Drug and Radiation Sensitivity Measurements of Successful Primary Monolayer Culturing of Human Tumor Cells Using Cell-adhesive Matrix and Supplemented Medium

Fraser L. Baker, Gary Spitzer, Jaffer A. Ajani, William A. Brock, John Lukeman, Sen Pathak, Barbara Tomasovic, Diva Thielvoldt, Marcia Williams, Charlotte Vines, and Philip Tofilon

ABSTRACT

The limitations of the agar suspension culture method for primary culturing of human tumor cells prompted development of a monolayer system optimized for cell adhesion and growth. This method grew 83% of fresh human tumor cell biopsy specimens, cultured and not contaminated, from a heterogeneous group of 396 tumors including lung cancer (33 of 114, 29%); melanoma (54 of 72, 75%); sarcoma (46 of 59, 78%); breast cancer (35 of 39, 90%); ovarian cancer (16 of 21, 76%); and a miscellaneous group consisting of gastrointestinal, genitourinary, mesothelioma, and unknown primaries (78 of 91, 86%). Cell growth was characterized morphologically with Papanicolaou-stained coverslip cultures and cytogenetically with Giemsa-stained metaphase spreads. Morphological features such as nuclear pleomorphism, chromatin condensation, basophilic cytoplasm, and melanin pigment were routinely seen. Aneuploid metaphases were seen in 90% of evaluable cultures, with 15 of 28 showing 70% or more aneuploid metaphases. Colony-forming efficiency ranged between 0.01 and 1% of viable tumor cells, with a median efficiency of 0.2%. This culture system uses a low inoculum of 25,000 viable cells per well which permitted chemosensitivity testing of nine drugs at four doses in duplicate from 2.2 x 10^5 viable tumor cells and radiation sensitivity testing at five doses in quadruplicate from 0.6 x 10^6 cells. Cultures were analyzed for survival by computerized image analysis of crystal violet-stained cultures. Drug sensitivity studies showed variability in sensitivity and in survival curve shape with exponential cell killing for cisplatin, Adriamycin, and etoposide, and shoulder survival curves for 5-fluouracil frequently seen. Radiation sensitivity studies also showed variability in both sensitivity and survival curve shape. Many cultures showed exponential cell killing, although others had shouldered survival curves. This method for growing cells from primary human biopsy specimens is more efficient than the agar culture method, enables easier and better biological analysis of the actual cells grown, and permits improved characterization of drug and radiation survival curves.

INTRODUCTION

The method currently in use for quantitative primary human tumor cell culturing is the agar suspension culture method (2). The main feature of this method is its selection of transformed cells for growth (3), although agar suspension cultures also support growth of normal hematopoietic cells (4), benign tumors (5), and anchorage-dependent cells if the medium is supplemented with high serum levels (6) or transforming growth factor (7). Culturing primary human tumor cells in soft agar has problems. More than 50% of tumors cannot be cultured adequately to enable routine testing of drug sensitivity (8, 9). Those specimens that can be tested for drug sensitivity routinely show low cloning efficiencies (less than 0.1%) (10, 11), unusual cisplatin resistance in ovarian cancer (12), and unusual radiation survival curves (13, 14). These findings may represent some of the true biology of human tumors such as low frequency of clonogenic cells in human tumors (15) and cell heterogeneity with tumors containing cells of varying drug sensitivities. Alternatively, the culture conditions may be so inadequate and subject to variability that the results are artifactual. Because of the technical difficulties associated with embedding the cells in agar, it is difficult to routinely prepare cultures for morphologic, cytogenetic and histochemical analysis. The agar suspension culture method also limits the periods of drug exposure either to a 1-hr preincubation, which is frequently accompanied by severe cell aggregation, or to continuous exposure.

Recognizing that cell attachment plays a major role in growth of normal and malignant epithelial and mesenchymal cell lines (16) and that the relationship between growth in agar suspension culture and cancer is not absolute (17, 18, 19), we developed a liquid monolayer culture system optimized for cell adhesion and cell growth.

