Acta Oncologica Vol. 35, No. 4, pp. 451–456, 1996

RADIOSENSITIVITY OF MESOTHELIOMA CELL LINES

ANNA-MAIJA HÄKKINEN, ANTERO LAASONEN, KAIJA LINNAINMAA, KARIN MATTSON and SEPPO PYRHÖNEN

The present study was carried out in order to examine the radiosensitivity of malignant pleural mesothelioma cell lines. Cell kinetics, radiation-induced delay of the cell cycle and DNA ploidy of the cell lines were also determined. For comparison an HeLa and a human foetal fibroblast cell line were simultaneously explored. Six previously cytogenetically and histologically characterized mesothelioma tumor cell lines were applied. A rapid tiazolyl blue microtiter (MTT) assay was used to analyze radiosensitivity and cell kinetics and DNA ploidy of the cultured cells were determined by flow cytometry. The survival fraction after a dose of 2 Gy (SF2), parameters α and β of the linear quadratic model (LQ-model) and mean inactivation dose (D_{MID}) were also estimated. The DNA index of four cell lines equaled 1.0 and two cell lines equaled 1.5 and 1.6. Different mesothelioma cell lines showed a great variation in radiosensitivity. Mean survival fraction after a radiation dose of 2 Gy (SF2) was 0.60 and ranged from 0.36 to 0.81 and mean α value was 0.26 (range 0.48 – 0.083). The SF2 of the most sensitive diploid mesothelioma cell line was 0.36: less than that of the foetal fibroblast cell line (0.49). The survival fractions (0.81 and 0.74) of the two most resistant cell lines, which also were aneuploid, were equal to that of the HeLa cell line (0.78). The α/β ratios of the most sensitive cell lines were almost an order of magnitude greater than those of the two most resistant cell lines. Radiation-induced delay of the most resistant aneuploid cell line was similar to that of HeLa cells but in the most sensitive (diploid cells) there was practically no entry into the G1 phase following the 2 Gy radiation dose during 36 h.

Mesothelioma is a rare tumor, closely related with exposure to asbestos. Its incidence is expected to increase in the forthcoming decades as a result of increased exposure to asbestos between the 1940s and 1970s and because of the long latency period of 30–40 years. The results of radiotherapy and chemotherapy have been poor, and the role of therapy is controversial (1). A few patients, however, may benefit from treatment.

Received 18 November 1994.
Accepted 5 December 1995.
From the Department of Oncology (A.M. Häkkinen, S. Pyrhönen), and Pulmonary Medicine, (K. Mattson), University Central Hospital, Helsinki, Department of Pathology, Central Hospital of Etelä-Pohjanmaa, Seinäjoki, (A. Laasonen) and Department of Industrial Hygiene and Toxicology, Institute of Occupational Health, Helsinki, (K. Linnainmaa), Finland.
Correspondence to: Dr. Anna-Maija Häkkinen, Department of Oncology, University Central Hospital, Haartmaninkatu 4, SF-00290 Helsinki, Finland.

Chromosomal karyotype analyses, as well as tumorigenicity and cytology in vitro studies have been performed previously (2–5). Flow cytometry (FCM) analyses have also been used to characterize malignant mesothelioma (4). The prognostic significance of DNA ploidy parameters was evaluated in 34 cases of malignant mesothelioma (6) and in another group in 70 cases of malignant mesothelioma (5). The results obtained in the former report (6) suggest that DNA ploidy may have some prognostic indicator, whereas the results of the other report in the larger group of patients (5) suggest that S-phase fraction (SPF) but not DNA ploidy may be a prognostic indicator. A few reports have connected the clinical radiosensitivity of human tumors with their ploidy: some aneuploid tumors have been found to be more radiosensitive than diploid ones, but other results have suggested the opposite (7–9). Even though mesothelioma is considered to be quite resistant to radiation clinically, there is in vitro evidence that mesothelioma cell lines may be radiosensitive (10).

