Journal of Cellular Biochemistry Supplement 24:152–159 (1996)

Radiation Biology of Lung Cancer

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Abstract The enormous problem that is lung cancer still defies satisfactory therapeutic strategy. This article summarizes some of the more important laboratory efforts directed at understanding the biology of this complex disease. The radiation sensitivities of established lung cancer cell lines are outlined. The effect of radiation dose rate and chemotherapy is explored. The emerging biology of oncogenetic alterations is explored as it relates to radiation sensitivity in general, and lung cancer in particular. Finally, novel therapeutic approaches including photodynamic therapy are introduced. © 1996 Wiley-Liss, Inc.*

Key words: lung cancer, radiation sensitivity, oncogenes, dose rate, chemosensitivity

The worldwide incidence of lung cancer continues to rise, such that between the United States and Europe, over 300,000 new cases are expected per year, and some 260,000 of these patients will die of their disease. Despite major advances in the understanding of the biology of lung cancer, no significant improvement in the survival has occurred in the past two decades. Human lung cancers are generally divided into two major classes: small-cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), which includes adenocarcinoma, epidermoid carcinoma, and large cell carcinoma [1,2]. Distinctions between these two classes have been based on clinical, histologic, biochemical, and chromosomal properties [1,3–6]. Whilst the best hope for survival in NSCLC is surgical resection of early stage lesions, SCLC is generally responsive although rarely cured by cytotoxic therapies, including chemotherapy and radiation therapy. Despite the poor overall results, there is obvious heterogeneity in the response of lung cancer patients to cytotoxic therapies. The variability in this response probably has many causes. Over the past two decades a systematic study of the biologic properties of lung cancer cells has led to a dramatic increase in our understanding of this complex problem. Important amongst these studies has been the use of fresh tissue and established tumor cell lines from patients with lung cancer in the investigation of radiation sensitivity and resistance. The following is a summary of the radiation biology of these cell lines.

RADIATION SENSITIVITY OF LUNG CANCER CELL LINES

The use of cell lines to establish radiation sensitivity dates back to the classic work of Theodore Puck who used the cervical carcinoma cell line (HeLa) to report the first mammalian X-ray survival curve [7]. Over the past decade or more, numerous investigators have reported on the in vitro radiation response of human tumor cell lines for a variety of malignant conditions [8–16]. The major advantages of using established human cell lines for such assays include the availability of large numbers of cells to perform survival, biochemical, cytogenetic, and molecular studies, as well as reproducibility and generally acceptable cloning efficiencies [15]. Such studies have confirmed a heterogeneity of in vitro response which often parallels the behavior of these diseases in the clinic. To illustrate this, the radiation survival curves for established cell lines of six different histologic types of lung cancer are shown in Figure 1A. The most striking feature observed amongst these curves is the variation in the initial shoulder as well as the terminal slope of the different lines, indicative of a differential cell survival. The mean surviving fraction at 2 Gy, thought to be a reliable indicator of radiation sensitivity, is displayed in Figure 1B and also demonstrates the heterogeneous response amongst the differing

Received January 19, 1996.

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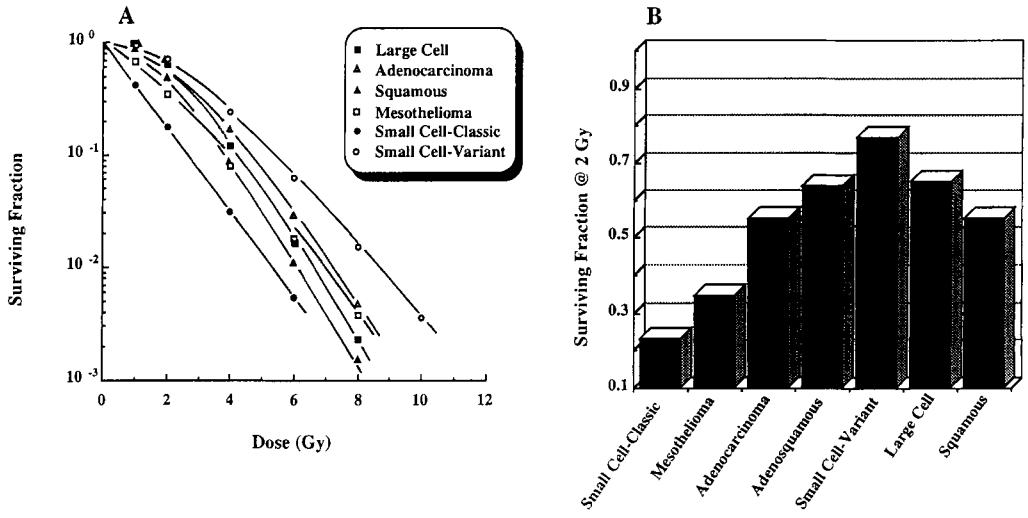

