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A Comparison of Clonogenic, Microtetrazolium and Sulforhodamine B Assays for Determination of Cisplatin Cytotoxicity in Human Ovarian Carcinoma Cell Lines

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An assay based upon quantitative staining of cellular protein by sulforhodamine B (SRB) has recently been adopted by the NCI for large-scale screening of new drugs. However, there are few data available regarding whether the SRB assay is comparable to other established methods. Cisplatin cytotoxicity was determined in 16 human ovarian carcinoma cell lines by both SRB and clonogenic assays, and by microtetrazolium (MTT) assay in seven cell lines. Cell lines were derived from untreated patients (some of which were selected for cisplatin resistance *in vitro*) and from patients clinically refractory to cisplatin-based chemotherapy. There was excellent linear correlation between SRB staining and cell number in all cell lines ($r = 0.972$–0.999). IC_{50} values obtained by the SRB and clonogenic assay ($r = 0.824$, $P = 0.000022$) were highly correlated, although values obtained in the SRB assay were uniformly higher. IC_{50} values obtained by SRB assay also correlated well with results obtained by MTT assay ($r = 0.906$, $P = 0.0010$). Overall, the SRB assay permitted rapid and reliable assessment of cisplatin sensitivity in these cell lines and compared favourably with clonogenic and MTT assays.
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INTRODUCTION

EVALUATION OF anticancer drugs and investigations of cancer cell biology depend upon quantitative, reliable and physiologically relevant *in vitro* assays of cell survival and proliferation. The human tumour stem cell clonogenic assay, described over a decade ago [1–4], was among the first of such assays. This assay, which is based on the capacity for substrate-independent growth, has been successfully applied to investigations of growth factors, new chemotherapeutic agents, ionising radiation, etc. However, clonogenic assays are labour-intensive, and plated cells often take weeks to grow into colonies of sufficient size to be enumerated. Manual colony counting can be tedious, and automated image analysis equipment is relatively expensive. Moreover, studies are limited by the requirement for substrate-independent growth. Normal cells and many malignant cells grow poorly in agar, if at all. In recent years, recognition of these potential problems has spurred development of several alternative assays, including the microtetrazolium (MTT) assay [5] ATP bioluminescence assay [6], thymidine uptake assay [7], dye exclusion assays [8], and others. Several of these assays have been evaluated by the National Cancer Institute (NCI) for applicability to large-scale screening of new drugs. The NCI recently adopted the sulforhodamine B (SRB) assay for this application.

Sulforhodamine B stains protein under weakly acidic conditions, a property which is exploited to provide a quantitative indication of cell number in this cytotoxicity assay. The SRB assay appears to compare favourably with the MTT assay for large-scale drug screening studies [9–11]. However, no data are presently available regarding the comparability of data obtained by SRB assay to those obtained by clonogenic assay. The present investigations were undertaken to compare cisplatin cytotoxicity data obtained by SRB, MTT, and clonogenic assays in human ovarian carcinoma cell lines from untreated patients, in cell lines selected for cisplatin resistance *in vitro*, and in cell lines from patients who were clinically refractory to cisplatin-based chemotherapy.

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MATERIALS AND METHODS

Ovarian carcinoma cell lines

A2780 and 1847 are ovarian cancer cell lines from untreated patients, originally derived by Dr S. Aaronson (NCI, Bethesda, Maryland, U.S.A.) [12]. Cell lines designated 2780^{CP} and 2780^{C} were produced by intermittent, incremental exposure of the sensitive parental A2780 cell line to various concentrations of cisplatin. The OVCAR 5 and 7 cell lines were developed from untreated patients, one of whom (OVCAR 7) responded to high-dose cisplatin, the other not evaluable for response. The remaining OVCAR cell lines were established from patients who were clinically refractory to platinum-based chemotherapy. The treatment status of patients from whom these cell lines were derived are summarized in Table 1. The characteristics of these cell lines have been reviewed in detail in previous publications [13].