 ISSN 0284-186X

In the present study a colorimetric tiazolyl blue microtiter (MTT) assay (11) was used to analyze the radiosensitivity of six previously cytogenetically and histologically characterized tumor cell lines which originated from fresh tumor tissue or pleural effusion from patients with asbestos-related malignant mesothelioma (2). A continuously growing cancer (HeLa) cell line (resistant) and a human foetal fibroblast (HFF) cell line (moderately sensitive to radiation) were studied at the same time for comparison. In addition radiation-induced changes in cell proliferation were determined using the bromodeoxyuridine (BrdUrd) method (12). Flow cytometry was used to determine the DNA ploidy of the cell lines.

Material and Methods

Cell lines. All the malignant mesothelioma cell lines were derived from patients diagnosed and treated at the Helsinki University Central Hospital. Histological diagnoses and subtyping were performed by the mesothelioma panels of the Finnish National Pathology Panel and the Lung Cancer Cooperative Group of the European Organization for Research and Treament of Cancer. The histology of original tumors as well as of the cell lines is described in more detail elsewhere (2).

We used cell lines M14K, M33K, M28K, M38K and M25K established from five fresh tumor specimens, and cell line M14 established from a pleural effusion (2). The specimens of tumor tissue were obtained before radio- or chemotherapy. For comparison, experiments were also performed on the previously documented radioresistent HeLa cell line (13) as well as on HFF cell line (moderately sensitive) (14). The cells were grown in 96-well plates (MTT-assay) or in 75 cm^2 cell culture flasks (Nunc A/S, DM). All the cell lines were cultured as monolayers in Eagle's medium (Gibco, Paisley, UK) as modified by Dulbecco supplemented with 10% FCS, 100 U/ml penicillin, 100 μg/ml streptomycin and 0.03% L-glutamine (all from Gibco), and were kept in a humidified atmosphere of 95% air and 5% CO_2 at 37°C. When used, the cells were in an exponential growth phase after subculture in completely fresh medium.

Irradiation. The exponentially-growing cultures of each cell line were irradiated by 6 MV photons from a linear accelerator at a dose rate of 2 GY/min. For each experiment, five to six different doses between 1 and 8 Gy were given. For each cell line, non-irradiated controls were established. Radiation was applied at room temperature, and the control cells were also kept at room temperature. The radiation survival data are presented as the mean ($\pm$SD) of 3–4 repeated experiments, each providing 16–32 parallel samples for the MTT-assay.

DNA index and cell cycle measurements (flow cytometry)

To allow the passage of the cells through the cell cycle to be determined, they were labelled with 10 μmol/l BrdUrd (Sigma Chemicals Co., St. Louis, MO) immediately before irradiation. After a 1-h incubation period, the cells were washed twice in phosphate-buffered saline (PBS) without calcium and magnesium ions. They were then recultured in fresh medium. For staining and analysis, the cells were removed from the flask with trypsin-EDTA (Gibco). Samples were collected every 2 h for 24 h. In order to determine the DNA index by flow cytometry, the cells were analyzed as described previously (13). The cells were prepared for analysis of DNA and BrdUrd content according to Dolbeare et al. (12). DNA analysis was performed using an EPICS C flow cytometer (Coulter Electronics, Hilaleah, FLA) equipped with a 2 W argon ion laser. Excitation of propidium iodine (PI) occurred at 488 nm with a light output at 200 mW. The fluorescent emission of PI was measured above 610 nm. The green fluorescence signal from FITC-conjugated BrdUrd antibody (Gibco) was measured at 515–530 nm. At least 20 000 cells were analyzed from each sample. Tumor cell lines were classified as aneuploid if a second G1 peak was observed in addition to the diploid G1 peak. Trout and chicken red blood cells were used as standards. The DNA index was calculated as the ratio of the aneuploid stemline G1 DNA peak channel to the diploid stemline G1 DNA peak channel.

