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Radiation Sensitivity of Human Lung Cancer Cell Lines*

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Abstract—*X-Ray survival curves were determined using a panel of 17 human lung cancer cell lines, with emphasis on non-small cell lung cancer (NSCLC). In contrast to classic small cell lung cancer (SCLC) cell lines, NSCLC cell lines were generally less sensitive to radiation as evidenced by higher radiation survival curve extrapolation numbers, surviving fraction values following a 2 Gy dose (SF2) and the mean inactivation dose values ($\bar{D}$) values. The spectrum of in vitro radiation responses observed was similar to that expected in clinical practice, although mesothelioma was unexpectedly sensitive in vitro. Differences in radiosensitivity were best distinguished by comparison of SF2 values. Some NSCLC lines were relatively sensitive, and in view of this demonstrable variability in radiation sensitivity, the SF2 value may be useful for in vitro predictive assay testing of clinical specimens.*

INTRODUCTION

HISTOLOGICALLY, lung cancer consists of a range of sub-types, which can be simply divided into small cell (SCLC) and non-small cell (NSCLC) cancers. The latter group accounts for 75% of all lung cancers, comprising of a variety of histological sub-types including adenocarcinoma, squamous carcinoma and large cell anaplastic carcinoma. Different cell types exhibit variability in clinical response to cytotoxic drugs and radiation, with SCLC the most sensitive [1].

Radiation therapy is an integral part of the management of a large number of lung cancer patients for both attempted cure and palliative purposes [2, 3]. Although SCLC is normally more sensitive to radiation treatment, some of these tumours, particularly on relapse, are relatively unresponsive. Cell lines derived from these tumours often exhibit the so-called variant phenotype, which is associated with loss of neuroendocrine differentiation, more rapid growth and increased expression of certain cellular oncogenes [4, 5].

The establishment of a panel of human lung cancer cell lines derived from the major histological sub-types would appear to be an ideal model for the study of parameters affecting the radiation response. The aims of this study were to perform a more extensive analysis of the radiosensitivity patterns of human lung cancer cell lines with emphasis on NSCLC cells. In addition, an assessment was made of the value of various parameters of the radiation response in distinguishing between histological sub-types of human lung cancer, and to predict radiosensitivity.

MATERIALS AND METHODS

Cell lines

Seventeen lung cancer cell lines were used for this study. There were 14 NSCLC cell lines, comprising four adenocarcinoma, three adenosquamous, two squamous, two mesothelioma and three large cell anaplastic cell lines. In addition, three SCLC cell lines were tested, including two lines exhibiting variant characteristics. All cell lines, apart from A549 and JMN, were isolated at the NCI-Navy Medical Oncology Branch and have previously been described [4, 5].

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Cell lines were grown in RPMI 1640 medium supplemented with 10% (v/v) foetal calf serum, with added penicillin and streptomycin. All cell lines grew as monolayers in culture, apart from two of the SCLC lines which grew as floating aggregates: NCI-H69 and NCI-H526. Stock cultures were maintained in exponential growth in a humidified atmosphere using 7% CO_2/93% air. Cell lines in general exhibited similar histology *in vitro* to that seen in the original tumour from which the cell line was isolated, except in three instances. NCI-H322 and NCI-H358 were both derived from patients diagnosed as having bronchiolo-alveolar carcinoma. However, following establishment of the cell lines, NCI-H322 has the *in vitro* appearance of an adenosquamous carcinoma with a similar appearance in nude mouse xenografts, and likewise NCI-H358 has the appearance of an adenocarcinoma. The third cell line NCI-H226 was derived from a patient diagnosed as mesothelioma. However, in xenografts and on cytology, the cell line now has the appearance of a squamous carcinoma. For the purposes of this study all cell lines were classified according to their *in vitro* appearance.

Irradiation

Cells were trypsinized where necessary, counted using an Elzone particle counter and plated in replicate tubes at a concentration of 10^6 cells/ml. They were irradiated using fractions of 1–12 Gy using a 6-MeV photon beam from a linear accelerator at a dose rate of 2 Gy/min.