All cell lines were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) (Gibco, Grand Island, New York, U.S.A.), 0.28 U/ml insulin (Squibb-Novo, Princeton, New Jersey, U.S.A.), 100 μg/ml streptomycin, 100 U/ml penicillin and 0.3 mg/ml glutamine. Cells were grown at

Table 1. Cell lines

Cell line	Treatment*	Comment
1847	Untreated	—
A2780	Untreated	—
2780-cp8	—	Derived from A2780
2780-cp20		*In vitro* resistance selected by
2780-cp70		intermittent, incremental
2780-c30		exposure to cisplatin
2780-c50		
2780-c80		
2780-c100		
2780-c200		
OVCAR-3	CTX/ADR/CDDP	Clinically refractory
OVCAR-4	CTX/ADR/CDDP	Clinically refractory
OVCAR-5	Untreated	—
OVCAR-7	Untreated	—
OVCAR-8	CDDP-based combination chemotherapy, CBDCA	Progressive disease after high-dose CBDCA (800 mg/m^2)
OVCAR-10	CDDP, CBDCA	Progressive disease after CDDP-based chemotherapy, high-dose CDDP (200 mg/m^2), and high-dose CBDCA (800 mg/m^2)

*Treatment status of patient at the time cell line was established. CTX, cyclophosphamide; ADR, doxorubicin; CDDP, cisplatin; CBDCA, carboplatin.

37°C, in a humidified atmosphere of 5% CO_2 in air. Cells were plated in maintenance media containing 10% FBS for clonogenic assays. Media for SRB assays was identical except that it contained only 5% FBS.

Chemicals/drugs

Chemicals and reagents were purchased from Sigma (St Louis, Missouri, U.S.A.). Cisplatin was furnished by Bristol-Myers Oncology Division, Bristol Laboratories (Evansville, Indiana, U.S.A.). The clinical formulation of cisplatin was reconstituted in sterile water and diluted to the desired concentrations immediately before use.

Cytotoxicity assays

Clonogenic assay. Cisplatin cytotoxicity was assayed by continuous treatment of cells suspended in soft agarose, as previously described [14]. Single-cell suspensions of 100 000–30 000 cells/ml in media and agarose to 0.3% (w/v), in the presence or absence of cisplatin, were plated over chilled 0.6% agarose feeder layers. Cells were plated at a density which yielded approximately 2000 colonies per 10 cm^2 in untreated controls. Colonies were counted with an Artek Omnicon FAS IV Image Analysis System following incubation of the plated cells under routine culture conditions for 7–21 days, by which time colonies greater than 60 μ in diameter were enumerated. Within experiments, percentage clonogenic survival was determined as the mean number of colonies from triplicate platings at each drug concentration relative to untreated controls.

MTT assay. The MTT assay was performed as described by Mosman [5], with minor modifications. Briefly, cells (1000–32 000 in 150 μl media per well) were plated in 96 well plates (0.16 cm^2; Corning) and allowed to attach overnight. Cisplatin was added at varying concentrations (0–300 μmol/l). After 3 days, 40 μl of 5 mg/ml 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyl-tetrazolium bromide (MTT) was added to each well. Following 2-h incubation, cells were then lysed overnight with 100 μl per well of extraction buffer [20% (w/v) sodium dodecyl sulphate, 50% *N*,*N*-dimethyl formamide, pH 4.7]. Absorbance at 570 nm was then measured with a Bio-Rad (model #3550) microplate reader, using wells without cells as blanks.

Sulforhodamine B assay. The SRB assay was performed as described by Skehan *et al.* [9], with minor modifications. Briefly, 5×10^3 cells in 0.2 ml media were plated in each well (0.16 cm^2) of 96-well plates and allowed to attach for several hours. Cisplatin was added to wells to produce the desired final concentrations, and plates were incubated at 37°C for 3 days. Cells were then fixed by gentle addition of 50 μl of cold (4°C) 50% trichloroacetic acid (TCA) to each well, followed by incubation at 4°C for 1 h. Plates were washed with deionised water five times and allowed to air dry. Cells were then stained by addition of 50 μl SRB solution [0.4% SRB (w/v) in 1% acetic acid (v/v)] to wells for 10 min. Following staining, plates were quickly washed five times with 1% acetic acid to remove unbound dye, and allowed to air dry. Bound dye was solubilised with 10 mmol/l Tris base (pH 10.5) prior to reading plates.