The changes in cell kinetics were examined in two different ways. First the durations of the G2/M phase and the irradiation-induced G2/M block were measured by monitoring the entry of cells into the G1 phase. The proportion of BrdUrd-labelled cells in the G1 phase was expressed as a percentage of the total population, and plotted against the time since irradiation. Second, the movement of the cells through the cell cycle (T_C) was monitored by calculating the mean BrdUrd content of mid S-phase cells and plotting this against the time since irradiation.

MTT assay. The MTT assay described by Mossman (11) was used. After irradiation, cells were plated in microwell plates at an appropriate density and the assays were run for 7–8 days, about 7–8 doubling times, to allow sufficient time for the cells to express any damage (15). After incubation, the plates were washed, the MTT solution was added and the plates incubated for a further 4 h. The cells were then detached and the medium centrifuged, aspirated by Pasteur pipette and the formazan crystals dissolved in dimethylsulfoxide (DMSO). The absorbance was measured with a spectrophotometer at a wavelength of 540 nm. There was no change in absorbance from 15–70 min after addition of DMSO. Absorbance was therefore measured 40–70 min after the solution had been prepared. The inoculation density was adjusted for the different cell lines to ensure adequate absorbance readings.

Analysis of the survival fraction data. The following linear-quadratic (LQ) model (16, 17) was used to analyze the survival fraction data:

$$S(D) = \exp(-\alpha * D - \beta * D^2)$$

where S is survival fraction, D is absorbed dose, and α and

Table 1

Survival parameters mean (±SD) for mesothelioma cell lines, HeLa and HFF foetal fibroblast cell lines

Cell line	Irradiation iNduced G2/M block		S(2 Gy)	α Gy^{-1}	β Gy^{-2}	α/β Gy	D_{MID} Gy
	2 Gy	6 Gy					
M38K	Nd	Nd	0.81 (±0.08)	0.089 (±0.015)	0.012 (±0.003)	7	5.4 (±0.8)
M28K	2	5	0.74 (±0.09)	0.083 (±0.015)	0.019 (±0.004)	4	4.8 (±0.6)
M33K	Nd	Nd	0.49 (±0.01)	0.46 (±0.15)	−0.04 (±0.04)	-	2.9 (±1.3)
M25K	5	$1.5 \times T_C$	Nd	Nd	Nd	Nd	Nd
M14K	$1.5 \times T_C$	$1.5 \times T_C$	0.62 (±0.09)	0.20 (±0.06)	0.007 (±0.005)	28	4.0 (±1.5)
M14	$1.5 \times T_C$	$1.5 \times T_C$	0.36 (±0.06)	0.48 (±0.04)	0.005 (±0.005)	92	2.0 (±0.3)
HeLa	3	7	0.78 (±0.09)	0.13 (±0.05)	0.007 (±0.005)	19	5.3 (±2.6)
HFF	5	$1.5 \times T_C$	0.49 (±0.04)	0.30 (±0.02)	0.016 (±0.011)	19	2.7 (±0.6)

Nd = no data, M25-cell line could be cultured only 25 passages.

β are constants. The mean inactivation dose D_{MID} is an appropriate parameter to measure radiosensitivity (18). This is measured as the area under the survival fraction curve and is thus inversely related to radioresponsiveness. The values of D_{MID} were calculated by numerical integration of the linear-quadratic equations of the survival data (Table 1). Malaise et al. (14) have selected a robust parameter, the survival fraction after 2 Gy (SF2), as a characteristic criterion of intrinsic radiosensitivity for radiation doses used in normal radiotherapy.

Results

The labelling index (Li) of the mesothelioma cell lines varied from 22% to 60%. The cell cycle (Tc(h)) lasted from 18 h to 23 h and the S-phase from 10.0–12.0 h (Table 2). The karyotypically near diploid cell lines M14K, M14, M25K, M33K show DNA indices equalling 1.0 whereas the karyotypically hypotetraploid cell lines M38K and M28K showed indices 1.5 and 1.6 respectively. The DNA indices obtained thus agree well with the karyotypic ploidy (Table 2).