Cell adhesion was optimized through the use of culture surfaces prepared from CAM, a preparation composed in part of fibronectin and fibrinopeptides. Cell growth was optimized through the use of hormone- and growth factor-supplemented culture medium.

We here report the successful growth of adhesive cells from biopsy specimens of human tumors. We document the malignant origin of cell growth, improved CFUs, and excellent morphologic and cytogenetic details. Survival curves determined for radiation and several common chemotherapy drugs, quantitated by automated image analysis of crystal violet-stained cultures, showed sensitivities and shapes expected for human tumor cells in vitro.
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MATERIALS AND METHODS

Materials

Cell-adhesive matrix was from Lifetec, Irvine, CA. Culture medium was Ham’s F-12 (K. C. Biological, Lenexa, KS) with: 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer (2.7 mg/ml; Sigma, St. Louis, MO); 10% swine serum (J. R. Scientific, Woodland, CA); and penicillin-streptomycin (100 units/ml) (GIBCO, Grand Island, NY) and supplemented with hormones (20, 21): transferrin (10 μg/ml); hydrocortisone (0.5 μg/ml); EGF (5 ng/ml); and insulin (5 μg/ml) (Collaborative Research, Lexington, MA). Attachment medium was the same formulation as the culture medium plus 0.6% Methylcellulose 4000 (Fisher Scientific, Houston, TX). Enzyme disaggregation medium was F-12 with 10% fetal calf serum, 0.075% collagenase type III (Cooper Biomedical, Malvern, PA), and 0.005% DNase (Sigma). Phosphate-buffered saline was Dulbecco’s (GIBCO). Culture vessels used were 24-well and 6-well multiwell plates (Costar, Cambridge, MA). Twenty-two-mm-square polyester coverslips were from Raylabcon, Shirley, NY.

Method for Primary Human Tumor Cell Culture

Culture Surface Preparation. CAM is a complex containing 50 to 70% fibronectin and fibrinopeptides supplied in solution at approximately 0.25 mg of protein per ml. Culture surfaces were prepared by coating the bottom of tissue culture dishes or wells by sequentially dispensing and aspirating the solution leaving a thin film of CAM. To produce an even coating in the small diameter wells of 24-well multiwell plates, the bottom of tissue culture dishes or wells by sequentially dispensing and aspirating the solution leaving a thin film of CAM. The culture surfaces were prepared by coating the bottom of tissue culture dishes or wells by sequentially dispensing and aspirating the solution leaving a thin film of CAM. The culture surfaces were prepared by coating the bottom of tissue culture dishes or wells by sequentially dispensing and aspirating the solution leaving a thin film of CAM.

Cell Preparation. Biopsy specimens of human solid tumors were obtained from the Department of Pathology of the University of Texas, M. D. Anderson Hospital and Tumor Institute at Houston, during routine diagnostic procedures, transported to the laboratory, and placed in tissue culture medium. Effusions were anticoagulated with preservative-free heparin (10 units/ml) immediately after aspiration. Solid specimens were minced with scalpels to 1-mm pieces. The solid- and fluid-derived tissues were disaggregated to single cells by incubating in enzyme disaggregation medium for 16 h with constant stirring.

Cell Culture Inoculation. The yield of trypan blue-viable cells was determined by a hemocytometer count of trypan blue-negative nucleated cells larger than 10 μm (excluding lymphocytes, granulocytes, and mesothelial cells) with a microscope fitted with a phase-gradient imaging apparatus (22) at a magnification of 400. The cell suspension was diluted with attachment medium to 25,000 cells/ml, and 24-well plates were inoculated with: (a) a cell inoculum titration consisting of 25,000, 12,500, 6,250, and 3,125 cells per well in the first column of four wells; and (b) 25,000 cells in each of the 20 remaining wells of each plate.