Clonogenic assays

Single cell suspensions were obtained from all cell lines, and cells were irradiated in suspension culture. The two floating cell lines were then cultured in medium containing 0.3% agarose, plated over an 0.6% agarose underlayer in 60 or 100 mm Petri dishes according to cell inoculum. The remaining cells were plated on plastic, although two cell lines (NCI-H322 and NCI-H520) required heavily irradiated feeder layers [6]. Plating efficiencies varied from 1 to 60%, with the lower plating efficiencies observed in classic SCLC lines. As the growth rate of the cell lines varied considerably, the cells were incubated for 2–4 weeks, allowing for a minimum of six cell doublings post irradiation. Cultures in agarose were examined and colonies counted using an inverted phase microscope. Adherent cultures were fixed in a solution of acetic acid and methanol 1:3 (v/v), stained with 0.1% (w/v) crystal violet and counted under × 8 magnification.

Survival curve analysis

Survival curves for each cell line were generated from a minimum of two experiments, with three replicates for each dose point. Survival curve data were fitted to the linear quadratic and single-hit, multitarget models using the program of Albright [7]. The mean inactivation dose was determined according to Fertil *et al.* [8].

RESULTS

Radiation survival curves for all 17 cell lines are shown in Figs. 1–6. As can be seen in Fig. 1, NCI-H69, a classic SCLC line, is sensitive to radiation with relative absence of a shoulder to the radiation survival curve. In contrast, the two variant SCLC lines NCI-H526, derived from a previously untreated patient, and NCI-H841, derived from a heavily pretreated patient, both have significant shoulders to their radiation survival curves. Figure 2 shows four adenocarcinoma cell lines exhibiting a range of sensitivity to radiation, from the sensitive NCI-H23 line to the highly resistant A549 cell line. Figure 3 illustrates the three large cell carcinoma lines all of which have large shoulders to their radiation survival curves, although one of the lines, NCI-H460 has a very low D_0. Variability in sensitivity to radiation is again observed, with one cell line NCI-H157 having less shoulder than other LCC cell lines. All adenosquamous and squamous carcinoma cell lines have a significant shoulder to their radiation survival curves as shown in Figs. 4 and 5. Radiation survival curves for the two mesothelioma cell lines are illustrated in Fig. 6, with both of these lines relatively sensitive to radiation.

These data are summarized in Table 1, showing the alpha and beta values, mean inactivation dose, extrapolation numbers, D_0 and SF2 values for all 17 cell lines, with data on five additional SCLC lines (NCI-H82, NCI-H187, NCI-H209, NCI-H249, NCI-H345) taken from previous studies reported by this laboratory [9, 10]. The five classic SCLC lines have low extrapolation numbers and low SF2 values. D_0 values were low in SCLC lines (0.8–1.17) but this parameter was of little value in distinguishing between histological sub-types.

The SF_2 value appeared to correlate well with predicted clinical response. Figure 7 illustrates this point with the SF2 value plotted against histological sub-type. This figure incorporates data from this and two previous studies [9, 10]. Classic SCLC lines are the most sensitive, with mesothelioma more sensitive than other NSCLC lines. Variability was observed in the adenocarcinoma cell lines, but other NSCLC lines were less responsive as were variant SCLC lines. The mean inactivation dose ($\bar{D}$) and the extrapolation number varied between subgroups and would appear to be of some value in predicting radiation sensitivity, but in contrast D_0 values were very variable. The shape of certain radiation survival curves appeared to better fit the linear quadratic model. Alpha values were higher in untreated SCLC lines, with low values in many