Fig. 1. **A:** Clonogenic cell survival assay for six different lung cancer cell lines as a function of radiation dose. Survival curves illustrate differing radiation sensitivities for the different histologies. The D_0s quoted in the text are representative of the terminal slope of the curves, whilst the extrapolation number ($\bar{n}$) gives an estimate of the size of the initial shoulder. **B:** The surviving fraction at 2 Gy for 22 lung cancer cell lines. Results are taken as the mean of the number of cell lines (n) for each histology: Classic SCLC (n = 5); variant SCLC (n = 3); adenocarcinoma (n = 4); squamous cell (n = 2); adenosquamous (n = 3); large cell (n = 3); mesothelioma (n = 2). Data adapted from [17].

histologies. A summary of the radiation survival curve parameters for selected lines evaluated from the NCI lung cancer cell panel [17], as well as the work of other investigators, confirms this heterogeneity and suggests that the response of lung cancer cell lines can be considered in three groups. Group 1 includes "classic" SCLC lines with D_0 s in the range of 0.76–1.24 Gy and extrapolation numbers ($\bar{n}$) in the 1.0–2.0 range. These were clearly the most radiosensitive. Both large cell carcinoma and "variant" SCLC lines could be considered as belonging to Group 2, with D_0s of 0.76–1.5 Gy and rather large $\bar{n}$ values of 4.6–17.7. Group 3 consists of the adenocarcinoma lines with D_0s in the 1.0–1.4 Gy and $\bar{n}$ of 1.2–6.8. Somewhat surprisingly, mesothelioma cell lines tested appeared radiosensitive with low $\bar{n}$ in the 1.0–1.8 range and D_0 1.3–1.86 Gy, whereas the clinical experience with this tumor would suggest a more radioresistant response.

Therefore the question may be asked, are the intrinsic radiation sensitivity values ($\bar{n}$, D_0, SF 2 Gy) of these cell lines generally predictive of clinical responsiveness or curability? Weichselbaum and his colleagues in studies of cell lines from a variety of malignancies found little or no correlation, while the data from the studies of lung cancer lines would appear predictive [15–17]. Classic SCLC is definitely responsive to radiation treatment in the clinic although rarely cured owing to its capacity for systemic spread [18]. Variant SCLC and NSCLC are clinically less responsive to radiation. Cell lines from responsive SCLC patients are characterized by low $\bar{n}$ values (1.0–2.0) and low SF 2 Gy values, whilst cell lines from variant SCLC or large cell NCSLC tend to have larger $\bar{n}$ values (4.6–17.7) and higher SF 2 Gy values. A large shoulder on the survival curve (or high $\bar{n}$ value) implies a considerable capacity for the repair of sublethal damage caused by the radiation, and thus a greater cell survival. Additionally, for a clinically relevant radiation dose of 2 Gy, the surviving fraction will usually be greater for those cell lines having a higher $\bar{n}$ value [17]. The absence of a shoulder on the SCLC cell lines has important dose-rate implications to be discussed below.

