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Radiosensitivity of New and Established Human Melanoma Cell Lines: Comparison of [^{3}H]Thymidine Incorporation and Soft Agar Clonogenic Assays

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Seven new low-passage melanoma lines were developed in this laboratory from clinical melanoma specimens and characterised for chromosome complement, DNA ploidy and S-phase content. The radiosensitivity of these lines was compared with that of eight established melanoma cell lines, FME, MM-96, SK-MEL-5, SK-MEL-28, SK-MEL-2, MALME-3M, M19-MEL and LOX-IMVI, using a 96-well microculture assay technique. Dose–response curves were determined using a 5-day incubation period and 6-h terminal [^{3}H]thymidine-labelling period. Radiation (^{60}Co source) was carried out under a lead wedge to provide a radiation dose range of 0–10 Gy, or by irradiating part of the plate (radiation dose 0 or 2 Gy). Data for a range of cell densities in a single 96-well plate were combined into a single regression equation incorporating linear quadratic terms for radiation dose and cell density. SF_2 values were defined as the amount of thymidine incorporated following a radiation dose of 2 Gy, expressed as a fraction of that of unirradiated cells, and varied from 0.36 to 0.93. The reproducibility in repeat assays, as defined by the standard error of determinations at different passage numbers, was ±0.04. The newly developed lines exhibited a similar range of radiosensitivity to that of the established lines, and melanin content did not correlate with resistance. For nine of the lines, radiation parameters were also determined using a modified Courtenay clonogenic soft agar assay technique, and the results compared with the thymidine incorporation results, and a significant linear correlation was found between SF_2 and SF_2' ($r = 0.89$). The linear (α) and quadratic (β) terms of the best-fit linear quadratic dose–response curves, were significantly correlated between the two assays. It is concluded for this series of human melanoma lines that proliferation assays in 96-well plates provide radiosensitivity parameters comparable to those using clonogenic assays.

Key words: thymidine incorporation, clonogenicity, cell culture, radiation
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INTRODUCTION

THE AVAILABILITY of rapid methods to predict the radiosensitivity of a clinical tumour prior to treatment would have many benefits for cancer patients. Although many *in vivo* factors influence response to therapy, intrinsic cellular radioresistance appears to be an important reason for treatment failure. *In vitro* assay systems to measure radiosensitivity and chemosensitivity, as recently reviewed [1], have employed colonies in soft agar [2–6], colonies on a cell-adhesive matrix [7–9] and cells growing in microcultures [10–13]. Of these assays, the Courtenay assay [3] has been the most widely used, and radiosensitivity, as determined by this assay, has been reported to correlate with clinical radioresponsiveness [14–19]. Despite this correlation, the clonogenic assay has not been adopted for routine *in vitro* screening of radioresponsiveness of human tumours. It is technically demanding, not all tumours can be grown in agar [20, 21], single cell suspensions must be obtained to ensure accurate radiation survival data [22], large numbers of cells are required for seeding to counter low plating efficiencies [21] and the presence of abortive colonies makes counting difficult [5].

Growth of cells recovered from freshly extirpated tumours in microcultures is advantageous in that the success of the assay is not dependent on complete disaggregation of tumours into a single cell suspension and comparatively few tumour cells are required. However, few studies have been carried out to compare radiosensitivity using microculture and clonogenic assays. Previous studies using cell lines derived from small cell lung cancer have shown a correlation between thymidine incorporation assays and clonogenic assays for high plating efficiency lines, but no correlation for low plating efficiency lines [10, 12].

We have previously developed a [^{3}H]thymidine incorporation

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microculture technique for the primary culture and chemosensitivity testing of tumour cells derived from patients' melanomas [23]. We have also applied this method to the measurement of radiosensitivity of a series of five murine and human cell lines, finding that thymidine incorporation assays correlate well with clonogenic assays on culture dishes [24]. In this report, we have used the method to determine the radiosensitivity of seven new low passage melanoma lines, developed from clinical samples, with that of eight established melanoma cell lines. For nine of the cell lines, we have compared radiosensitivity determined by thymidine incorporation with that using clonogenic assays in soft agar of a type used in the clinical correlations.