In the mesothelioma cell lines studied, significant differences in radiosensitivity were found, as expressed by the survival fraction at 2 Gy (SF2) or α (Fig. 1 and Table 2). The mean SF2 was 0.60 (range 0.36–0.81) and corresponding mean α was 0.26 Gy^{-1} (range 0.48–0.083 Gy^{-1}). The aneuploid cell lines M28K and M38K were resistant (SF2 = 0.74 and 0.81 or $\alpha = 0.083$ Gy^{-1} and 0.089 Gy^{-1} correspondingly), like the HeLa cells (SF2 = 0.78 or $\alpha = 0.13$ Gy^{-1}). There was a considerable amount of variation in the shapes of the survival curves. The α/β ratio was about 4 for M28K and 7 for M38K but 19 for the HeLa cell line. The three diploid cell lines were moderately

Table 2

Characterization of cell lines and cell cycle parameters for mesothelioma, HeLa and human foetal fibroblast (HFF) cells

Cell line	Histological subtype	No. of chr	DNA index	Li%	$T_{C(h)}$	$T_{G1(h)}$	$T_{S(h)}$	$T_{G2/M(h)}$
M38K	Mixed/IIB	65–80	1.6	Nd	Nd	Nd	Nd	Nd
M28K	Epithelial/IIA	70–90	1.5	22*	23*	5*	12*	6*
M33K	Mixed/IV	46–49	1.0	Nd	Nd	Nd	Nd	Nd
M25K	Epithelial/IIIB	43–47	1.0	60	18	2	11	5
M14K	Epithelial/IIB	41–45	1.0	41	19	4	10	5
M14	Epithelial/IIB	44–46	1.0	28	21	6	10	5
HeLa	Epithelial	Nd	2.0	37	22	5	12	6
HFF	Fibroblastic	Nd	1.0	35	23	7	10	6

Li = labelling index, Nd = no data, * diploid and aneuploid cells are partly overlapped.

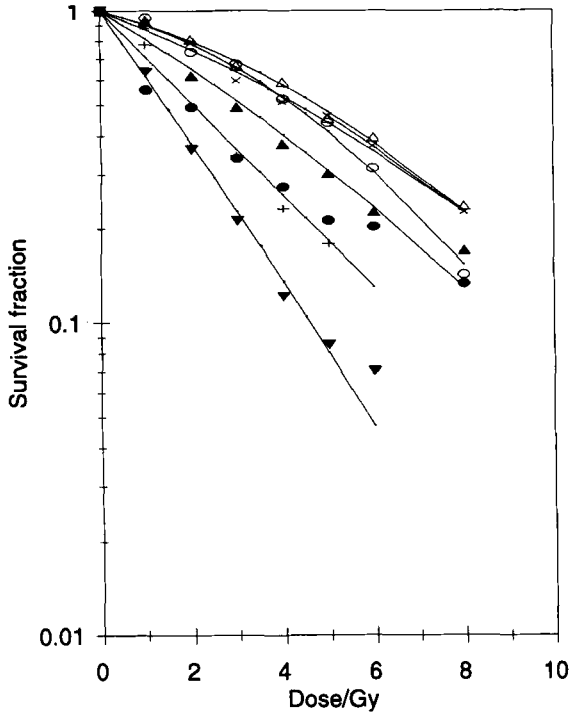

Fig. 1. Cell survival fractions for M38K △, M28K ○, M33K ●, M14K ▲, M14 ▼, HeLa ×, and HFF fibroblast +.

radiosensitive: M14 (SF2 = 0.36 and $\alpha = 0.48$ Gy^{-1}) was even more sensitive than the HFF cells (SF2 = 0.49 and $\alpha = 0.30$ Gy^{-1}). The sensitivity of M14K layed between that of HeLa cells and HFF cells. The diploid M14K and M14 cell lines showed no clear shoulder. The β values were almost an order of magnitude smaller than those for the aneuploid cell lines, and the α/β ratio was greater for the diploid than for the aneuploid cell lines. The range of survival fraction values for M33K partly overlapped the values for HFF, but the shape of the M33K survival curve was clearly different. For this cell line, the survival curve was biphasic. D_{MID} (Table 1) was dominated by the effect of the parameter α and followed the same trend as for SF2. In separate experiments, the robust parameter SF2 varied less than the other parameters.