ONCOGENES AND RADIATION SENSITIVITY

Over the past decade there has been an explosion in our understanding of the molecular and genetic events important in the biology of lung cancer [19]. Initial studies focussed on the amplification and/or overexpression of members of the myc family of oncogenes (e.g., c-myc, N-myc, and L-myc). Such alterations were predominantly noted in SCLC [20–22]. Oncogene amplification was more commonly found in established cell lines rather than freshly harvested tumor, and especially so in specimens from heavily pre-treated patients. C-myc amplifica-

tion was particularly noted in variant cell lines of SCLC [23]. In addition, with transfection of a c-myc gene into a classic SCLC line not initially expressing c-myc, the transfected (and now overexpressing) c-myc cells take on the growth and morphologic characteristics of the c-myc amplified SCLC lines [24]. Recently another oncogene, c-jun has been identified at high levels in both SCLC and NSCLC. The c-jun product appears to be a transcription factor and may exert tumor promoting effects [25]. In NSCLC, the ras family of oncogenes has been associated, particularly with adenocarcinoma [26–29]. In one recent study, K-ras oncogene activation was shown to be an independent prognostic marker in adenocarcinoma of the lung [30]. Furthermore, experimental studies have shown that transfecting mutated ras gene into a SCLC cell line, changes its phenotype to one suggesting NSCLC [31]. Further recent work has identified alterations in the tumor suppressor gene (p53) on chromosome 17 as the most frequent genetic change in lung cancer [32–36]. This important finding in the majority of cases of lung cancer thus far studied, allows the potential for exciting novel therapeutic approaches such as gene transfer to be evaluated in the future.

Can specific genetic alterations be shown to be associated with radiation resistance? Abnormalities of ras and myc oncogenes have been associated with poor cancer prognosis and radioresistant cell lines [15–39]. Similarly the raf oncogene family has been implicated in radiation resistance [40,41]. A cell line transformed by ras was shown to have a D_0 greater than the parent line when irradiated at standard, though not at low dose rates [42]. Sklar transfected mutated ras oncogenes into NIH 3T3 cells and significantly increased the intrinsic radioresistance (D_0) [43]. McKenna and colleagues using a primary rat embryo cell (REC) model, suggested that transfection with H-ras oncogene alone conferred little alteration in radiation resistance; whereas, the presence of a co-transfected cooperating oncogene (v-myc) induced a dramatic increase in radioresistance [44–46]. However, these results need to be interpreted with caution, since transfection of REC and human cell lines with non-oncogenic DNA sequences alone (pSV2neo, encoding only for neomycin resistance) and subjecting them to subsequent clonal selection, can also yield clones with increased resistance to radiation [47]. Likewise, considerable heterogeneity in radiation response exists among different clones of the NIH 3T3 cells as used by Sklar, such that when ras, raf, and myc genes were transfected, no clear modification of radiation sensitivity was attained when compared with the non-transfected parent lines [48]. It is clear therefore that although the use of cell lines expressing different genetic features is an important mode of evaluating radiation sensitivity and resistance, caution must be used in interpreting these early results. Further work clarifying the role of clonal heterogeneity in radiation response is required.

DOSE RATE EFFECTS AND LUNG CANCER

It has long been known that *dose rate* is an important determinant of mammalian cell survival in response to radiation [49–51]. Clinical and experimental studies have clearly shown a marked sparing effect in the lung as a result of fractionating radiation doses [52–54]. This process is also known to occur during protracted lung irradiation at low dose rates [55]. In vivo work in the mouse model showed a marked sparing effect of low dose rate irradiation in terms of the development of radiation pneumonitis [53]. Lung carcinoma lines in culture exhibit a marked heterogeneity in response to varying dose rates, as shown in Figure 2. While NSCLC lines (NCI-H460 and A549) show increased survival at low dose rates, the SCLC line (NCI-H69) shows no such variation in response. The absence of an initial shoulder on the SCLC acute dose radiation survival curve probably represents an inability to repair sublethal damage. Thus, it would be expected that the use of low dose rate irradiation (1–5 cGy/min) in SCLC would bring about cell killing equivalent to standard/high dose rate irradiation (100–200 cGy/min), with less toxicity to normal tissue (e.g., lung and esophagus). This strategy has been recently brought to clinical trial at the NCI and early tumor responses are encouraging, with a suggestion of less acute toxicity. Should tumor response rates be equivalent to standard radiation responses, for example, with less acute esophageal toxicity, this might allow for the possibility of escalating the total radiation dose and/or treating greater tumor volumes.