Adherent Cell Harvest. After 24 h of incubation, the attachment medium was aspirated, and the adherent cells were washed with phosphate-buffered saline and refed with culture medium, drugs, or experimental conditions as described below. The number of adherent cells harvested from the inoculum was counted using an inverted microscope fitted with a phase-gradient apparatus (22) at magnifications of 100 or 200. The yield of adherent cells per well was calculated by multiplying the average field count (N = 5) by 82 or 330 for × 100 or × 200 magnification, respectively.

Controls. Columns 1 and 2 were reserved for control and EGF studies. Column 1 consisted of the inoculum titration described in “Cell Culture Inoculation.” The second 4-well column consisted of: one well fixed with 95% ethanol after 24-h incubation to provide a record of the starting cell population; one well with culture medium to provide two control cultures per plate; one well with 5 μCi of tritiated thymidine per ml to provide a record of the background cell population; and one well cultured without added EGF to determine EGF sensitivity.

Drug Responses. Drug survival studies were carried out in the remaining four columns of four wells. Four drugs were tested each at four concentrations over a narrow concentration range which bracketed the IC50 of GM-CFC (23). The drugs were added to duplicate cultures after 24 h of incubation and removed after 6 days of incubation, resulting in a 5-day exposure period. After an additional 7 days of incubation (13-day incubation period), the cultures were stained, and survival at each dose was determined quantitatively by image analysis.

Radiation Response. Each of the 24 wells of multiwell plates was inoculated with 25,000 cells. A 3-mm lead shield was made that permitted a 250-kV X-ray source to irradiate a single column of four wells, thus permitting quadruplicate cultures, consisting of a control set and five other sets each receiving doses of 1.0, 2.0, 3.0, 4.0, and 6.0 Gy. The cultures were irradiated after they had been washed and refed after 24 h of incubation. After incubation for a total of 13 days, the cultures were
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Fig. 2. A and B, Day 1 and Day 13 photographs, respectively. Cultures of melanoma. The Day 1 culture showed pleomorphic cells with dendritic-like cytoplasmic processes. Two of the three cells shown were broadly spread. Only one of the two spread cells was pigmented. The Day 13 culture showed pleomorphic fusiform cells with elongated cytoplasmic processes that contained melanin granules. Nuclei were oval and contained one to three small round nucleoli. × 400.

Fig. 3. Histogram of the yield of adherent cells per 16-mm well in 80 consecutive assays.

stained, and survival at each dose was determined by quantitative image analysis. The data were then computer fitted to a linear quadratic model for radiation survival, using least-squares regression analysis.

Incubation. Cultures were incubated in a humidified atmosphere of 5% CO₂ in air for 13 days. All cultures were refed by 100% medium exchange after 6 days of incubation.

Fixing and Staining. The cultures were washed with phosphate-buffered saline, fixed in 70% ethanol for 20 min, and stained with 0.5% crystal violet. The multiwell plates were processed four at a time in 2-litter tubs of reagent to reduce intratest staining variability.

Quantitation of Cell Growth and Survival. A Nikon/Joyce-Loeble Magiscan image analysis system was used to determine the absorbance of crystal violet-stained cultures integrated over the surface of the monolayer cultures. The resulting parameter, IA, reached maximum values exceeding 3000 units relative to the Day 1 fixed cultures. Cultures with an IA of 300 units or less above background were not evaluable. Growth in such cultures was approximately 15 to 20 colonies per control well or 0.06 to 0.08% colony-forming efficiency. Overall variability of the culture system appears to be 3 to 4%, as an analysis of a 24-well plate in which all wells were treated as controls showed a mean IA of 2295 units and a SD of 84 units, resulting in a coefficient of variation of 3.7%.

