hallmarks of cancer [review]. *Cell* 2000; **7**:57–70.

13 Andreotti PE, Cree IA, Kurbacher CM, Hartmann DM, Linder D, Harel G, *et al.* Chemosensitivity testing of human tumors using a microplate adenosine triphosphate luminescence assay: clinical correlation for cisplatin resistance of ovarian carcinoma. *Cancer Res* 1995; **55**:5276–5282.
14 Sharma S, Neale MH, Di Nicolantonio F, Knight LA, Whitehouse PA, Mercer SJ, *et al.* Outcome of ATP-based tumor chemosensitivity assay directed chemotherapy in heavily pre-treated recurrent ovarian carcinoma. *BMC Cancer* 2003; **3**:19.
15 Cree IA, Kurbacher CM, Untch M, Sutherland LA, Hunter EM, Subedi AM, *et al.* Correlation of the clinical response to chemotherapy in breast cancer with ex vivo chemosensitivity. *Anticancer Drugs* 1996; **7**: 630–635.
16 Hunter EM, Sutherland LA, Cree IA, Dewar JA, Preece PE, Wood RA, *et al.* Heterogeneity of chemosensitivity in human breast carcinoma: use of an adenosine triphosphate (ATP) chemiluminescence assay. *Eur J Surg Oncol* 1993; **19**:242–249.
17 Hartley JA, O'Hare CC, Baumgart J. DNA alkylation and interstrand cross-linking by treosulfan. *Br J Cancer* 1999; **79**:264–266.
18 Peters GJ, Ruiz van Haperen VW, Bergman AM, Veerman G, Smitskamp-Wilms E, van Moorsel CJ, *et al.* Preclinical combination therapy with gemcitabine and mechanisms of resistance. *Semin Oncol* 1996; **23**: 16–24.
19 Knight LA, Conroy M, Fernando A, Polak M, Kurbacher CM, Cree IA. Pilot studies of the effect of zoledronic acid (Zometa) on tumor-derived cells *ex vivo* in the ATP-based tumor chemosensitivity assay. *Anticancer Drugs* 2005; **16**:969–976.
20 Guppy AE, Nathan PD, Rustin GJ. Epithelial ovarian cancer: a review of current management. *Clin Oncol* 2005; **17**:399–411.
21 Gabra H, Redman C, Byrom J. Ovarian cancer. *Clin Evid* 2003; **10**: 2170–2183.
22 National Centre for Clinical Excellence. *Paclitaxel, pegylated liposomal doxorubicin hydrochloride and topotecan for second-line or subsequent treatment of advanced ovarian cancer*. NICE Technology Appraisal 2005; 91. http://www.nice.org.uk/pdf/TA091guidance.pdf.
23 Konecny G, Crohns C, Pegram M, Felber M, Lude S, Kurbacher C, *et al.* Correlation of drug response with the ATP tumor chemosensitivity assay in primary FIGO stage III ovarian cancer. *Gynecol Oncol* 2000; **77**:258–263.
24 See HT, Kavanagh JJ, Hu W, Bast RC. Targeted therapy for epithelial ovarian cancer: current status and future prospects. *Int J Gynecol Cancer* 2003; **13**:701–734.
25 Dunton CJ. New options for the treatment of advanced ovarian cancer. *Semin Oncol* 1997; **24**:S5–S11.
26 Miglietta L, Amoroso D, Bruzzone M, Granetto C, Catsafados E, Mammoliti S, *et al.* Paclitaxel plus ifosfamide in advanced ovarian cancer: a multicenter phase II study. *Oncology* 1997; **54**:102–107.
27 Lincet H, Poulain L, Remy JS, Deslandes E, Duigou F, Gauduchon P, *et al.* The p21(cip1/waf1) cyclin-dependent kinase inhibitor enhances the cytotoxic effect of cisplatin in human ovarian carcinoma cells. *Cancer Lett* 2000; **161**:17–26.
28 Rose P, Mossbruger K, Fusco N, Smrekar M, Eaton S, Rodriguez M. Gemcitabine reverses cisplatin resistance: demonstration of activity in platinum- and multidrug-resistant ovarian and peritoneal carcinoma. *Gynecol Oncol* 2003; **88**:17–21.
29 Nagourney RA, Brewer CA, Radecki S, Kidder WA, Sommers BL, Evans SS, *et al.* Phase II trial of gemcitabine plus cisplatin repeating doublet therapy in previously treated, relapsed ovarian cancer patients. *Gynecol Oncol* 2003; **88**:35–39.
30 Zhao J, Kim JE, Reed E, Li QQ. Molecular mechanism of antitumor activity of taxanes in lung cancer [review]. *Int J Oncol* 2005; **27**:247–256.
31 McDaid HM, Johnston PG, McDaid HM, Johnston PG. Synergistic interaction between paclitaxel and 8-chloro-adenosine 3′,5′-monophosphate in human ovarian carcinoma cell lines. *Clin Cancer Res* 1999; **5**:215–220.
32 Di Nicolantonio F, Mercer SJ, Knight LA, Gabriel FG, Whitehouse PA, Sharma S, *et al.* Cancer cell adaptation to chemotherapy. *BMC Cancer* 2005; **5**:78.
33 Duan Z, Lamendola DE, Duan Y, Yusuf RZ, Seiden MV. Description of paclitaxel resistance-associated genes in ovarian and breast cancer cell lines. *Cancer Chemother Pharmacol* 2005; **55**:277–285.