Plates were read on a Bio-Rad (model #3550) microplate reader. Optical density (O.D.) increases linearly with increasing dye concentration, up to 1.8–2.0 O.D. units, and absorbance is maximal at 564 nm [9]. Plates were, therefore, read using a 570 nm filter (nearest available filter to absorbance maximum) when all samples within an experiment stained within the linear range. Otherwise, plates were read at the next available alternative wavelength (595 nm) which provided the greatest sensitivity within the linear range, as per Skehan *et al.* [9]. At either 570 or 595 nm, 5×10^3 cells could readily be detected above background staining.

Data analysis

The IC_{50} of cisplatin in individual cell lines was determined for each experiment by median effects analysis, using commercially available software [15]. Data used for median effects analysis fit the regression lines used for the analysis with correlation coefficients ≥ 0.95. Data in tables and figures represent the mean (± S.D.) of two to four individual experiments, unless otherwise indicated. Standard (Pearson) correlation coefficients were calculated for staining intensity (O.D.) vs. cell number. Cytotoxicity (IC_{50}) data generated by each assay were analysed by Spearman rank correlation.

RESULTS

SRB assay optimisation and calibration

Prior to cytotoxicity studies, the staining protocol was optimised with respect to staining time, staining volume and background staining. Cells were plated at densities of 10^3–10^5 cells per well, and allowed to attach for several hours. Staining times of up to 30 min were tested, in increments of 5 min. Staining was maximal by 10 min; longer times only served to minimally increase background staining. Similarly, staining volumes of 50 μl were optimal. Smaller volumes did not entirely cover the well surface area, while larger volumes were associated with increased background staining. Background staining was substantially influenced by the serum concentration of the media. Media containing 10% FBS produced background staining nearly 2-fold higher than media containing 5% FBS

(0.118 ± 0.012 vs. 0.060 ± 0.005 O.D. units at 595 nm, respectively). Media containing 5% FBS did not produce significantly greater background staining over that observed in wells to which neither cells nor media had been added. In addition, cisplatin (3–300 μmol/l) did not increase background staining. These observations were incorporated into the staining protocol described above.

Linearity of staining with respect to cell number was established by plating 10^3–10^5 cells per well. Cells were allowed to attach for several hours, then fixed and stained as described. There was excellent correlation between cell number and staining (r = 0.972–0.999). Even at 595 nm, 5 × 10^3 cells (the initial number of cells plated per well in cytotoxicity assays) could easily be detected. Linearity of staining with respect to cell number was maintained at this wavelength.

Cytotoxicity assay comparison

Cytotoxicity assays were performed as previously described. Cisplatin produced concentration-dependent cytotoxicity in all cell lines. Representative survival curves generated with SRB assay data are shown in Fig. 1a. Survival curves obtained for all cell lines, with all three assays, were similar in appearance and the cytotoxicity data are summarised in Tables 2 and 3. Resistance in both tables is arbitrarily calculated relative to the A2780 cell line to facilitate comparison between cell lines, despite the fact many of the cell lines tested were not derived from A2780. Cytotoxicity data from both assays were very reproducible, as indicated by the small standard deviations of the mean IC_{50}.

Cisplatin cytotoxicity in the SRB assay was influenced by the number of cells plated. When 2 × 10^4 cells were plated in each well, less cytotoxicity was observed in all cell lines at given cisplatin concentrations (Fig. 1b, same cell lines as Fig. 1a). However, untreated control cells plated at this density grew past confluence before the conclusion of the assay. Therefore, a lower initial cell number was considered optimal, as cells would then remain in log growth throughout the assay.

Cisplatin IC_{50} values obtained by SRB assay were uniformly higher than those obtained by clonogenic assay. However, both assays identified a similar pattern of resistance in these cell lines. A scatterplot of IC_{50} data from all cell lines (Fig. 2) shows good overall agreement between the two assays. The Spearman correlation coefficient for these IC_{50} data was 0.824 (P = 0.000022).