METHODS AND MATERIALS

Cells and culture conditions

FME [25] and MM-96 [26] lines were provided by Drs K. Tveit (Norwegian Radium Hospital, Oslo, Norway) and R. Whitehead (Ludwig Institute, Melbourne, Australia), respectively. SK-MEL-28, SK-MEL-2, SK-MEL-5, M19-MEL, MALME-3M and LOX-IMVI were provided by Mr Richard Camalier, National Cancer Institute (Division of Cancer Treatment, NCI, Bethesda, U.S.A.). The basic culture medium used was α-modified minimal essential medium supplemented with fetal bovine serum (FBS; 5 or 10% vol/vol), penicillin (100 U/ml) and streptomycin (100 μg/ml) to which insulin (2 μg/ml) and hydrocortisone (200 ng/ml; Sigma Chemical Co., U.S.A.) were added for MM-96 cells. Insulin (10 μg/ml), transferrin (10 μg/ml) and selenium (10 ng/ml; ITS) were added for NZM5.

New Zealand melanoma lines

The New Zealand melanoma cell lines (designated NZM) were developed from pathologically-confirmed metastatic malignant melanomas. Formal consent was obtained from all patients under Auckland Area Health Board Ethics Committee guidelines. None of the patients from whom tissue had been obtained had received prior radiotherapy or chemotherapy. Melanoma tissue specimens were placed into α-modified minimal essential medium supplemented with ITS, together with antibiotics (penicillin 100 U/ml; streptomycin 100 μg/ml; amphotericin B 5 μg/ml) and disaggregated immediately or after overnight storage at 4°C in those instances where the tumour was received late in the day. Normal, adipose and grossly necrotic material were removed, and the tumour tissue finely minced using crossed scalpels. Cells were released by digestion of tissue with collagenase (1 mg/ml) and DNAase (50 μg/ml) with continuous stirring at 37°C for 1–2 h. Cells were centrifuged at 400 *g* for 5 min, washed twice in medium and counted using a haemocytometer. Cytospins were prepared using a Shandon cytospin (Shandon Scientific Ltd, U.K.) and stained with Leishman's stain. The presence of malignant cells was confirmed histologically and 10^6 tumour cells seeded into 25-ml plastic culture flasks. Cultures were fed weekly and passaged when approaching confluence. Cultures were classified as cell lines when they could be regularly and reproducibly subcultured. Cytospin preparations of the cells were made at this stage and examined by a pathologist. They were confirmed to contain only cells with cytological characteristics consistent with malignant melanoma, with no evidence of stromal cell contamination. Content of melanin granules was assessed cytologically.

Cytogenetic analysis

Colchicine added to 10 ng/ml in sub-confluent monolayers in 25-cm^2 culture flasks and the cells incubated for 3 h. Cells were released from the plastic by trypsinisation in 0.25% trypsin in phosphate-buffered saline (PBS) containing 20 μg/ml phenol red and 180 μg/ml EDTA, centrifuged and resuspended in potassium chloride (0.4% w/v). The cells were then fixed in methanol/glacial acetic acid (3:1 vol/vol), air dried, GTG-banded and analysed for chromosome abnormalities [27].

Flow cytometric analysis

Flow cytometry was performed using a combination of published methods [28]. Cultured cells were labelled for 60 min with bromodeoxyuridine (BUdR; 10 μM; Amersham International, U.K.). Labelled cells were fixed in 70% aqueous ethanol at −20°C, centrifuged, washed, resuspended in PBS/2% FBS and incubated with a solution of pepsin (0.2 mg/ml) in 2M HCl (1 ml) at 37°C for 30 min. The cells were washed twice with PBS/FBS (10 ml) and once with PBS/0.5% Tween 20 (2 ml), resuspended in PBS/0.5% Tween 20 and stained with a 1:10 dilution of anti-BUdR rat antibody (Seralab, U.K.) for 1 h at room temperature. Cells were then washed, incubated with sheep anti-rat IgG fluorescein conjugated antibody (Silenus, Victoria, Australia; 1:100 dilution), washed once with PBS and resuspended in PBS containing propidium iodide (16 μg/ml) and ribonuclease (1 mg/ml). After 15 min the cell suspension was analysed on an Epics Profile (Coulter Electronics, Florida, U.S.A.) flow cytometer, employing correction for cell doublets. Data were analysed using MULTIPLUS software (Phoenix Flow Systems, San Diego, California, U.S.A.).