Survival was calculated as the fraction of IA of treated cultures relative to the IA of control cultures. We have reported that the surviving fraction of an established cell line, measured by a standard clonogenic assay, was in close agreement to results obtained with this method over the radiation dose range of 0 to 6 Gy (24). It was also found that radiation-induced growth delay and growth kinetics were not significantly different from control across the dose range used. Even though this assay is not a direct clonogenic assay, the results suggest that survival calculated from IA measurements closely resembles the true clonogenic potential of the irradiated cells.

Morphology. Cultures for morphological analysis were set up on two 22-mm plastic coverslips coated with CAM prior to use. The coverslips were placed in 35-mm wells which were inoculated with 100,000 cells in 4 ml. After 20 to 24 h of incubation, a coverslip was removed from a well, washed, clipped to a microscope slide, and stored in 95% ethanol. After 2 wk of incubation, the second coverslip was removed, washed, and clipped to the same microscope slide. The Day 1 and Day 13
Fig. 4. Photographs of typical cell dose titrations of 25,000 (A), 12,500 (B), 6,250 (C), and 3,125 (D) viable cells per well, showing merged colonies at the 25,000-cell inoculum level, decreasing cell density with decreasing inoculum, and individual colonies at the 6,250- and 3,125-cell inocula levels.
Successful Human Tumor Cell Growth in Vitro

Cytogenetic Analysis. Cultures for cytogenetic analysis were also set up on CAM-coated 22-mm coverslips. After 11 days of incubation, the medium was exchanged to increase the harvest of metaphase cells 2 days subsequent. After 13 days of incubation, cultures were harvested in situ for cells in metaphase by incubating with 1% hypotonic citrate for coverslip cultures were stained with Papanicolaou stain.

RESULTS

Effect of CAM and EGF. The ability of CAM to support cell adhesion and growth was demonstrated by comparing monolayer cell growth over culture surfaces prepared from agarose with and without CAM. Agarose culture surfaces are nonadhesive. Cells did not attach toagarose and were lost from the coverslips cultures were stained with Papanicolaou stain.

Improvement in growth was also shown in 47 of 115 primary human tumor cell cultures by the addition of EGF to the culture medium. This effect was primarily on colony size with increases from 50 to 200 cells routinely seen with no change in colony number (25). This high response to EGF was frequently observed in melanoma cultures; the one shown in Fig. 2 responded to EGF with a 4-fold increase in cell growth.

Adherent Cell Yield. Routinely, 25,000 viable cells were inoculated into 16-mm wells, which have a surface area of one-fourth that of a 35-mm dish. Fewer cells than this adhere to the culture surface and remain in the culture. The yield of adherent cells varied from 0 to approximately 25,000 cells per well, with a median harvest of 3,000 cells per well. The distribution of the yield of adherent cells from an inoculum of 25,000 viable cells over 80 consecutive assays is shown in Fig. 3. The yield of adherent cells increased linearly with increased cell inoculum. The yield of adherent cells was not correlated with the content of tumor cells in the specimen.

CFE. Inoculating 25,000 viable cells into a 16-mm well generally resulted in merged colonies so that individual colonies were not scorable. To evaluate CFE, the colony count was done at the dilution of the cell inoculum titration (25,000, 12,500, 6,250, and 3,125 cells per well) at which individual colonies were discernible. Fig. 4 shows a typical cell inoculum titration after 13 days of incubation, illustrating merged colonies with the highest inoculum, a decrease in cell density over the culture surface with decreasing inoculum, and very large individual colonies at the lower inocula. CFE (number of colonies per 25,000 viable cells) was calculated by multiplying the actual colony count by a factor depending on the dilution at which the count was made. The number of colonies per 25,000 viable cells varied from 5 to over 200, reflecting a CFE of 0.01 to 1%. The distribution of CFE is shown in Fig. 5.

Colony size, an evaluation much more appreciated in monolayer culture, routinely exceeded 100 cells and often exceeded 250 cells. Occasionally, colony size was small, 8 to 32 cells per colony.