Table 2. Cisplatin cytotoxicity in A2780 and resistant sublines

Cell line	Clonogenic assay	SRB assay	MTT assay
A2780: IC_{50} (μmol/l)	0.15 ± 0.03	0.30 ± 0.05	0.19
Relative resistance (fold)*	1	1	1
2780-cp8: IC_{50} (μmol/l)	1.22 ± 0.05	3.38 ± 0.65	—
Relative resistance (fold)	8	11	
2780-cp20: IC_{50} (μmol/l)	1.67 ± 0.91	8.78 ± 4.78	5.2
Relative resistance (fold)	11	29	27
2780-cp70: IC_{50} (μmol/l)	6.40 ± 2.34	7.98 ± 1.39	5.5
Relative resistance (fold)	43	29	29
2780-c30: IC_{50} (μmol/l)	25.55 ± 7.38	102.80 ± 16.2	54
Relative resistance (fold)	170	343	284
2780-c50: IC_{50} (μmol/l)	25.99 ± 2.02	120.90 ± 10.4	100
Relative resistance (fold)	173	403	526
2780-c80: IC_{50} (μmol/l)	20.83 ± 2.14	142.13 ± 14.4	115
Relative resistance (fold)	139	474	605
2780-c100: IC_{50} (μmol/l)	23.87 ± 7.38	163.50 ± 29.3	125
Relative resistance (fold)	159	545	658
2780-c200: IC_{50} (μmol/l)	38.67 ± 9.36	133.00 ± 16.7	205
Relative resistance (fold)	258	443	1078

IC_{50} values are expressed as mean ± S.D. *Relative to A2780.

Results from both assays were also compared to data obtained by MTT assay in a limited number of cell lines (Table 2). These data have recently been communicated in a separate publication [16]. Correlation coefficients for IC_{50} data obtained by all three assays were high (MTT vs. SRB, ρ = 0.905, P = 0.0010; MTT vs. clonogenic assay, ρ = 0.810, P = 0.0074). The correlation between all three assays is also apparent in Fig. 2.

DISCUSSION

Clonogenic assays have been used extensively for *in vitro* investigations of anticancer drug cytotoxicity. The clonogenic assay was designed to select for substrate-independent growth, a condition favourable to growth of malignant cells but not fibroblasts and normal cells. This selection was advantageous

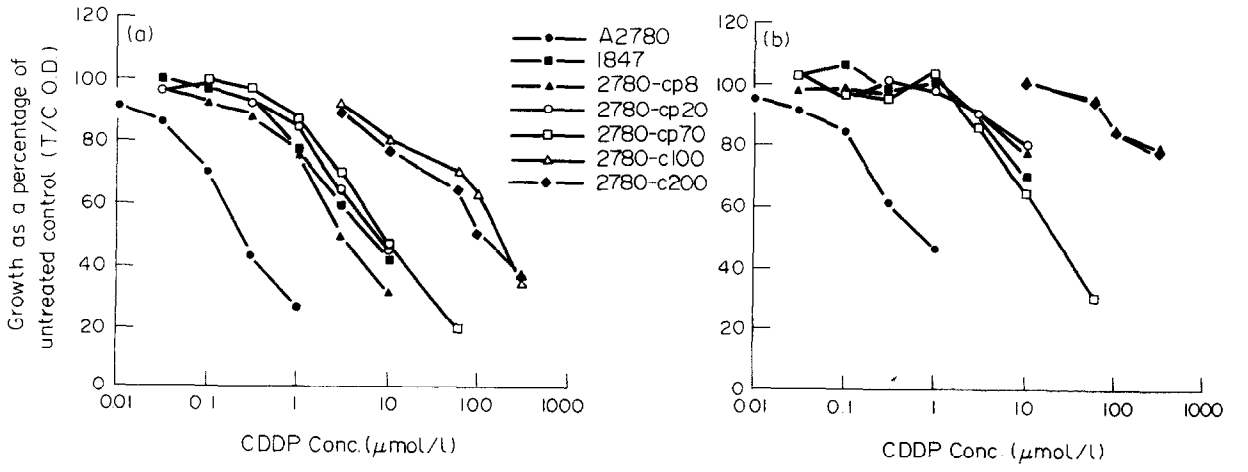

Fig. 1. (a) Cisplatin cytotoxicity in representative human ovarian carcinoma cell lines by SRB assay, following initial plating of 5 × 21^3 cells per well. (b) Cisplatin cytotoxicity by SRB assay in the same cell lines as in (a), except that 2 × 10^4 cells were initially plated per well.