Microculture assays

Sub-confluent monolayers were dissociated enzymatically (0.07% trypsin in citrate saline). Cells were collected by centrifugation, resuspended in fresh medium and seeded into microculture plates (150 μl per well) at a range of cell densities. Irradiation was carried out immediately after plating. Cultures were incubated for 5 days at 37°C in a humidified atmosphere of 5% CO_2 in air. 5-[Methyl-^{3}H]thymidine (20 Ci/mmol, 0.04 μCi/well; Dupont, NEN, U.S.A.), together with unlabelled thymidine and 5-fluoro-2'-deoxyuridine (each at a final concentration of 0.1 μM) in medium were added to the wells (20 μl/well) for the last 6 h of culture. Cells were released from the plastic by addition of pronase (0.5 mg/ml final concentration; Calbiochem, U.S.A.) and sodium EDTA (final concentration 2 mM) at 37°C for 1 h and deposited on filter paper (Wallac OY, Finland) using a multi-well automated cell harvester (Wallac OY). The filter paper was dried overnight and the amount of tritium retained was measured by scintillation counting.

Clonogenic assays

Cell survival was measured using a modification of a soft agar assay method [3]. Briefly, 2 ml of 2× concentrated medium containing 25 or 50% FBS were added to 2 ml of 0.75% w/v Bacto agar (Difco Laboratories, U.S.A.). Sub-confluent monolayers were dissociated enzymatically (sequentially 0.07% trypsin in citrate saline and 50μg/ml DNAase). Feeder cells (0.5 ml) consisting of 40 Gy irradiated autologous cells for NZM3 and NZM4 or 100 Gy irradiated FME cells for the remaining cell lines were added to the soft agar, followed by the tumour cell suspension (0.5 ml). The optimal range of tumour cells seeded and the optimal concentration of FBS were determined in a preliminary experiment. Aliquots of 1 ml from the above were then dispensed in 15-ml polystyrene tubes. Cultures were irradiated immediately after dispensing and were then incubated at 37°C for 3–5 weeks in an atmosphere of 5% CO_2 in air. Culture

medium (2 ml) containing 10 or 20% FBS (depending on the requirements of the cell line, as determined in preliminary experiments) was added to the top of the agar cultures 3–5 days after seeding. One half of the medium was removed each week and was replaced with fresh medium. Colonies containing at least 50 cells or colonies greater than 125 μm in diameter were counted using a stereomicroscope with a graduated eyepiece graticule. Replicate cultures held at room temperature were checked for the presence of cell clumps within 1 week of seeding but these were not seen. Plating efficiency was calculated from the number of colonies counted and the number of morphologically-viable single cells seeded.

Irradiation

Microculture plates were irradiated for 8.7 min at room temperature from a ^{60}Co source (Eldorado, Atomic Energy of Canada Limited), using a lead wedge designed to produce a dose gradient across the plate of 0.22 to 1.15 Gy/min [24]. Control cells were added to the plates after irradiation. A block method was also utilised to provide a radiation dose of 2 Gy. The microculture plate was placed under the ^{60}Co source so that the first five rows of the plate were within the radiation field. Control wells (the last three rows outside the field) received less than 0.1 Gy. Radiation doses were determined by a modified Fricke dosimetry technique [24].

Data analysis

All data points derived from the 96-well tray (or all test tubes in the soft agar assays) were analysed (SigmaStat, Jandel Scientific, San Rafael, California, U.S.A.) using a single regression equation of the following type:

$$\log_e(D) = \alpha r - \beta r^2 + as + bs^2$$

D represents the experimental data, expressed either as disintegrations per minute (d.p.m.) or colonies per plate, r is the radiation dose in Gy and s is the seeding cell density (per microculture well or per plate). α and β are the coefficients of the linear quadratic radiation dose curve, while a and b are the coefficients of the linear quadratic cell number dependence curve. The value for α was allowed to be zero or positive, while the sign for β was not restricted. SF_2 (survival at 2 Gy) and SF_2' (thymidine radioactivity as a proportion of control following irradiation at 2 Gy) were calculated from α and β, and the standard error of the mean (S.E.M.) was determined from the S.E.M. values for α and β in the regression equation. A similar treatment, omitting terms for radiation dose, was used for the determination of SF_2' in the block irradiation experiments.