Radiation-induced changes in cell kinetics, measured by the BrdUrd method, are presented in Figs 2 and 3 and in Table 1. After a 2-Gy dose, the progression of the labelled S-phase cells through the G1 phase was delayed (G2-block) in all the cell lines compared with the activity pattern of the non-irradiated cells. In the most sensitive DNA diploid cell lines, M14 and M25, practically no cells entered the G1 phase after 2 Gy, during 36-h follow-up. Inhibition of the progression of the labelled S-phase cells was even more complete after 6 Gy. After 2 Gy irradiation of M25 and HFF cells there was only slight movement in the S-phase and after 6 Gy practically no progression into the G1 phase during the 36-h follow-up. In the aneuploid cell line M28, there was a delay after 2 Gy irradiation and an even greater one after the 6 Gy dose.

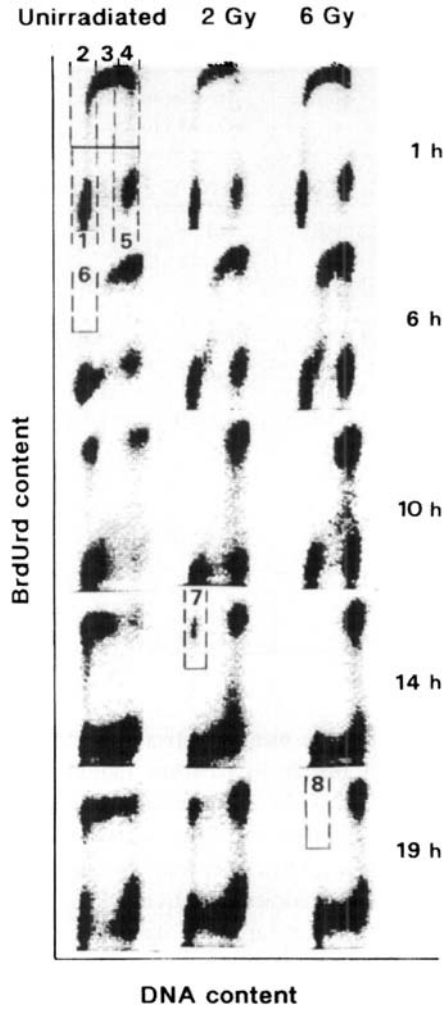

Fig. 2. The progression of M14 cells in the cell cycle before (column unirradiated) and after irradiation (columns 2 Gy and 6 Gy). x-axis is DNA content in a linear scale; y-axis bromodeoxyuridine (BrdUrd) content in a logarithmic scale. The analysis was performed 1 h, 6 h, 10 h, 14 h and 19 h after a 1 h BrdUrd pulse. BrdUrd-labelled cells are seen above the solid line (column irradiated at 1 h). Dotted lines separate the G1, S and G2/M phases on the bases of their DNA content. Box 1 = unlabelled cells (BrdUrd-negative) G1 phase cells: Box 2 = labelled early S-phase cells; Box 3 = labelled mid-S phase cells; Box 4 = labelled late S-phase cells; Box 5 = unlabelled G2/M phase cells. Unirradiated cells that were initially labelled in S-phase have progressed into G1 phase within 6 h (Box 6), whereas irradiated cells arrive in G1 within 14 h (2 Gy, Box 7) and 19 h (6 Gy, Box 8).