Because we were unable to count colonies over the cell inoculum titration at the inoculum used in this monolayer assay, we analyzed the linearity of cell growth over the dose response using the Magiscan2 image analysis system. With this instrument we quantitated the IA of crystal violet-stained cultures over the cell-dose titration and found it to be linear as shown by three cases in Fig. 6.

Morphology. Cells spread very broadly over culture surfaces of CAM and showed morphologies that varied from polygonal to fusiform, often with long cytoplasmic processes.

Fig. 2 shows a melanoma culture, as evidenced by the predominance of brown pigment granules in the cytoplasm of the Day 1 culture and in the majority of cells in the Day 13 culture. Melanoma cells grew in a predominantly fusiform morphology, with round to oval nuclei, multiple small irregular nucleoli, and pale-staining chromatin. The Day 1 cell and the cells that formed in culture after 13 days of incubation appeared similar in terms of overall morphology, nuclear structure, and pigmentation. A sarcoma is shown in Fig. 7. The Day 1 culture showed large fusiform cells with intense cytoplasmic granularity, an irregular nucleus with prominent nucleoli, and coarse chromatin. The Day 13 culture grew colonies of similar cells.

A lung adenocarcinoma culture is shown in Fig. 8. The Day 1
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Fig. 7. A and B, photographs of Day 1 and Day 13 cultures, respectively, of a sarcoma. The Day 1 culture showed a large fusiform cell with an irregular nucleus and many cytoplasmic organelles. The Day 13 culture showed growth of large fusiform cells with irregular nuclei and many prominent cytoplasmic organelles. x 400.

culture showed polygonal and broadly spread cells with large, round nuclei; prominent round to irregular nucleoli; thick nuclear rims; and clumped, densely stained chromatin. The Day 13 culture shows polygonal cells featuring nuclei similar to that of the Day 1 cells.

Day 1 and Day 13 cultures of a lung squamous cell cancer are shown in Fig. 9. The Day 1 culture shows a fusiform and a polygonal cell, each with a similar elongated nucleus, two small round nucleoli, thin nuclear rims, and pale chromatin. The culture grew large pleomorphic cells with pleomorphic elongated nuclei; multiple, often irregular nucleoli; and pale-staining, slightly granular chromatin.

Cell cultures from Days 1, 5, 9, and 13 from a fluid specimen from a transitional cell cancer are shown in Fig. 10. The Day 1 culture showed the cell population to be composed predominantly of well-spread, round cells with foamy cytoplasm and a small nucleus that were considered histiocytes. Elongated cells with long cytoplasmic processes and large nuclear:cytoplasmic ratio were seen and were considered as tumor cells. The time sequence of photographs showed rounding up and diminished content of histiocytes, increased content of elongated cells, and their development of epithelial morphology by the Day 13 culture.

Cytogenetics. Thirty-eight % of cultures yielded evaluable metaphases. Photographs of Giemsa-stained metaphase spreads harvested from cultures of colon, ovarian, renal, and lung tumor cells are shown in Fig. 11. The colon metaphase showed 69 chromosomes. The ovarian metaphase had a very high number of chromosomes, with chromosome fragments and individual chromatids. The renal cell metaphase showed 52 chromosomes, and the lung cancer culture showed 71 chromosomes coated with amorphous substance, some of which had spiral structures.

Twenty-eight samples were processed for chromosome counting. Aneuploid metaphases were observed in 90% of the cultures evaluated. Fifteen assays showed greater than 90% aneuploid metaphases, 11 assays showed 10 to 30% diploid metaphases, and 2 assays showed a predominance of diploid normal-appearing metaphases. Pathological analysis of the biopsy specimen for one of the diploid assays showed a mixed tumor composed of an ovarian fibroma and adenocarcinoma. The other predominantly diploid culture was a melanoma that rapidly grew in the assay (forming 85 cell colonies after 10 days of incubation) and showed resistance to drugs. The patient experienced progressive disease and died 3 mo after the assay.