RESULTS

Derivation of cell lines

Seven new melanoma lines were developed, all displaying the morphological characteristics of malignant melanoma. Three were developed from primary cultures that had been previously characterised for chemosensitivity: NZM1 was derived from patient 1 specimen 1a, NZM2 from patient 1 specimen 1b and NZM4 from patient 12 [23]. Cytogenetic analysis showed that each of the seven lines displayed abnormal karyotypes containing only human chromosomes with all but one being hyperdiploid (Table 1). S-phase proportions were determined by flow cytometry (Table 2). Cell lines were used between passage 3 and 25, depending on the cell line.

Determination of radiosensitivity using microculture plates and thymidine incorporation

Initial experiments were carried out with several cell lines to determine the relationship between time of culture, radiation dose and thymidine incorporation. A typical result is shown in Figure 1. A time of 5 days was selected to provide conditions allowing continuous growth of untreated cells as well as maximal expression of the effects of radiation (as detected by a reduction of thymidine incorporation). For each of the 15 cell lines, a preliminary experiment was carried out to determine the best range of cell densities. The response to radiation doses between 0 and 10 Gy was then determined at four cell densities. A representative result is shown in Figure 2. Data were analysed in a single regression equation which was fitted by linear quadratic equations in radiation dose and cell density. For the range of lines, values for α (linear term) ranged from 0 to 0.49 while those for β (quadratic term) ranged from -0.05 to 0.04 (Table 2). As an index of radiation response, the term SF_2' was calculated from the regression line as the thymidine incorpor-

Table 1. Characteristics of melanoma cell lines

Cell line	Pigment	Patient sex	Patient age (years)	Melanoma site	Chromosome number
NZM1 (1a)*	+	M	47	Obturator lymph node	71–78
NZM2 (1b)	–	M	47	Deep iliac lymph node	71–78
NZM3	–	M	69	Cervical lymph node	78–95
NZM4 (12)	+	M	56	Malignant ascites	110–120
NZM5	+	M	79	Axillary lymph node	40–43
NZM6	–	F	72	Small bowel	79–88
NZM7	+	M	36	Cervical lymph node	55–59

*NZM1 and NZM2 were developed from the same patient whose malignant lymph nodes were excised 4 weeks apart. Primary cultures (from [22]) from which the lines were prepared are indicated in parentheses.

Table 2. Radiation parameters of melanoma cell lines

Cell line	Plating efficiency (%)	S-phase content (%)	SF_2' microculture	SF_2 clonogenicity	Microculture α	Microculture β	Clonogenicity α	Clonogenicity β
NZM1	7	10	0.59 ± 0.01	0.50 ± 0.08	0.28	−0.009	0.31	0.021
NZM2	2	11	0.73 ± 0.03	0.88 ± 0.01	0.12	0.018	0	0.032
NZM3	19	27	0.87 ± 0.02	0.91 ± 0.06	0.04	0.018	0	0.044
NZM4	60	22	0.93 ± 0.01	0.89 ± 0.05	0	0.023	0	0.028
NZM5	57	23.5	0.53 ± 0.04	0.36 ± 0.15	0.33	0.007	0.55	−0.020
NZM6	9.4	38.5	0.46 ± 0.06 0.36 ± 0.02*	0.15 ± 0.09	0.49	−0.051	0.94	0
NZM7		24	0.79 ± 0.08*	ND	ND	ND	ND	ND
FME	25	32	0.53 ± 0.03	0.72 ± 0.03	0.28	0.021	0.11	0.028
MM-96	66	40	0.46 ± 0.06	0.58 ± 0.03	0.35	0.016	0.24	0.016
SK-MEL-5	ND	24	0.72 ± 0.10	ND	0.15	0.007	ND	ND
SK-MEL-28	47	28	0.84 ± 0.06	0.74 ± 0.01	0.08	0.007	0.08	0.035
SK-MEL-2	ND	28	0.66 ± 0.02	ND	0.12	0.044	ND	ND
MALME-3M	ND	27	0.80 ± 0.10	ND	0.09	0.009	ND	ND
LOX/IMVI	ND	43	0.68 ± 0.02	ND	0.17	0.013	ND	ND
M19-MEL	ND	30	0.77 ± 0.02	ND	0.11	0.011	ND	ND

ND, not determined. *Radiation block method.

ation at 2 Gy as a fraction of that in unirradiated cells. SF_2' values ranged from 0.36 to 0.93 (Table 2).