RELATIONSHIP OF RADIATION SENSITIVITY TO CHEMOSENSITIVITY

The in vitro sensitivity of lung cancer cell lines to a large number of chemotherapeutic agents in common usage in the clinic has been

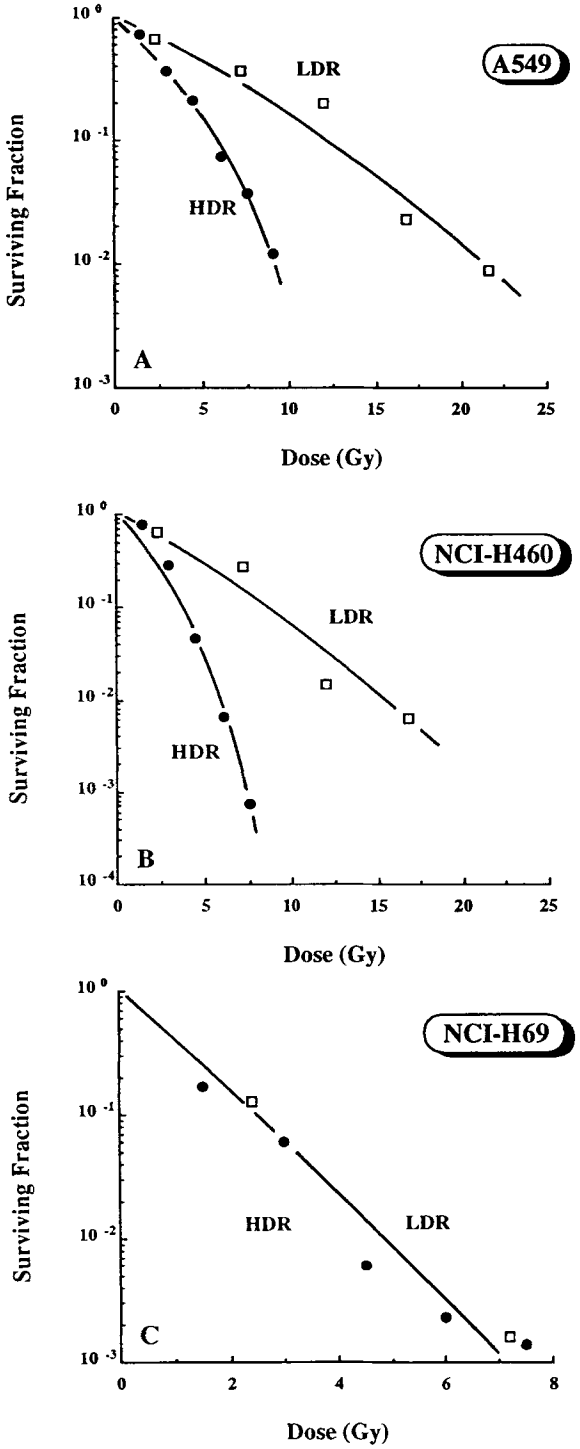

Fig. 2. Clonogenic cell survival assay illustrating the effect of low dose rate (1 cGy/min) vs. high dose rate (200 cGy/min). The upper two panels (**A,B**) show the NSCLC cell lines A549 and NCI-H460. Marked survival advantage is seen at the low dose rates. The lowermost C shows the survival curves generated for the classic SCLC cell line NCI-H69. No significant survival differences are seen between the low and high dose rates; note also the relative lack of an initial shoulder on the SCLC survival curve.

assessed [56]. As is true of radiation sensitivity, there is a wide histologic variance in response. In addition, there was a strong correlation between the prior treatment status of the patient and subsequent response. This in vitro assay of drug response seemed better able to detect tumor cell resistance than sensitivity. The suggestion is made in the clinical literature that patients relapsing following initial chemotherapy seem to be less sensitive to radiation than de novo irradiated patients [57]. Thus, we reviewed our data to see if any correlation exists between measured sensitivity to irradiation and chemotherapy. Furthermore, since modulation in intracellular levels of glutathione has been shown to relate to the cells' ability to survive radiation as well as chemotherapeutic agents [58], we likewise analyzed the data for any such association. We compared the radiation sensitivity of 22 lung cancer cell lines as estimated by surviving fraction at 2 Gy (SF 2 Gy), with the inhibitory concentration (IC_{50}) of the anthracycline, adriamycin, and the alkylator, melphalan [17,56]. Figure 3 shows that there is little if any association between radiation sensitivity and the two drugs assayed in these cell lines. Generally it may be appreciated that classic SCLC and mesothelioma cell lines show sensitivity to both, while NSCLC and the variant SCLC lines are relatively resistant to both. However, no strong correlation is seen in either case. Figure 4 looks for any association between glutathione (GSH) levels with radiation and chemosensitivity [17,56,59]. Again, little correlation is noted, indicating that modulations of glutathione levels are not predictive of radiation, melphalan, or adriamycin response in these particular cell lines.