An additional 11 randomly selected cultures were processed for evaluation of sister chromatid exchange, and simultaneously chromosome number was also determined. Of 200 metaphases examined in this group, extreme variation in chromosome number was seen. Only two diploid metaphases were seen in this study (1%).

Radiation Survival Curves. Radiation survival curves were constructed by plotting the log of the survival versus the radiation dose. Survival curves from a melanoma and a sarcoma are shown in Fig. 12. Variation in sensitivity and in the degree of shouldering in the survival curves between cultures was observed. We have analyzed over 40 irradiated cultures thus far,
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Fig. 8. A and B, photographs of Day 1 and Day 13 cultures, respectively, of lung adenocarcinoma. The Day 1 culture showed oval cells with thin, occasionally vacuolated cytoplasm and round nuclei with one or two prominent nucleoli. The Day 13 culture showed numerous similarly appearing cells with prominent single nucleoli. Occasional giant multinucleated cells were seen. × 400.

and the range of sensitivities varies but falls within the range expected for cell cultures. The calculated range of survival after 2 Gy was from 20 to almost 90%.

Although a constant inoculum of 25,000 cells was used, the yield of adherent cells varied between assays as did the final cell density in the control cultures. High-density cultures may be subject to inhibition of cell growth by density-dependent inhibition, to depleted medium, or both. To study whether variation in the final cell density affected the measurement of radiation sensitivity, we determined radiation survival curves at four cell inocula: 50,000; 25,000; 12,500; and 6,250 cells per well. The survival curves obtained at the lower inocula showed similar radiation sensitivities; however, cultures with the highest inoculum showed decreased sensitivity and an increased shoulder (Fig. 13). These results show that it is necessary to use lower cell inocula numbers so that the cells in the unirradiated controls are still growing at the end of the culture period.

Drug Survival Curves. Drug survival curves were constructed by plotting the log of the survival versus the drug dose. Examples from melanoma and squamous cell lung cancer biopsy specimens with different sensitivities are shown for each drug (cisplatin, Adriamycin, etoposide, and 5-fluorouracil) in Fig. 14. Exponential responses were observed for cisplatin, Adriamycin, and etoposide, and a shouldered response was observed for 5-fluorouracil in the sensitive assay. A shouldered response was observed for all the other drugs in the resistant assay. In over 110 to 170 responsive drug sensitivity assays, the IC₅₀ varied from 0.03 to 0.7 μg/ml for cisplatin, 0.0014 to 0.028 μg/ml for Adriamycin, 0.025 to 0.3 μg/ml for etoposide, and 0.11 to 0.6 μg/ml for 5-fluorouracil.

Performance. The performance rate of a drug assay based on this culture system was determined. Of 475 specimens received by the laboratory, 328 drug sensitivity reports were made reflecting an overall performance of 69% (Table 1). The performance rate according to the number of assays actually set up and not contaminated reached 83% (Table 2). This performance was fairly consistent over a spectrum of tumor types (Table 2). Hematopoietic neoplasms are nonadherent and are lost from the culture system.

DISCUSSION

We have described a monolayer cell culture system for adherent cells that is 70 to 80% efficient in growing colonies from tumors of diverse histology and origin. The CFE (0.01 to 1%; median, 0.2%) of this system suggests improvement over the Hamburger and Salmon (2) agar suspension culture method, although agar suspension culture shows improved efficiency when cultured at low densities (26) or with the modifications described by Courtney (27). Colony size routinely exceeded 100 cells and frequently exceeded 250 cells after 13 days of incubation.