Intra-experiment variation, which was minimised by analysing data at all cell densities in one regression equation, was estimated by the average standard error in SF_2 values to be ±0.04. Inter-experiment variation, determined in nine of the cell lines from the average standard error in one or more repeated assays (at different passage number) of SF_2 values, was also ±0.04, demonstrating good reproducibility.

Some thymidine incorporation experiments were carried out to determine whether radiation at a single dose of 2 Gy (block irradiation) would provide better estimates of SF_2 as compared with those for the wedge assay. The survival curve of NZM7 is shown in Figure 3. The intra-experiment variation was similar to that of the wedge assay (Table 2).

Determination of radiosensitivity using clonogenic assays

Preliminary experiments were carried out for 7 of the 15 cell lines to determine the optimal conditions for colony formation using clonogenic assays. Irradiated feeder cells greatly improved the plating efficiencies for all seven cell lines. Three problems arose with the use of feeder cells. Firstly, some melanoma cell cultures irradiated to 40 Gy were able to undergo several cell divisions, thereby creating a background of small colonies and making counting more difficult. Secondly, feeder cell numbers influenced tumour cell colony development; low numbers did not stimulate colony formation while too many inhibited colony growth. Thirdly, the preparation of sufficient feeder cells,

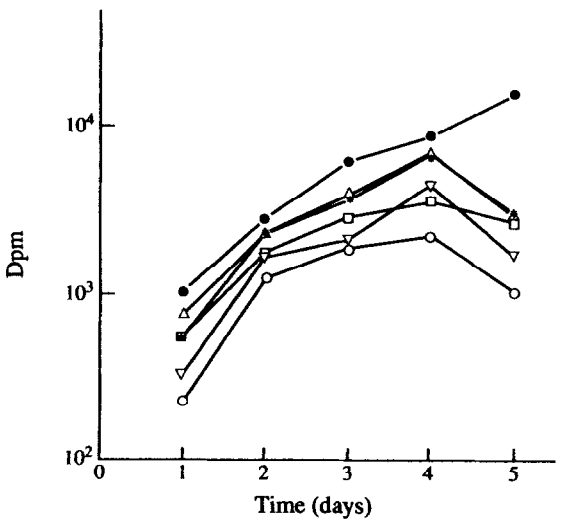

Figure 1. Growth of NZM3 cells in microcultures as assessed by [³H]thymidine incorporation. Cells were seeded at 940 cells per well (6.3 × 10^3/ml) and irradiated at room temperature. Control cells were seeded after irradiation. ● Control (unirradiated); * 1.5 Gy; △ 2.18 Gy; □ 3.61 Gy; ▽ 5.59 Gy; ○ 7.84 Gy.

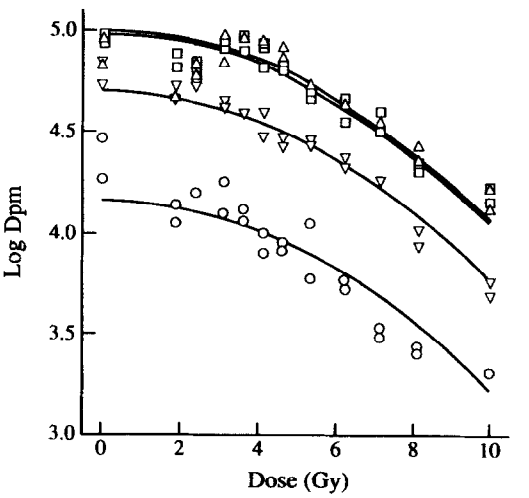

Figure 2. Dose–response curves for NZM4 cells as measured by [³H]thymidine incorporation. Each point on the graph represents one well. Symbols are △ 2000 cells per well, □ 1000 cells per well, ▽ 500 cells per well, ○ 250 cells per well.