Table 3. Cisplatin cytotoxicity in parental cell lines

Cell line	Clonogenic assay	SRB assay
A2780: IC_{50} (μmol/l)	0.15 ± 0.03	0.30 ± 0.05
Relative resistance (fold)*	1	1
1847: IC_{50} (μmol/l)	0.71 ± 0.35	5.28 ± 0.66
Relative resistance (fold)	5	18
OVCAR 3: IC_{50} (μmol/l)	0.12 ± 0.01	5.49 ± 0.32
Relative resistance (fold)	1	18
OVCAR 4: IC_{50} (μmol/l)	0.40 ± 0.01	3.18 ± 0.64
Relative resistance (fold)	3	11
OVCAR 5: IC_{50} (μmol/l)	0.46 ± 0.19	7.47 ± 0.26
Relative resistance (fold)	3	25
OVCAR 7: IC_{50} (μmol/l)	0.13 ± 0.02	5.79 ± 2.30
Relative resistance (fold)	1	19
OVCAR 8: IC_{50} (μmol/l)	0.68 ± 0.40	6.10 ± 1.42
Relative resistance (fold)	5	20
OVCAR 10: IC_{50} (μmol/l)	6.10 ± 1.15	11.18 ± 2.76
Relative resistance (fold)	41	37

IC_{50} values are expressed as mean ± S.D. *Relative to A2780.

for the initial application of the assay, namely comparison of the *in vitro* chemosensitivity of clinical specimens with clinical response data. However, the selective properties of the clonogenic assay may be less relevant to the evaluation of established cell lines. Moreover, clonogenic assays are not suited to studies of normal cells or malignant cells which do not grow in agarose. In addition, the assay is fairly labour-intensive, and cells may take several weeks to form large colonies.

Despite these potential problems, clonogenic assays have been widely used. One reason for their popularity may be that clonogenic assays appear to have some biological relevance with respect to clinical chemosensitivity. Overall, clonogenic assays have a negative predictive value of 91% and a positive predictive value of 69% [17]. This correlation between lack of response *in vitro* and clinical resistance to chemotherapy suggests that clonogenic assays may be valid model systems for drug resistance studies.

Alternative assays have generally been less labour-intensive and yielded more rapid results. The NCI recently adopted the SRB assay for large-scale screening of potential anticancer drugs.

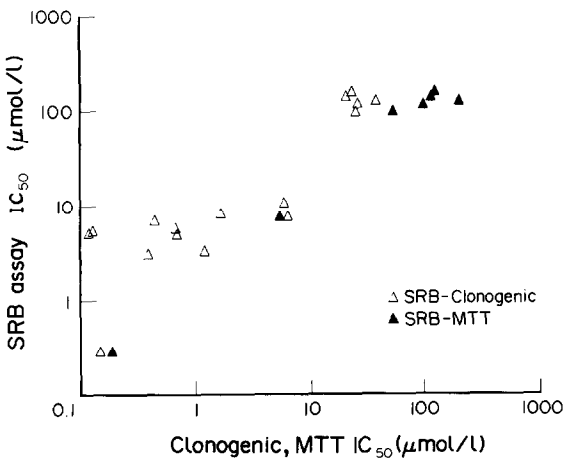

Fig. 2. Scatterplot of IC_{50} data generated by SRB, clonogenic and MTT assays for all cell lines.

SRB had superior sensitivity (signal: noise ratio), relative to several other protein/biomass stains and to tetrazolium dye reduction (MTT or XTT) assays [9]. The SRB assay also demonstrated better correlation between staining and cell number than the other assays [9, 11]. Explanations for these differences were not apparent.

Our optimised staining protocol was similar to protocols developed for over 60 cell lines at the NCI [9, 10]. We observed linear SRB staining relative to cell number (correlation coefficient range 0.972–0.999). The excellent correlation between staining and cell number and the sensitivity of the method to detect relatively small numbers of cells support the use of SRB staining as an endpoint for rapid cell-proliferation assays.

We determined cisplatin cytotoxicity by both clonogenic and SRB assays in 16 human ovarian carcinoma cell lines which encompassed a wide range of sensitivity. The cisplatin sensitivity of seven cell lines was also determined by MTT assay. IC_{50} values obtained in these assays were highly correlated, although IC_{50} data obtained by the SRB and MTT assays were uniformly higher than in clonogenic assays. All three assays identified similar groups of cells which could be categorised as having either low, moderate or high levels of resistance.