The morphology of the cells grown in this culture system varies from polygonal to fusiform. The expression of fusiform
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Fig. 9. A and B, photographs of Day 1 and Day 13 cultures, respectively, of lung squamous carcinoma. The Day 1 culture showed well-spread cells with elongation of the cytoplasm that resembled fibrous cells. Nuclei were oval with multiple small nucleoli. The Day 13 culture showed growth of the elongated fibrous-like cells, which had delicate thin cytoplasm and pleomorphic oval nuclei with up to five irregular nucleoli, × 400.

morphology in these primary human tumor cell cultures raises the concern that growth of fibroblasts may occur. However, the finding of morphological features of cancer, such as pigment in melanoma cells, large nucleus:cytoplasm ratio, nuclear pleomorphism, prominent nucleoli, basophilic cytoplasm, and cording (28) in many cultures showing fusiform cell morphology, suggests that any potential contamination of the cultures with fibroblast growth is small.

To ascertain the cancer of the cells grown, ploidy was determined by counting the number of Giemsa-stained chromosomes in expanded metaphases. The distribution of chromosome number was generally broad, with aneuploid metaphases seen in 95% of evaluable cultures. Frequently, both hypodiploid and hyperdiploid populations were seen in the same culture, suggesting a high incidence of aneuploidy and heterogeneous DNA populations in the cultured cells. In a study determining the frequency of sister chromatid exchange in primary human tumor cells cultured in this system, 11 randomly selected cultures were evaluated for chromosome number, and in over 200 metaphases examined, diploid metaphases were rarely seen. In a total of 39 cultures examined for chromosome number, only 2 (5%) were predominantly diploid. These results suggest that, when cells from biopsies of malignant tumors are cultured in this culture system, predominantly malignant cells grow and that normal cell growth, if it occurs, is a minority proportion of the culture.

The end point for the measurement of growth in our method of analysis is the absorbance of crystal violet-stained cells integrated over the culture surface. This end point, IA, has been correlated with the traditional colony assay by us (24) and others (29). The plating of nonviable aggregates and debris does not contribute error in IA as they do not attach and are removed from the culture during medium exchange. Error in this end point due to the plating of viable aggregates of CFCs appears minimal, as an aggregate of CFCs grows into a larger colony that contributes proportionally more to the IA of the culture. This contrasts with the agar suspension clonogenic assay, in which a colony derived from an aggregate of CFCs is counted as one regardless of its size. This error, inherent in the agar suspension clonogenic assay, places a stringent need for the inoculum into the agar culture system to be a pure single cell suspension, a criterion that may not be attainable due to the propensity for tumor cells to aggregate. Aggregates of CFCs would be expected to produce plateaus in survival curves and limit the dose response of the agar suspension clonogenic assay, particularly in the measurement of radiation sensitivity.

The adhesive tumor cell culture system produced a wide range of radiation sensitivities, all in the range expected for in vitro cultures. The ability of the adhesive tumor cell culture system to produce classical radiation survival curves suggests that the system may have potential usefulness in determining the sensitivity of human tumors to radiation. Reviews of published radiation survival curves of established human tumor cell lines showed that there are significant differences in survival curves at 2.0 Gy, but not in D0 (30, 31). Small differences in survival at 2.0 Gy
Fig. 10. A to D, photographs of Day 1, 5, 9, and 13 cultures, respectively, of a fluid specimen from a transitional cell cancer. The Day 1 culture showed two cell populations. The predominant population was round, was very well spread, had a foamy cytoplasm, and had a small bean-shaped nucleus. This population resembled histiocytes. The minor population was very elongated, having a prominent nucleus and nucleolus. The Day 5 culture showed decreased numbers and degree of spreading by the histiocytes and increased numbers of the elongated cells. The Day 9 culture showed a dramatic increase in the number of elongated cells and further loss of histiocytes. The level of cell growth in the Day 13 culture was similar to the Day 9 culture, however, the Day 13 culture showed marked development of epithelial morphology and giant cells with multiple heterogeneous nuclei. x 100.
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Fig. 11. Metaphase spreads from colon (A), ovarian (B), renal (C), and lung (D) cancer. The colon cancer metaphase was hyperdiploid with 72 chromosomes. The ovarian cancer metaphase also was hyperdiploid with numerous chromosomes and chromosome fragments. The renal cancer metaphase contained 52 chromosomes, and the lung cancer culture showed 71 chromosomes covered with amorphous material and having spiral morphology.
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Fig. 12. Radiation-survival curves of cells from a melanoma (○) and a sarcoma (□) biopsy specimen showing differences in sensitivity. Bars, SE.