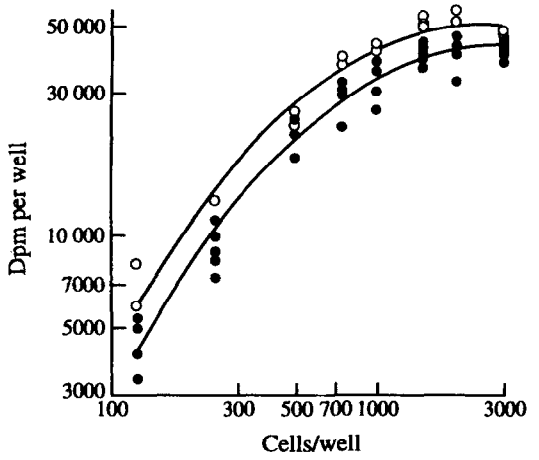

Figure 3. Dose–response curves for NZM7 for replicate microculture experiments using the block irradiation microculture assay. ○ Control cells, ● 2 Gy irradiated cells. Each point on the graph represents one well.

particularly for melanoma lines with long doubling times, was time-consuming and labour-intensive. Colony size and number was improved and background colonies eliminated, when FME, MM-96, NZM1 and NZM2 cells were grown in the presence of heavily irradiated (100 Gy) FME cells (10^4 per tube). However, NZM3 and NZM4 would not form colonies in the presence of such heavily irradiated FME feeders and colony growth was, therefore, measured with homologous feeders irradiated to 40 Gy, with the colony counts being corrected for background in cultures of feeders alone. NZM5, NZM6 and NZM7 lines cells were found to form good colonies when plated at high cell number in the absence of feeder cells. Plating efficiencies ranged from 2% for NZM2 to 66% for MM-96 (Table 2). No significant correlation was found between SF_2 and either plating efficiency, chromosomal number or proportion of S-phase, G_1- or G_2/M-phase cells.

Survival parameters for nine cell lines were determined by clonogenic assay (Table 2) and six of the survival curves are shown in Figure 4. Curves were fitted by linear quadratic equations, the parameters of which (α and β) correlated significantly with those obtained for the thymidine incorporation assays ($r = 0.89$ for α; $r = 0.77$ for β; Figure 5). Derived SF_2 values were also linearly correlated significantly with SF_2' values ($r = 0.89$).

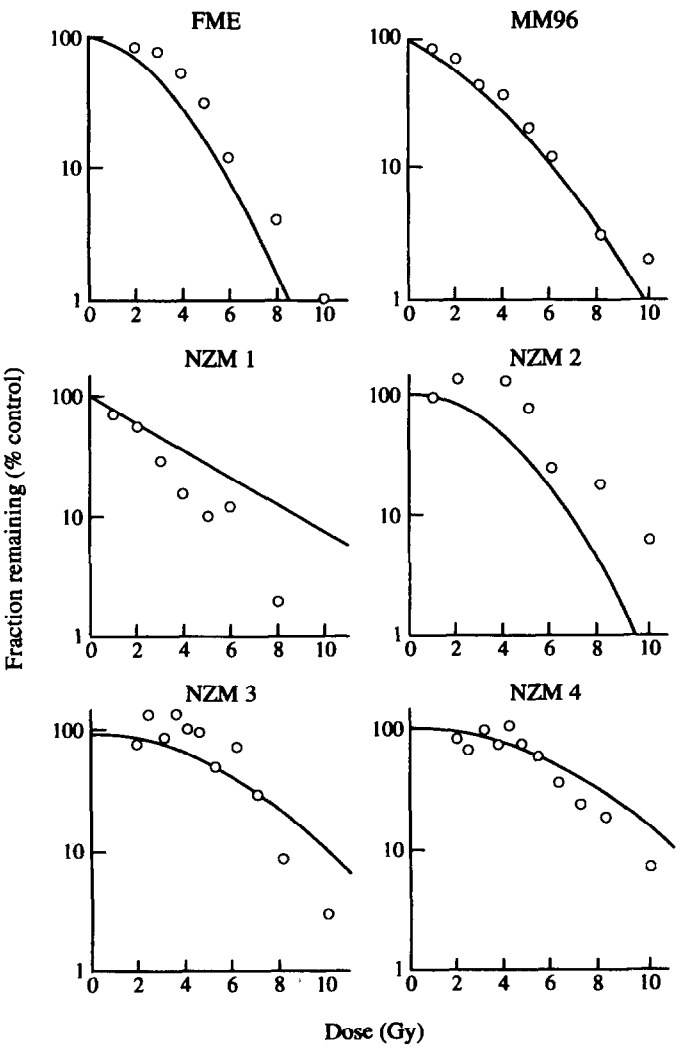

Figure 4. Comparison of dose–response curves for clonogenic assays (○) and [^{3}H]thymidine incorporation assays (shown as a regression line) for six cell lines.