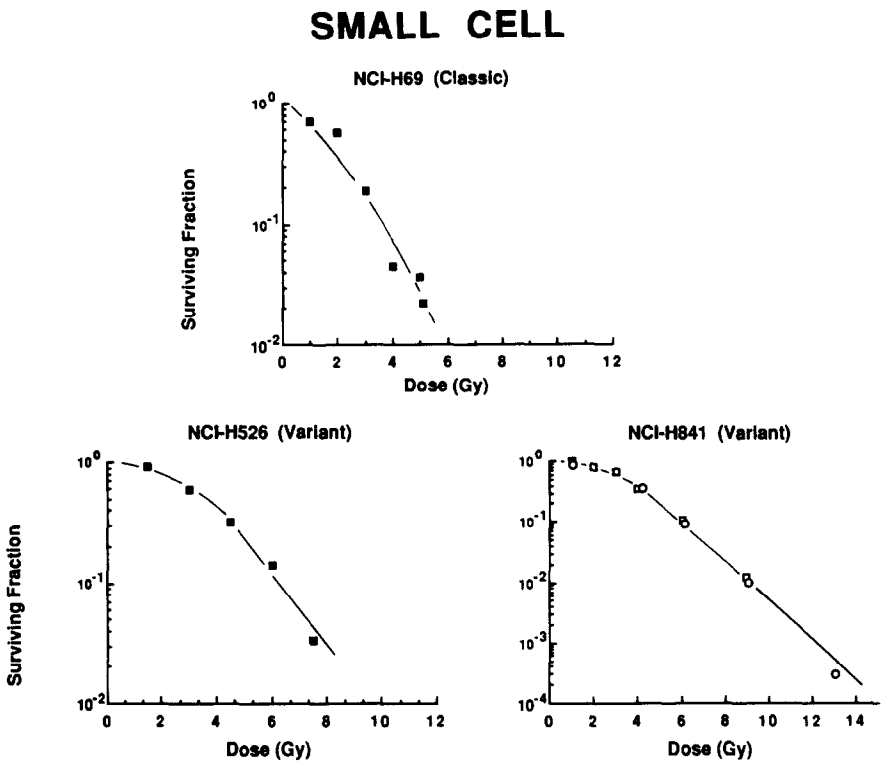

Fig. 1. *Radiation survival of three human small cell lung cancer cell lines using a clonogenic assay. NCI-H69, a classic SCLC line and two variant lines: NCI-H526, derived from a previously untreated patient and NCI-H841 derived from a patient heavily pretreated with chemotherapy. Each point represents the mean of three replicates, with different symbols indicating different experiments.*

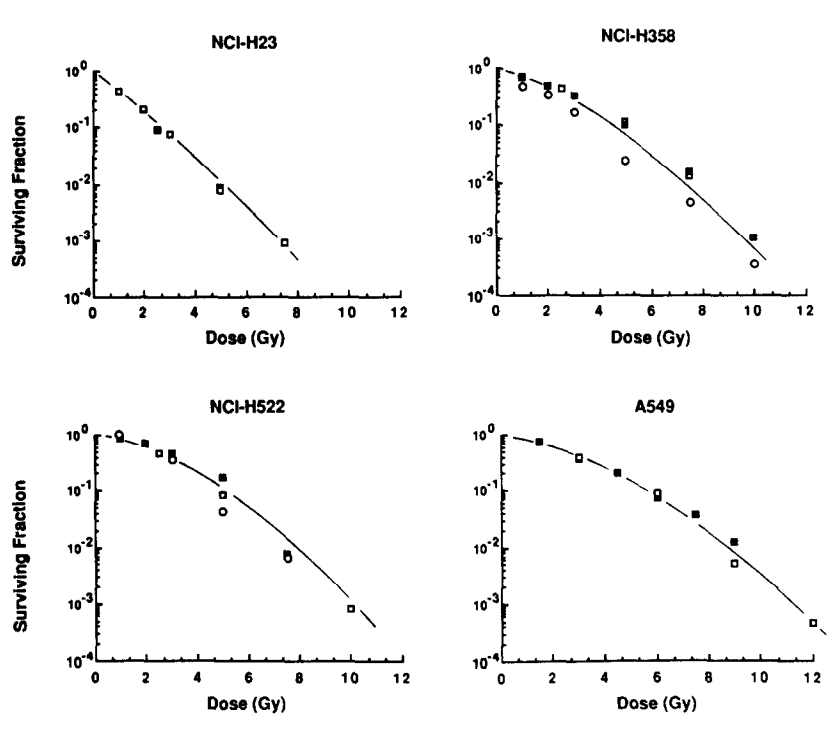

Fig. 2. *Radiation survival of four human lung adenocarcinoma cell lines derived using a clonogenic assay. Different symbols indicate separate experiments.*