Discussion

The newly established (passages 4–25) malignant mesothelioma cell lines (epithelial or mixed cell type) from different patients showed great differences in in vitro (intrinsic) radiosensitivity, as expressed by the survival frac-

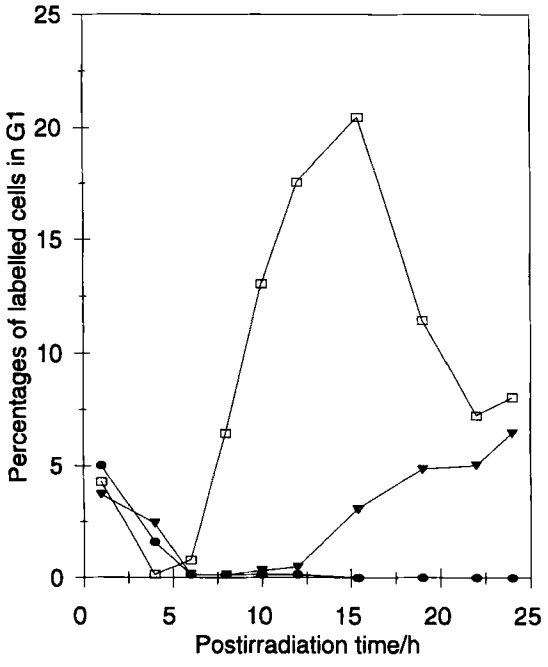

Fig. 3. Irradiation-induced G2/M block monitored by the entry of labelled S phase cells into the G1 phase for M14 cell line. The measurement points present mean of two experiments. □ Unirradiated, ▼ 2 Gy, ● 6 Gy.

tion at 2 Gy (SF2) or by the parameters α and β of the LQ model. The mean survival fraction of the mesothelioma cells varied from 0.36 to 0.81 compared to e.g. radioresistant HeLa cells (0.75) and moderately sensitive HFF fibroblast (0.49). Wide variations in in vitro radiosensitivity have also been reported for established cell lines of head and neck cancers, sarcomas and melanomas (19–23).

The clonogenic survival assay has been a widely used method for determining the radiosensitivity or drug sensitivity of cultured tumor cells. It is, however, laborious and time-consuming. During the last decade, more automated assays have been developed. In the present study we used a colorimetric tiazolyl blue microtiter (MTT) assay (11). Different assays may show differences in survival values after irradiation (24, 25), but the survival figures we found for HeLa and HFF cells agree with those previously reported (14, 26, 27) although survival fractions are somewhat greater.

The robust parameter, the survival fraction after 2 Gy (SF2) allows a rough estimate of the differences in radioresponsiveness of these mesothelioma cell lines to be made. SF2 seems to discriminate between moderately sensitive and resistant tumor types as Deacon and others have observed (28–30).

The parameters α and β (and D_{MID}) reflect characteristics of the survival curves and show increased radiosensitivity in the DNA diploid M14, M14K and M33 cell lines and a poorly defined shoulder (small β value, and a large α/β ratio) in the survival curve.

The survival curves of two mesothelioma cell lines described by Carmichael et al. (10) also showed moderate radiosensitivity and almost no shoulder in the curves. Survival curves steeper than normal initial slope (greater α) has been previously reported for fibroblasts derived from patients with unusual clinical responses to radiation (27). The biphasic-shape of the survival curve for the M33 cell line and the negative β value (which is biologically impossible), may be explained by differing sensitivities of the cells in heterogenous populations. The best fit of the survival data requires a curve more complex than that described by the linear quadratic equation. However, the distal part of the survival curve (partly described by parameter β) is thought to be particularly dependent on the experimental conditions (29). It may be mentioned that the growth requirements of M33 cell line are also different.

The BrdUrd results in the aneuploid cell line M28 show radiation-induced growth delays similar to those reported previously for the HeLa cell line (3.2 h after 2 Gy and 7.2 h after 6 Gy) (13). Delays of 4–6 h after a dose of 2 Gy were previously observed for breast and lung carcinoma (31). Remarkable radiation-induced growth delays have also been reported previously for neuroblastoma and squamous cell cacinoma of the tongue in clinical studies (32). The results in the M14, M14K and M33 cell lines (with DNA indices 1.0) suggest significantly longer radiation-induced delays ($\sim 1.5 \times T_C$) or reduced cell proliferation. Longer G1 delay, increased G2 blocks may give greater chance for DNA repair, as observed with the use of radiation protectors. On the other hand decrease of blocks caused by caffeine is observed to increase radiosensitivity in HeLa and Chinese hamster cells (33). Some sensitive tumors have previously been reported to have good repair capacity (21). The results from the BrdUrd method, however, suggest that the underlying molecular bases of radiosensitivity in the moderately sensitive (DNA diploid) mesothelioma cells and the resistant (aneuploid) cells are different.