Fig. 13. Radiation survival curves at inocula of 50,000 (○), 25,000 (■), 12,500 (■), and 6,250 (□) cells per well show similar sensitivities for the lower three inocula and decreased sensitivity at the highest inoculum. (Standard errors were omitted for clarity).

Fig. 14. Drug survival curves of cells from melanoma (●) and a squamous cell lung cancer biopsy (♦) specimen for cisplatin (a), Adriamycin (b), etoposide (c), and 5-fluorouracil (d) for two assays showing different sensitivities. Exponential responses were observed for cisplatin, Adriamycin, and etoposide in the sensitive assay. A shoulder in the response was observed for 5-fluorouracil in the sensitive assay and for all the drugs in the resistant assay.

Table 1

| Performance of 475 primary cell cultures from biopsy specimens of human tumors in the adhesive tumor cell culture system |
|--------------------|-----------------|-----------------|
| Specimens/assays    | No.             |                  |
| Specimens assessed  | 475 (100)*      |                  |
| Specimens with inadequate yield of cells | 44 (9)           |                  |
| Assays cultured     | 436 (86)        |                  |
| Assays contaminated | 33 (7)          |                  |
| Assays with low growth | 58 (12)     |                  |
| Assays with technical error | 12 (3)       |                  |
| Completed assays    | 328 (69)        |                  |

* Numbers in parentheses, percentage.

would result in great differences in survival after several fractions, assuming equal effect per fraction. Because this measurement could have important diagnostic value, we plan to test for a possible correlation between survival at 2.0 Gy and clinical outcome in radiotherapy patients with this assay.

Cisplatin, Adriamycin, and etoposide showed simple exponential cell killing, typical of in vitro responses for these drugs (32), and shouldered responses in more resistant tumors. The sensitivity of different tumors to these drugs varied. The IC50 rates reported herein are similar to the dose range reported for these drugs by Hill et al. (33), using cell lines from human tumors. For myelosuppressive drugs, the drug dose range showing toxicity to human tumor cells falls within the range of toxicity to normal marrow GM-CFC, confirming the findings of Hug et al. (34).

The adhesive tumor cell culture system offers advantages over the agar culture system for primary cultures of human tumor cells. It is efficient and grows most tumor types. Morphological and cytogenetic analyses suggest that the system grows malignant cells, and the CFE rate suggests that the majority of proliferating cells in tumors are grown. The drug and radiation survival curves are classical and show sensitivities expected of cells in vitro. This method is relatively easy to perform, and the cultures can be read by image analysis systems. It appears capable of assaying human tumors for drug and radiation sen-
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Table 2
Performance of adhesive tumor cell culture system by tumor type relative to assays not contaminated

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>No. of assays set up</th>
<th>No. of assays completed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>114</td>
<td>93 (82)*</td>
</tr>
<tr>
<td>Breast</td>
<td>39</td>
<td>35 (89)</td>
</tr>
<tr>
<td>Sarcoma</td>
<td>59</td>
<td>48 (78)</td>
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<tr>
<td>Melanoma</td>
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<td>59 (82)</td>
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<tr>
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<td>16 (76)</td>
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<tr>
<td>Gastrointestinal</td>
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<td>20 (87)</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>21</td>
<td>18 (86)</td>
</tr>
<tr>
<td>Totals</td>
<td>396</td>
<td>327 (83)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, percentage.

Sensitivity, which is potentially useful in predicting clinical response and in screening new chemotherapy agents.

REFERENCES

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