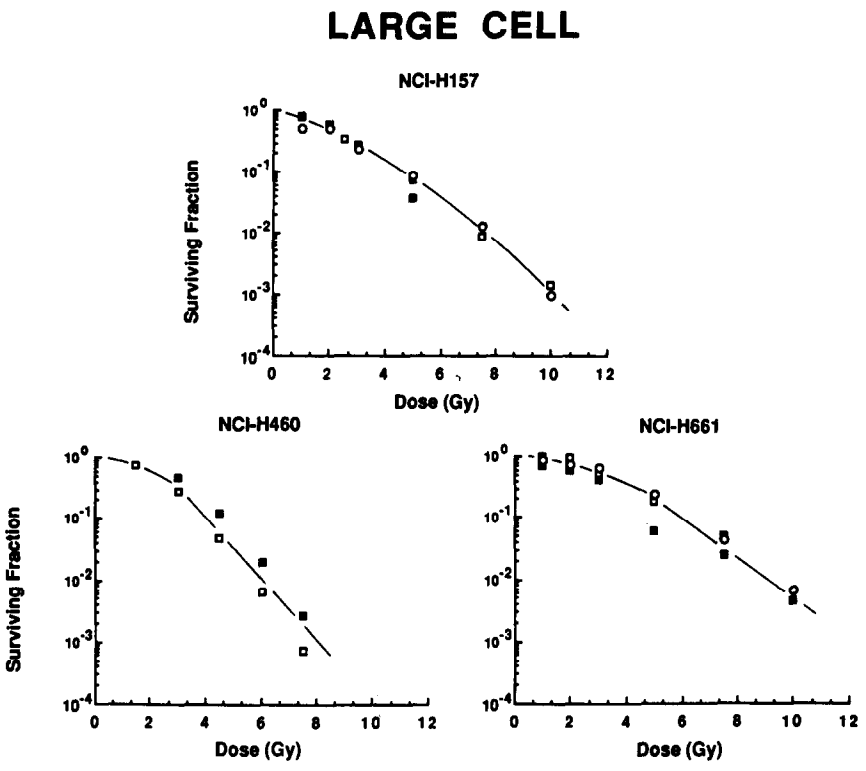

Fig. 3. Radiation survival in three human large cell anaplastic carcinoma cell lines derived by clonogenic assay.

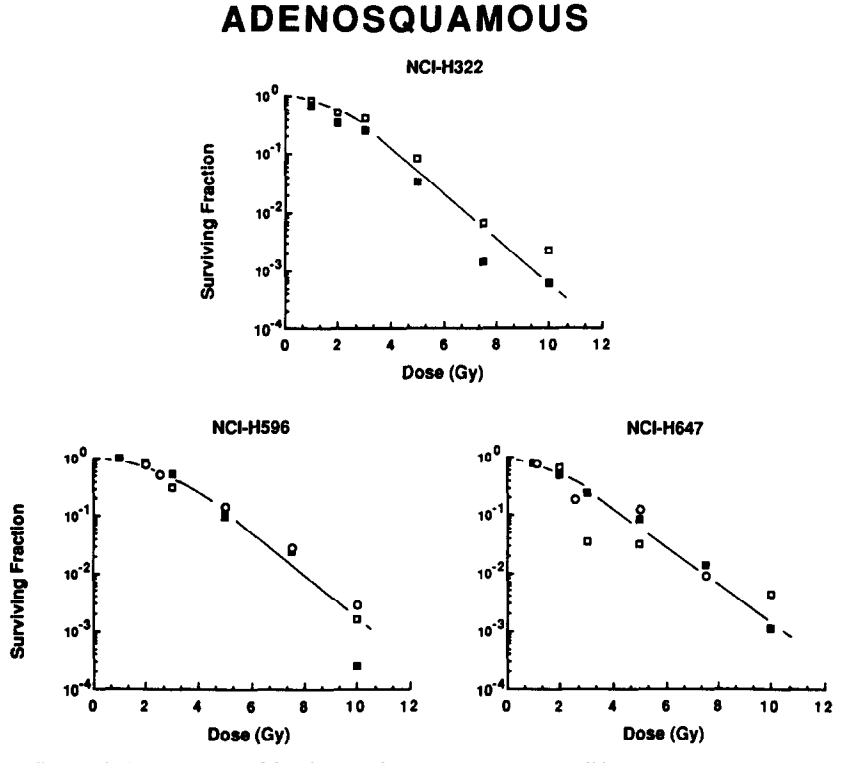

Fig. 4. Radiation survival of three human adenosquamous carcinoma cell lines using a clonogenic assay.

NSCLC and previously treated SCLC cell lines suggesting good correlation well with clinical response to radiation.