PHOTOBIOLOGY AND PHOTODYNAMIC THERAPY OF LUNG CANCERS

Given the aforementioned poor responses in lung cancer to standard treatment approaches, there is much need for novel effective therapies in this disease. Several groups have evaluated photodynamic therapy (PDT) as possible treatment for lung cancer in various clinical situations [60–62]. Despite increasing use of PDT for relief of endobronchial by primary and metastatic tumors [63,64], relatively little in vitro work had been done to evaluate this approach. Initial work with the NSCLC cell line A549, revealed substantial sensitivity of these cells to PDT. While neither sensitizer nor light alone were toxic, increasing cell kill was observed with

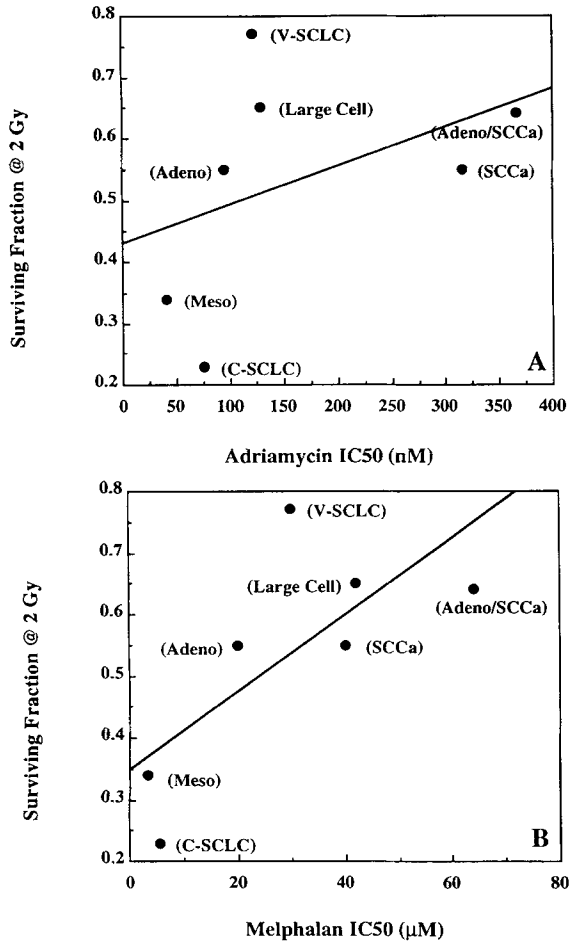

Fig. 3. The comparison between the radiation sensitivity (SF 2 Gy) and the chemosensitivity (adriamycin, **A**; melphalan, **B**) as judged by IC_{50}. Results are reported for 22 cell lines. The SF 2 Gy and the IC_{50} are taken as the mean of the cell lines assayed. The n for each histology is as shown in Figure 1. The correlation coefficients for A and B was 0.18 and 0.52, respectively.

increasing concentrations of sensitizer, and with increasing sensitizer exposure times [65]. In addition, there was a suggestion that low light fluences were associated with increasing cell survivals, indicative of a possible dose rate effect. This was further evaluated [66] and confirmed with an enhancement ratio of 1.6, at the 50% survival level. It should be noted, however, that there is no general acceptance of such an effect with PDT. Gomer et al. were unable to show a dose rate effect in their PDT system [67]. Further work is required to clarify this question. Perry et al. evaluated a panel of six lung cancer cell lines and demonstrated a heterogeneous response among the different histologys [68]. The survival parameters revealed a range