DISCUSSION

We have shown that a thymidine incorporation microculture system can provide reproducible radiosensitivity data (SF_2 values) for human melanoma cell lines, correlating with SF_2 values determined by clonogenic assay and thus capable of predicting such values. The thymidine incorporation and clonogenicity assays produce similar dose–response curves (Figure 4), with significantly correlated α and β coefficients (Figure 5). Although there is some divergence between the two assays at higher radiation doses (Figure 4), good agreement is obtained over the clinically-relevant lower radiation doses.

The use of a regression equation to combine data obtained at several seeding densities greatly improves the accuracy of determination of radiosensitivity parameters, and minimises the potential problem that at higher cell densities, proliferation of control cells is slightly suppressed (Figure 2, 3). The lead wedge method provides a range of radiation doses, while the block method might be expected to provide better statistics at a single radiation dose (2 Gy). However, the wedge method appears to be of comparable accuracy and also provides the radiosensitivity parameters α and β.

The choice of incubation time for microculture measurement of radiosensitivity is somewhat arbitrary, but is important for obtaining SF_2' values predictive of SF_2. If the incubation time is too long, cells must be inoculated at very low densities to allow continued growth over the whole incubation period. If the incubation time is too short (less than 5 days), thymidine incorporation by lethally-damaged cells, which retain a limited capacity for DNA synthesis (as seen in the time course in Figure 1), will result in underestimation of radiosensitivity and in tailing of the dose–response curve. Some evidence of tailing is seen in the present results, where the NZM1, NZM5 and NZM6 lines all show slightly negative β values (indicative of tailing) and SF_2' values which are somewhat lower than the measured SF_2 values.

Most of the cell lines tested are radioresistant, with only three cell lines exhibiting SF_2' values of less than 0.5 (Table 1). The average SF_2' value for the newly established lines (0.69) is similar to that of the established cell lines (0.68), and among the new lines there is no significant difference between the average

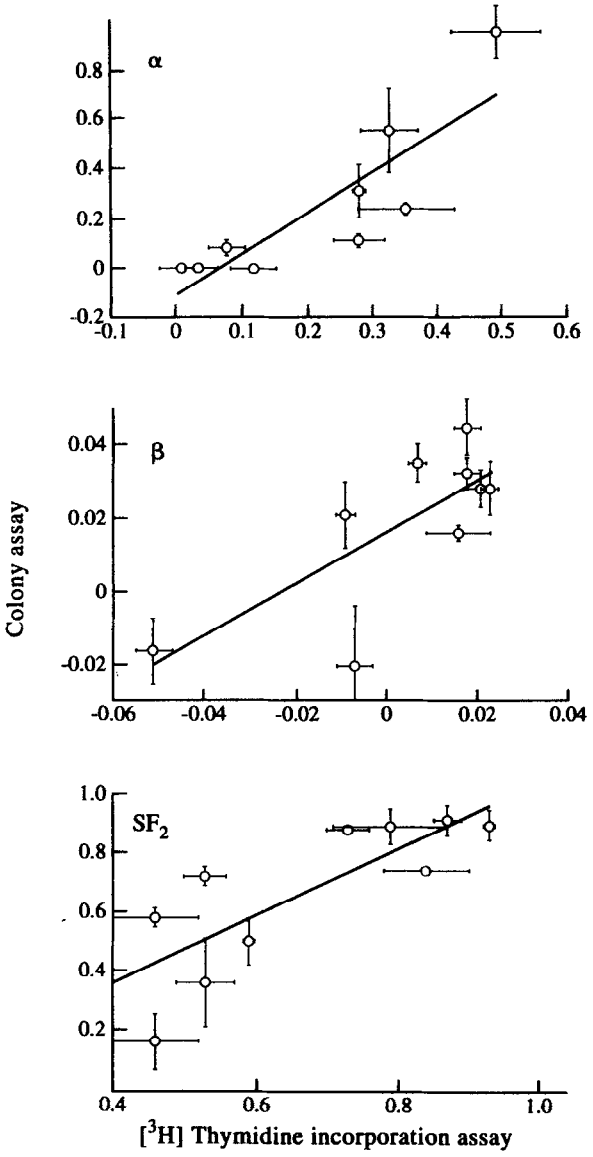

Figure 5. Linear regression lines for radiosensitivity parameters in clonogenic assays versus [^{3}H]thymidine incorporation assays α, β and SF_2. The correlation coefficient for α values is 0.89, for β values is 0.77 and for SF_2' versus SF_2 is 0.89. Vertical and horizontal bars represent S.E.M.