DISCUSSION

The present study extends our radiosensitivity assessment to include 29 human lung cancer cell lines. With this panel of cell lines patterns of radiosensitivity have clearly emerged. Classic SCLC lines were found to be the most sensitive of all the groups tested, as reported in previous studies [9–11]. There was great variation in the sensitivity of NSCLC lines, although, surprisingly, both mesothelioma cell lines were remarkably sensitive to

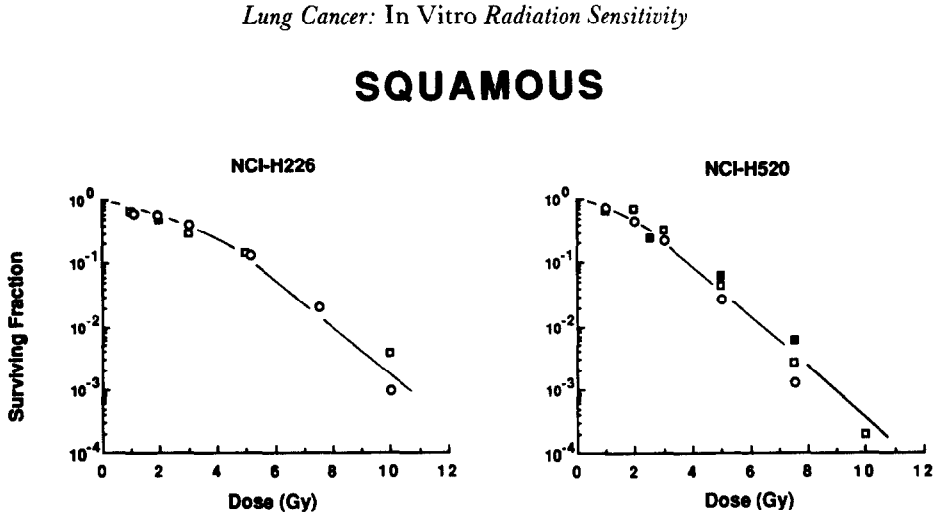

Fig. 5. Radiation survival, as derived by clonogenic assay, in two human squamous carcinoma of lung cell lines.

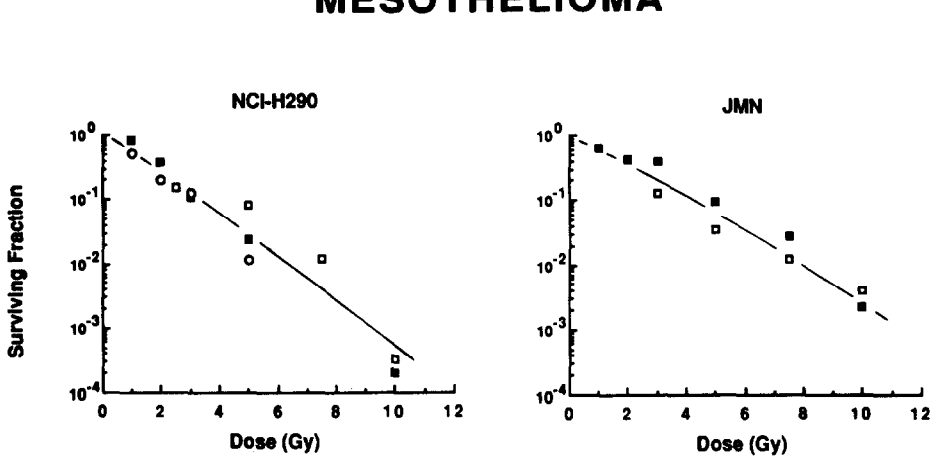

Fig. 6. Radiation survival of two human mesothelioma cell lines using a clonogenic assay.

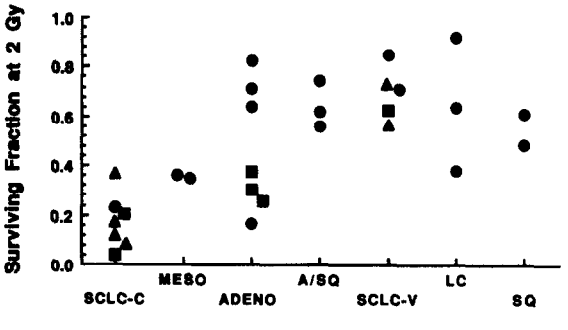

Fig. 7. Surviving fraction at 2 Gy *expressed according to histological sub-type of* 29 *human lung cancer cell lines. SCLC-C, classic small cell; MESO, mesothelioma; ADENO, adenocarcinoma; A/SQ, adenosquamous; SCLC-V, variant small cell; LC, large cell anaplastic and SQ, squamous carcinoma. These data include results derived from two additional studies: Morstyn* et al. [10] *and Carney* et al. [9].

radiation. Both squamous and all three adenosquamous cell lines were relatively resistant to radiotherapy, as were variant SCLC lines. In contrast, there was wide intra-group variation between the adenocarcinoma and large cell carcinoma cell lines, with a small group relatively sensitive to radiation, as shown in Fig. 7.