The higher IC_{50} values obtained in the SRB and MTT assays, relative to the clonogenic assay, are consistent with the kinetics of cisplatin-induced growth arrest and cell death. Cells treated at cytotoxic cisplatin concentrations may remain arrested at one or more steps of the cell cycle for up to several days prior to cell death [18]. Membrane integrity is maintained during growth arrest. In addition, it is possible that cells may undergo a limited number of divisions before growth arrest occurs. It is, therefore, likely that cells which lack sufficient reproductive potential to form colonies in agarose remain sufficiently intact during the time frame of the MTT and SRB assays to reduce tetrazolium or to stain with SRB. Further, one would expect protein integrity to be maintained for a longer time than mitochondrial function in cells destined to die. This contention is consistent with the lower IC_{50} values obtained with the MTT assay vs. the SRB assay, in the majority of cell lines tested.

Cisplatin cytotoxicity determined by the SRB assay appears to depend on the initial number of cells plated. This observation is consistent with reports demonstrating a similar effect of cell number on the linearity of colony formation, the distribution of colony size, and the chemosensitivity of cells plated in clonogenic assays [19]. Similarly, chemosensitivity tends to decrease with increasing cell number/density in MTT assays [20].

The MTT assay depends on the reduction of tetrazolium salt to formazan in the mitochondria of viable cells, a reaction which correlates with cell number and with incubation time. However, the reaction appears to be sensitive to concentrations of glucose, hydrogen ion, NADH and NADPH [21, 22]. Moreover, it is conceivable that drugs might potentially affect mitochondrial metabolism independently from growth. These factors could contribute to over- or underestimation of the cytotoxicity of some agents by the MTT assay. When MTT assays are used, it may be necessary to consider appropriate controls to exclude chemical reactions of agents being tested which would tend to enhance or inhibit tetrazolium reduction. These caveats aside, initial reports suggest that MTT chemosensitivity data appear to correlate with clinical response in ovarian cancer patients [23].

The SRB assay would appear to have some advantages over both clonogenic assays and the MTT assay. The SRB assay provides more rapid results than clonogenic assays, and it

permits investigation of cells which do not grow in agarose. In addition, the cost of equipment and supplies are lower for the SRB assay than for clonogenic assays. In contrast to the MTT assay, the SRB assay appears to be free from potentially confounding metabolic considerations, and results are not dependent on a time-sensitive reaction. Of course, the SRB assay is not suitable for applications which require substrate-independent growth. These considerations may be of more hypothetical than actual significance for cytotoxicity studies. The SRB assay appears to provide consistent results which may complement those obtained by other methods.

One potential criticism of the SRB assay is its inability to distinguish viable cells from dead cells. We have circumvented this problem by using growth relative to control as our ultimate endpoint. In this respect, our endpoint is very similar to that in clonogenic assays, i.e. cell proliferation, except that the SRB assay does not select for substrate-independent growth.

Overall, the SRB assay permitted rapid and reliable assessment of the cisplatin sensitivity of 16 human ovarian carcinoma cell lines. Results compared favourably to those obtained by clonogenic assay and by MTT assay. Moreover, the excellent correlation observed between cell number and staining suggests that SRB staining may be useful in other situations where quantitation of cell number is necessary. The assay should also be readily adaptable to studies of schedule-dependent drug effects. We anticipate that the SRB assay will be increasingly used for these and other applications.