The results of this study suggest that mesothelioma tumor cells from different patients may exhibit remarkable differences in radiosensitivity. In addition to the cell kinetic and other biological parameters, differences in in vitro radiosensitivity between the mesothelioma cell lines may be relevant when the results of therapy are interpreted and treatment strategies designed. Even if data are very scarce, the results suggest an increase of the radioresistance by the increase of aneuploidy. The rapid MTT assay seems to be suitable for radiosensitivity testing in established cell lines. Further studies defining a possible role of DNA index and cell kinetic parameters in estimating the outcome of radiotherapy are warranted.

ACKNOWLEDGEMENTS

The authors thank Ms. Päivi Laurila, Ms. Tuula Sariola and Ms. Madeleine Mattson for skilled technical assistance. The work was supported by the Finnish Cancer Foundation.

REFERENCES

1. Albers AS, Falkson G, Goedhals L, Vorobiof DA, Van Der Merwe CA. Malignant pleural mesothelioma: a disease unaffected by current therapeutical manouvers. J Clin Oncol 1988; 6: 527–35.
2. Pelin-Enlund K, Husgafvel-Pursiainen K, Tammilehto L, et al. Asbestos-related malignant mesothelioma: growth, cytology tumorigenicity and consistent chromosome findings in cell lines from five patients. Carcinogenesis 1990; 11: 673–81.
3. Tiainen M, Tammilehto L, Rautonen J, Tuomi T, Mattson K, Knuutila S. Chromosomal abnormalities and their correlations with asbestosis exposure and survival in patients with mesothelioma. Br J Cancer 1989; 60: 618–26.
4. Pyrhönen S, Tiainen M, Rautonen J, et al. Comparison of DNA and karyotype ploidy in malignant mesothelioma. Cancer Genet Cytogenet 1992; 60: 8–131.
5. Pyrhönen S, Laasonen A, Tammilehto L, et al. Diploid predominance and prognostic significance of S-phase cells in malignant mesothelioma. Eur J Cancer 1991; 27: 197–200.
6. Dejmek A, Strömberg C, Wikström B, Hjerpe A. Prognostic importance of the DNA ploidy pattern in malignant mesothelioma of the pleura. Analyt Quant Cytol 1992; 14: 217–21.
7. Dyson JED, Joslin CAF, Quirke P, Rothwell RI, Bird CC. Quantitation by flow cytofluorometry of response of tumours of the uterine cervix to radiotherapy. Br J Radiol 1985; 58: 41–50.
8. Kelland LR, Bingle L, Edwards S, Steel GG. High intrinsic radiosensitivity of a newly established and characterised human embryonal rhabdomyosarcoma cell line. Br J Cancer 1989; 59: 160–4.
9. Girinsky T, Lubin R, Pignon JP, et al. Predictive value of in vitro radiosensitivity parameters in head and neck cancers and cervical carcinomas. Preliminary correlations with local control and overall survival. Int J Radiat Oncol Biol Phys 1992; 25: 3–7.
10. Carmichael J, DeGraff WG, Gamson J, et al. Radiation sensitivity of human lung cancer cell lines. Eur J Cancer Clin Oncol 1989; 25: 527–34.
11. Mossman T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxity assays. J Immunol Methods 1983; 65: 55–63.
12. Dolbeare F, Grazner HG, Pallvincini MG, Gray JW. Flow cytometric measurements of total DNA content and incorporate bromodeoxyuridine. Proc Natl Acad Sci USA 1983; 80: 5573–7.
13. Laasonen A, Pyrhönen S, Kouri M, Räty J, Holsti LR. The effect of fractionated irradiation on cell kinetics. Acta Oncol 1991; 30: 51–6.