SF_2' value of the melanotic (0.71) and amelanotic (0.69) melanomas. The ranges of SF_2 values (0.36–0.93) and SF_2 values (0.15–0.91) are comparable with the published ranges of radiosensitivity in melanoma cells of 0.22–0.77 [15] and 0.15–0.70 [29].

Cultures in 96-well plates offer a number of advantages over soft agar colony-forming assays: cell yields are higher (since tumour cells are not required to be disaggregated to a single cell suspension), cell–cell contacts are maintained in the small clusters of cells cultured, an end-point is reached after a short time (approximately 1 week) and semi-automated cell harvesting and scintillation counting allows high throughput. Because a relatively small number of cells (approximately 8×10^5) are needed for radiosensitivity testing, other assays may also be performed. We have also obtained chemosensitivity testing data for 16 anti-neoplastic drugs with these cell lines, finding no correlation between radiosensitivity and chemosensitivity to each of the drugs [30].

Although no correlation with clinical response of the patients from whose tumours the cell lines were derived is possible, it is interesting that of the 2 patients in this study who received radiotherapy, the first had no clinical response to radiotherapy and the derived cell line (NZM7) was radioresistant. The second had a partial response and the derived cell line (NZM5) was relatively radiosensitive (Table 2). We are now extending the thymidine incorporation method to the determination of the radiosensitivity of primary cultures of tumour cells from patients. The use of such assays may be particularly suited to malignant cutaneous melanoma tissue since cells grow well from clinical samples in primary culture [23]. However, melanoma is infrequently treated by radiotherapy and our current aim is to develop culture methods for tumours, such as cancer of the cervix, where irradiation is commonly used in therapy.

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Measurement of Radiation Survival Using the MTT Assay

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There is increasing interest in the development of rapid assays of radiosensitivity which can be used on clinical specimens. Unfortunately, the measurement of radiation survival using clonogenic assays, which are the established standard, can be difficult and time consuming. We have used the MTT assay to measure the radiation survival of four lymphoblastoid cell lines with low plating efficiencies. We measured surviving fractions both when the irradiated cells had regained exponential growth and when the non-irradiated cells had undergone four or more doublings. The results were compared to surviving fractions measured by clonogenic assay. We found both methods could be used successfully to rank the cell lines in order of radiosensitivity. However, cells exposed to the higher radiation doses in the MTT assay did not always regain exponential growth, limiting the dose range for which the assay was useful. We also found the best correlation between the two assays was sometimes obtained by using the MTT surviving fractions from different days for different radiation dose levels. Thus, although the MTT assay can be used to measure radiation survival in relation to other cell lines, its use can be complicated by restrictions on radiation dose ranges and difficulties with data interpretation.

Key words: MTT assay, clonogenic assay, radiation survival, lymphoblastoid cell lines
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INTRODUCTION

THE RADIATION survival of cells has traditionally been measured by clonogenic assay [1, 2]. However, such assays require reliable cell growth in culture media, and can be difficult and time consuming to perform in cell lines with low plating efficiencies [3]. The search for alternative methods to measure radiation survival has resulted in several evaluations of the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay for this purpose [4–7]. The MTT assay quantifies the ability of viable cells to reduce a yellow tetrazolium salt to a purple formazan crystal using the mitochondrial enzyme, succinate dehydrogenase [8]. The optical density of the solution resulting from the solubilisation of the formazan precipitate with an organic solvent can be detected using a multiwell spectrophotometer [9]. The MTT assay is thus semi-automatable and rapid, and has been used successfully to test the chemosensitivity of established cell lines [10, 11] and fresh tumor samples [12–14].

The use of the MTT assay for the measurement of radiation