These data correlate well with clinical response to radiotherapy, where SCLC is generally more sensitive, although a small sub-group of patients with this histological sub-type do badly. On the other hand, the NSCLC lines were, as expected, more resistant, although a small number were relatively sensitive *in vitro*. Surprisingly, both mesothelioma lines were sensitive, in contrast to the clinical situation where this tumour type is relatively unresponsive to radiation [12]. This finding is very interesting, although its aetiology remains uncertain. Many clinical factors could be involved, including decreased tissue oxygenation and impaired blood flow to the tumour. Likewise, growth factors secreted by neighbouring fibroblasts could modify the tumour response and this finding is certainly worthy of further study.

A number of these cell lines have increased expression of one or more oncogenes [5, 13, 14]. Some cell lines exhibiting shoulders to their radi-

Table 1. Radiation survival curve parameters for human lung cancer cell lines

Cell line	Type	Extrapolation number (n)	D_0	Mean inactivation dose (D)	Surv. fract. @2 Gy	α (Gy^{-1}) × 10	β (Gy^{-2}) × 100
NCI-H 23	Adeno	1.2 (0.3)*	1.02 (0.03)	1.10	0.17	8.9 (0.7)	1.0 (0.9)
NCI-H358		4.9 (1.6)	1.20 (0.06)	2.29	0.64	2.7 (0.6)	4.6 (0.7)
NCI-H522		6.8 (2.5)	1.10 (0.06)	2.16	0.57	3.4 (1.6)	3.4 (1.7)
A549		6.3 (3.5)	1.40 (0.12)	2.91	0.82	1.8 (0.5)	3.7 (0.5)
NCI-H322	Adenosq.	5.5 (3.4)	1.10 (0.10)	1.93	0.62	3.7 (1.6)	4.6 (1.9)
NCI-H596		5.2 (1.3)	1.35 (0.07)	2.72	0.74	1.8 (0.7)	4.6 (0.9)
NCI-H647		2.7 (0.8)	1.50 (0.11)	2.39	0.56	3.0 (0.9)	3.0 (1.1)
NCI-H226	Squamous	2.8 (1.2)	1.60 (0.15)	2.57	0.61	2.6 (0.7)	3.1 (0.8)
NCI-H520		4.6 (2.0)	1.00 (0.08)	1.76	0.49	4.3 (1.3)	4.7 (1.7)
NCI-H157	LCC	2.0 (0.8)	1.30 (0.10)	1.77	0.38	4.9 (0.8)	2.3 (0.9)
NCI-H460		7.0 —	1.00 (0.04)	2.08	0.64	1.8 (0.1)	10.2 (1.8)
NCI-H661		9.5 (2.3)	1.40 (0.05)	3.38	0.93	0.8 (0.3)	4.5 (0.4)
NCI-H290	Mesoth.	1.8 (1.0)	1.30 (0.20)	1.60	0.35	5.9 (1.6)	1.2 (2.2)
JMN		1.0 (0.6)	1.86 (0.20)	1.93	0.34	5.1 (1.5)	0.2 (0.1)
NCI-H 69	C-SCLC	1.2 (0.4)	1.24 (0.08)	1.31	0.23	7.5 (1.0)	0.5 (1.7)
NCI-H187†		1.0 (0.5)	1.15 (0.30)	1.15	0.18	8.7 (2.4)	0.0 —
NCI-H209†		1.5 (0.6)	1.50 (0.30)	1.93	0.37	3.4 (0.9)	5.8 (2.3)
NCI-H249†		2.0 (0.6)	0.76 (0.06)	1.05	0.14	8.0 (0.7)	8.5 (1.8)
NCI-H345‡		2.0 —	0.91 —	1.36	0.21	—	—
NCI-H 82†	V-SCLC	17.7 (10)	0.76 (0.50)	1.94	0.73	2.4 (1.6)	9.7 (2.2)
NCI-H526		4.6 (3.0)	1.40 (0.30)	2.11	0.72	3.4 (2.8)	13.8 (3.9)
NCI-H841		6.3 (1.3)	1.50 (0.25)	3.02	0.85	1.6 (0.2)	3.8 (0.2)