1. Hamburger AW, Salmon SE. Primary bioassay of human tumor stem cells. *Science* 1977, **197**, 461–463.
2. Salmon SE, Hamburger AW, Soehlen B, Durie BGM, Alberts DS, Moon TE. Quantitation of differential sensitivity of human tumor stem cells to anticancer drugs. *N Engl J Med* 1978, **298**, 1321–1327.
3. Courtenay VD, Mills J. An *in vitro* colony assay for human tumors grown in immune-suppressed mice and treated *in vivo* with cytotoxic agents. *Br J Cancer* 1978, **37**, 261–268.
4. Courtenay VD, Selby PJ, Smith IE, Mills J, Peckham MJ. Growth of human tumor cell colonies from biopsies using two soft-agar techniques. *Br J Cancer* 1978, **38**, 77–81.
5. Mosman T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods* 1983, **65**, 55–63.
6. Grewal HS, Ahmann FR, Schifman RB, Celniker A. ATP assay: ability to distinguish cytostatic from cytocidal anticancer drug effects. *J Natl Cancer Inst* 1986, **77**, 1039–1045.
7. Sanfilippo O, Silvestrini R, Zaffaroni N, Piva L, Pizzocaro G. Application of an *in vitro* antimetabolic assay to human germ cell testicular tumors for the preclinical evaluation of drug sensitivity. *Cancer* 1986, **58**, 1441–1447.
8. Weisenthal LM, Marsden JA, Dill PL, Macaluso CK. A novel dye exclusion method for testing *in vitro* chemosensitivity of human tumors. *Cancer Res* 1983, **43**, 749–757.
9. Skehan P, Storeng R, Scuderio D, *et al.* New colorimetric cytotoxicity assay for anticancer drug screening. *J Natl Cancer Inst* 1990, **82**, 1107–1112.
10. Rubenstein LV, Shoemaker RH, Paull KD, *et al.* Comparison of *in vitro* anticancer drug screening data generated with a tetrazolium assay versus a protein assay against a diverse panel of human tumor cell lines. *J Natl Cancer Inst* 1990, **82**, 1113–1118.
11. Keepers Y, Piazo P, Peters G, van Ark-Otte J, Winograd B, Pinedo H. Comparison of the sulforhodamine B protein and tetrazolium (MTT) assays for *in vitro* chemosensitivity testing. *Eur J Cancer* 1991, **27**, 897–900.
12. Eva A, Robbins K, Anderson P, *et al.* Cellular genes analogous to retroviral onc genes are transcribed in human tumor cells. *Nature* 1982, **295**, 116–119.
13. Hamilton T, Lai G, Rothenberg M, Fojo A, Young R, Ozols R. Mechanism of resistance to cisplatin and alkylating agents. In Ozols R, ed. *Drug Resistance in Cancer Therapy*. Boston, Kluwer Academic Publishers, 1989, 151.
14. Perez R, O'Dwyer P, Handel L, Ozols R, Hamilton T. Comparative cytotoxicity of CI-973, cisplatin, carboplatin, and tetraplatin in human ovarian carcinoma cell lines. *Int J Cancer* 1991, **48**, 265–269.
15. Chou J, Chou T. *Dose-effect Analysis with Microcomputers*. Cambridge, UK, Biosoft, 1987.
16. Godwin AK, Meister A, O'Dwyer PJ, Hamilton TC, Anderson ME. Acquired resistance to cisplatin in human ovarian cancer cell lines is associated with altered glutathione synthesis. *Proc Natl Acad Sci USA* 1992, **89**, 3070–3074.
17. Von Hoff D. He's not going to talk about *in vitro* predictive assays again, is he? *J Natl Cancer Inst* 1990, **82**, 96–101.
18. Sorenson C, Eastman A. Mechanism of *cis*-diamminedicloroplatinum(II)-induced cytotoxicity: role of G_2 arrest and DNA double strand breaks. *Cancer Res* 1988, **48**, 4484–4488.
19. Thompson SP, Buckmeier JA, Sipes NJ, Meyskens FL, Hickie RA. Colony size, linearity of formation, and drug survival curves can depend on the number of cells plated in the clonogenic assay. In Salmon SE, Trent JM, eds. *Human Tumor Cloning*. Orlando, Grune and Stratton, 1984, 37–51.
20. Shimoyama Y, Kubota T, Watanabe M, Ishibiki K, Abe O. Predictability of *in vivo* chemosensitivity by *in vitro* MTT assay with reference to the clonogenic assay. *J Surg Oncology* 1989, **41**, 12–18.
21. Jabbar SAB, Twentyman PR, Watson JV. The MTT assay underestimates the growth inhibitory effects of interferons. *Br J Cancer* 1989, **60**, 523–528.
22. Vistica DT, Skehan P, Scuderio D, Monks A, Pittman A, Boyd MR. Tetrazolium-based assays for cellular viability: a critical examination of selected parameters affecting formazan production. *Cancer Res* 1991, **51**, 2515–2520.
23. Wilson JK, Sargent JM, Elgie AW, Hill JG, Taylor CG. A feasibility study of the MTT assay for chemosensitivity testing in ovarian malignancy. *Br J Cancer* 1990, **62**, 189–194.

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