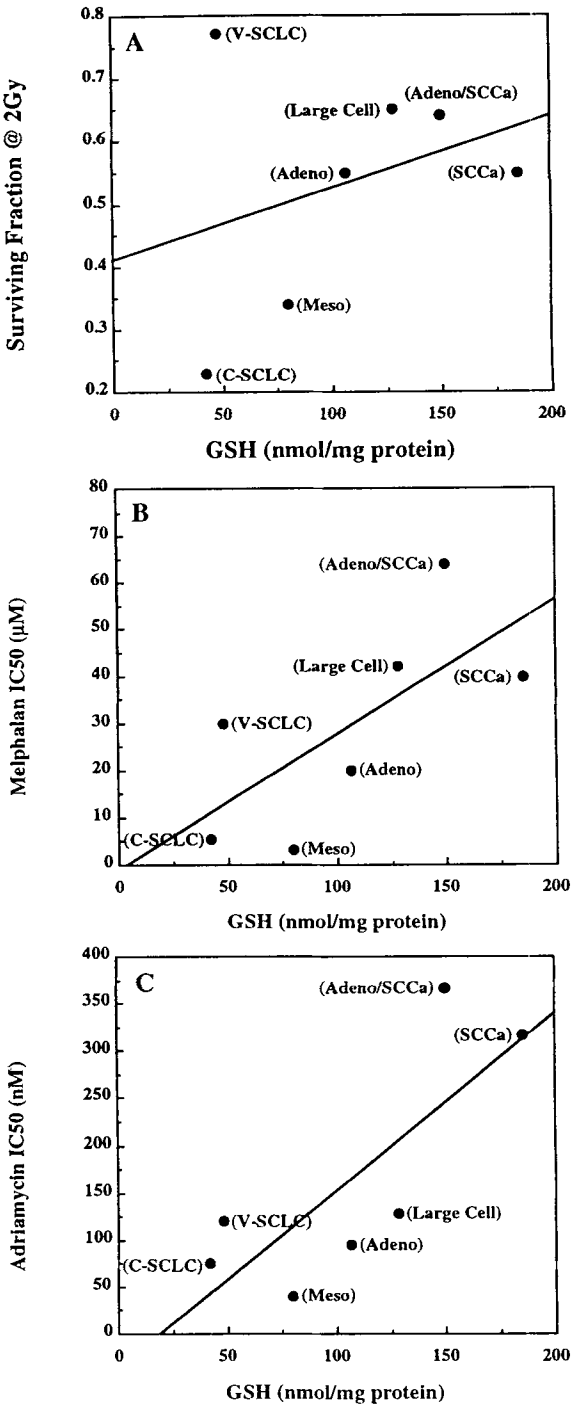

Fig. 4. The comparison between glutathione (GSH) levels and radiation sensitivity (SF 2 Gy) (**A**), chemosensitivity (IC_{50}) to melphalan (**B**) and adriamycin (**C**). Results are reported for 22 cell lines. The SF 2 Gy and the IC_{50} are taken as the mean of the cell lines assayed. The n for each histology is as shown in Figure 1. The correlation coefficients for A, B, and C were 0.10, 0.49, and 0.62, respectively.

of responses, with extrapolation numbers ($\bar{n}$) in the 1.2–51.3 range, and D_0 ranging from 194–378 J/m^2. Since little correlation is seen between the differential histologic sensitivity to either chemotherapy or radiation, a distinctly different mechanism of cytotoxicity is likely. This suggests that PDT may be a potentially useful adjunct to standard therapies with a complimentary mode of cell kill.

SUMMARY

The human lung cancer cell line panel developed by the NCI-Navy Medical Oncology Branch has contributed much to our understanding of the radiobiology of lung cancer. Enhanced methodology allowing for the establishment of large numbers of cell lines from human cancers has provided a potent tool in the quest to understand malignancy, and design rational strategies for its eventual elimination. The yield in relevant biologic information has already been immense. This article has summarized those issues relating to the radiation biology of lung cancer. As the complex molecular biology of these cell lines is further realized, our understanding of radiation damage repair and the factors involved will have relevance not only for this disease, but for cell biology and the treatment of cancer in general. Much important work is yet to be done.

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