*Standard deviation.
†Carney *et al.* [9].
‡Morstyn *et al.* [10].

ation survival curves have previously been shown to be amplified for the c-myc oncogene [5], with this considered as a potential factor in their radiation resistance. Increased expression of c-myc, n-myc, l-myc and p53 message in many of these cell lines has recently been demonstrated (Vinocour, personal communication). Some cell lines that exhibit shoulders on their radiation survival curves showed high levels of expression of c-myc message, while others with similar shoulders had no evidence of increased expression for this oncogene. Likewise, NCI-H526 was shown to express high levels of n-myc message, but NCI-H249 which has no shoulder, expresses higher levels. Expression of p53 was also variable, with no direct relationship with the shoulder on the radiation survival curve. Increases in expression of these oncogenes may well be responsible for changes in growth rate and clonogenicity of the lines, but would not appear to have any direct causal relationship to the differences observed in radiosensitivity, either between classic and variant SCLC lines, or between SCLC and NSCLC lung cancer cell lines. The role of oncogene expression in radiation sensitivity could best be approached by transferring specific oncogenes into the radiosensitive classic SCLC cell lines that do not have or do not express these genes. Assessment of the radiosensitivity of cells with different levels of gene expression may shed greater insight on this question, and is presently underway in our laboratory. Such an approach was recently taken to assess the role of amplified ras oncogene in the inherent sensitivity of mouse NIH-3T3 cells [15]. While transfection of the ras oncogene did increase the radiation survival curve slope (D_0) as compared to the control cell line, there was little change in the extrapolation number or SF2 [15]. The increased 'resistance' attributed to the ras oncogene reported in the study is probably not relevant to clinical radiotherapy as no correlations to date have been associated with *in vitro* derived D_0 values and clinical responsiveness to radiation [16–18]. These cell lines form part of a panel of lung cancer cell lines that have been analysed for chemosensitivity to a range of cytotoxic drugs [19]. Glutathione levels had been measured in these cell lines and, in addition, activities of a number of detoxification enzymes have been estimated [20]. Classic SCLC lines were shown to maintain their relative chemosensitivity *in vitro* and were found to have lower glutathione and detoxification enzyme levels [20]. Variant SCLC lines were more resistant to chemotherapy *in vitro* [19],

and were shown in this study to maintain radiation resistance *in vitro*. However, no differences were observed comparing variant and classic SCLC lines with respect to glutathione levels or to detoxification enzyme activity [20], thus questioning the role of these compounds in inherent radiation sensitivity.

Differences were observed in the radiation sensitivity of these cell lines, both between the major sub-groups (SCLC vs. NSCLC) and also within each histological sub-type. As predictors of radiation response, it was found that the extrapolation number was a good indicator, as was the SF2. This is illustrated in Fig. 7, showing marked differences in radiation sensitivity between classic SCLC lines and the other histological categories, confirming previous reports [9, 10]. With regard to the relationship between *in vivo* sensitivity and the SF2 value *in vitro*, these results are in agreement with previous analyses [16–18]. However, the relationship between *in vitro* radiosensitivity of particular histological sub-types using established cell lines and *in vivo* sensitivity has been controversial, although some studies suggest that cell lines do retain the radiobiological properties of their cell of origin [17, 18, 21, 22]. Of interest, three of the non-small cell lines which proved to be relatively radiosensitive, NCI-H23, NCI-H290 and JMN, have also been shown to be sensitive to a number of cytotoxic drugs [19]. This suggests that a small sub-group of NSCLC patients may be sensitive to either radiotherapy or cytotoxic drugs, although identification of this sub-group remains impossible at present. One possibility is that these sensitive NSCLC tumours are the ones that express neuroendocrine markers, and this is currently under investigation.

In view of the observed differences in radiation sensitivity, *in vitro* predictive testing may be of clinical value for the detection of patients with sensitive tumors. Although the aetiology of the shoulder to the radiation survival curve in NSCLC and variant SCLC cell lines remains unknown, this panel of human lung cancer cell lines offers an extremely valuable model for the study of factors involved.

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