14. Malaise EP, Fertil B, Deschavanne PJ, Chavaudra N, Brocks WA. Initial slope of radiation survival curves is characteristic of the origin of primary and established cultures of human tumor cells and fibroblasts. Radiat Res 1987; 111: 319–33.
15. Carmichael J, DeGraff WG, Gazdar AF, Minna JD, Mitchell JB. Evaluation of a tetrazolium-based semiautomated colorimetric assay: assessment of radiosensitivity. Cancer Res 1987; 7: 943–6.
16. Chadwick KH, Leenhouts HP. The repair of potentially lethal damage: An alternative approach. Radiat Environ Biophys 1975; 11: 319–25.
17. Deschavanne PJ, Fertil B, Malaise EP, Lachet B. Radiosensitivity and repair of radiation damage in human HF19 fibroblast. Int J Radiat Biol 1980; 38: 167–89.
18. Fertil B, Dertinger H, Courdi A, Malaise EP. Mean inactivation dose: A useful concept for intercomparison of human cell survival curves. Radiat Res 1984; 99: 73–84.
19. Brock WE. Cellular radiosensitivity of primary head and neck squamous cell carcinomas and local control. Int J Radiat Oncol Biol Phys 1990; 18: 1283–6.
20. Kelland LR, Bingle L. Radiosensitivity and characterization of a newly established cell line from an epithelial sarcoma. Br J Cancer 1988; 58: 322–5.
21. Peters LJ. Inherent radiosensitivity of tumor and normal tissue cells as a predictor of human tumor response. Radiother Oncol 1990; 17: 177–90.
22. Rofstad EK, Wahl A, Brustad T. Radiation sensitivity in vitro of cells isolated from human tumor response. Cancer Res 1987; 47: 106–10.
23. Grenman R, Carey TE, McClatchley KD. In vitro radiation resistance among cell lines established from patients with squamous cell carcinoma of the head and neck. Cancer 1991; 67: 2741–7.
24. Begg AC, Mooren E. Rapid fluorescence-based assay for radiosensitivity and chemosensitivity testing in mammalian cells in vitro. Cancer Res 1989; 49: 565–9.
25. Schwachöfer JHM, Crooijmans RPMA, van Gasteren JJM, et al. Radiosensitivity of different human tumor cell lines grown as multicellular spheroids determined from growth curves and survival data. Int J Radiat Oncol Biol Phys 1989; 17: 1015–20.
26. Hall EJ, Marchese T, Hei TK, Zaider M. Radiation response characteristics of human cells in vitro. Radiat Res 1988; 114: 415–24.
27. Loeffler JS, Harris JR, Dahlberg WK, Little JB. In vitro radiosensitivity of human diploid fibroblast derived from women with unusually sensitive clinical responses to definitive radiation therapy for breast cancer. Radiat Res 1990; 121: 227–31.
28. Deacon J, Peckham MJ, Steel GG. The radioresponsiveness of human tumours and the initial slope of the cell survival curve. Radiother Oncol 1984; 2: 317–23.
29. Deschavanne PJ, Malaise EP. The relevance of α/β ratios determined in vitro for human cell lines to the understanding of in vivo values. Int J Radiat Biol 1989; 56: 539–42.
30. West CML, Davidson SE, Hunter RD. Evaluation of surviving fraction at 2 Gy as a potential prognostic factor for the radiotherapy of carcinoma of the cervix. Int J Radiat Biol 1989; 56: 761–5.
31. Matthews JHL, Meeker BE, Capman JD. Response of human tumor cell lines in vitro to fractionated irradiation. Int J Radiat Biol 1989; 16: 133–8.
32. Deacon JM, Wilson PA, Peckham MJ. The radiobiology of human neuroblastoma. Radiother Oncol 1985; 3: 201–9,
33. Labanowska J, Beetham KL, Tolmach LJ. The action of caffeine on x-irradiated HeLa cells. Radiat Res 1990; 123: 228–31.